

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**22-352**

**CLINICAL PHARMACOLOGY AND  
BIOPHARMACEUTICS REVIEW(S)**



|            |                                           |           |
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## 1 Executive Summary

### 1.1 Recommendation

The submission is acceptable from a Clinical Pharmacology and Biopharmaceutics perspective provided that a mutually satisfactory agreement can be reached between the sponsor and the Agency regarding the language in the package insert.

### 1.2 Phase IV Commitments

None

### 1.3 Summary of Clinical Pharmacology Findings

Mutual Pharmaceutical Company submitted a 505(b)(2) NDA 22-352 for Colstat, colchicine tablets, for the \_\_\_\_\_ (FMF) and \_\_\_\_\_ in adults and children up to 4 years of age. The sponsor is relying on literature for clinical pharmacology in special populations, safety and efficacy data in pediatric and adult FMF patients. Because of rare incidence of this disease, its treatment is an orphan indication. Sponsor conducted relative bioavailability studies comparing Colstat with Col-probenecid, a generic drug by Watson Labs approved for treating acute gout flares.

b(4)

Familial Mediterranean fever (FMF) is a rare hereditary disease characterized by recurrent fever, abdominal and chest pain, arthralgia, and erysipeloid erythema. Some affected individuals may also experience secondary amyloidosis, a potentially life-threatening complication that could result in renal failure. Publications of three randomized double-blind, placebo-controlled, crossover studies involving oral colchicine as prophylaxis of acute attacks of FMF support clinical safety and efficacy in FMF patients. Colchicine formulation, strength indicated in the publications is mentioned below. Several publications suggest that colchicine has anti-inflammatory properties. However, the exact mechanism of the anti-inflammatory actions is unknown and it is an active area of research.

| Publication                                            | Formulation, strength and Dosing           |
|--------------------------------------------------------|--------------------------------------------|
| Zemer et al., N Engl J Med 1974, 291:932-34.           | 0.5 mg colchicine tablet twice daily       |
| Dinarello et al., N Engl J Med 1974;291:934-937.       | 0.6 mg colchicine tablet three times a day |
| Goldstein and Schwabe, Ann Intern Med 1974;81:792-794. | 0.6 mg colchicine tablet three times a day |

Mutual has conducted four clinical PK studies in healthy volunteers to describe single dose and multiple dose pharmacokinetics of colchicine and its metabolites, two drug interaction studies with clarithromycin and oral contraceptives.

In healthy adults, mean maximum plasma colchicine levels after administration of 0.6 – 1.8 mg Colstat are noted around 1.5 -2 hours (see table below). The elimination half-life as calculated following a single oral dose is approximately 5 hours.

**PK parameters of colchicine from different studies.**

|                                                                | C <sub>max</sub><br>(ng/mL) | T <sub>max</sub> <sup>1</sup><br>(h) | AUC <sub>0-t</sub><br>(ng·h/mL) | AUC <sub>∞</sub><br>(ng·h/mL) | Vd/F (L)       | CL/F<br>(L/hr) | Ke <sup>-1</sup><br>(h <sup>-1</sup> ) | t <sub>1/2</sub><br>(h) |
|----------------------------------------------------------------|-----------------------------|--------------------------------------|---------------------------------|-------------------------------|----------------|----------------|----------------------------------------|-------------------------|
| <b>Colchicine 0.6-mg Single Dose (N=13) from study # 1004</b>  | 2.45<br>(28.7)              | 1.50<br>(1.0 – 3.0)                  | 10.5<br>(33.8)                  | 12.3<br>(36)                  | 341<br>(54.3)  | 54.05<br>(31)  | 0.183<br>(32.4)                        | 4.95<br>(89.5)          |
| <b>Colchicine 1.8 mg Single Dose (N=13) from study # 1003</b>  | 6.19<br>(39.3)              | 1.81<br>(1 – 2.5)                    | 43.79<br>(26.1)                 | 52.07<br>(26.3)               | 1188<br>(26.9) | 36.95<br>(27)  | 0.0326<br>(31)                         | 23.6<br>(39)            |
| <b>Colchicine 4.8 mg over 6 hours (N=15) from study # 1002</b> | 6.84<br>(19)                | 4.47<br>(3.12- 7.5)                  | 104.95<br>(23.45)               | 118.2<br>(22)                 | 1876<br>(24.3) | 43<br>(29.8)   | 0.0242<br>(36.6)                       | 31.38<br>(26.6)         |

Study #1001 was conducted to comparative bioavailability of Colstat with Col-probenecid and also to determine food effect on colchicine PK. The pharmacokinetic profile of colchicine was compared following administration of Mutual's 0.6 mg Colstat tablets with Col-Probenecid (Watson Laboratories), containing colchicine 0.5 mg (in addition to probenecid 500 mg) under fasting condition. After dosage adjustment, Colstat tablets produced 25% higher Cmax and 37% higher AUC compared to Col-Probenecid. Dose-adjusted Cmax and AUC for colchicine after Colstat administration and the 90% confidence intervals (CI) were not within the bioequivalence interval of 80 to 125% (See table below).

**PK parameters and Dose-Adjusted Statistical Comparison of Mutual's Colchicine Tablets USP, 0.6 mg (Dose Adjusted to 0.5 mg) and Col-Probenecid, Containing Colchicine 0.5 mg**

| Parameter (units)                  | Colchicine 0.6 mg [Mutual] (N=25) | Colchicine 0.5 mg [Col-Probenecid] (N=25) | Ratio of Dose-adjusted Geometric Means* | 90% CI         |
|------------------------------------|-----------------------------------|-------------------------------------------|-----------------------------------------|----------------|
| C <sub>max</sub> (ng/mL)           | 2.50 (28.85)                      | 1.71 (32.87)                              | 125.10                                  | 111.97, 139.76 |
| AUC <sub>0-t</sub> (ng·h/mL)       | 12.59 (48.62)                     | 8.09 (75.55)                              | 137.43                                  | 122.50, 154.18 |
| AUC <sub>∞</sub> (ng·h/mL)         | 14.11 (39.65)                     | 8.48 (33.36)                              | 137.13                                  | 124.46, 151.09 |
| T <sub>max</sub> <sup>1</sup> (hr) | 1.50 (0.5-2.0)                    | 1.02 (1.0-2.0)                            |                                         |                |
| t <sub>1/2</sub> (hr)              | 6.36 (73.85)                      | 3.83 (33.13)                              |                                         |                |

<sup>1</sup>Tmax is presented as Median

Following coadministration of Colstat with high fat breakfast, the peak plasma colchicine levels were similar but AUC was 15% lower compared to fasting condition.

|  | Arithmetic Mean (%CV) | Statistical Comparison Fed vs. Fasted (n=25) |
|--|-----------------------|----------------------------------------------|
|  |                       |                                              |

| Parameter (units)            | Fasted (N=25)  | High-Fat Meal (N=25) | Ratio of Geometric Means | 90% CI        |
|------------------------------|----------------|----------------------|--------------------------|---------------|
| C <sub>max</sub> (ng/mL)     | 2.50 (28.85)   | 2.50 (27.84)         | 100.37                   | 89.84, 112.14 |
| T <sub>max</sub> (hr)        | 1.50 (0.5-2.0) | 1.50 (0.5-4.0)       | --                       | --            |
| AUC <sub>0-t</sub> (ng·h/mL) | 12.59 (48.62)  | 10.49 (48.62)        | 87.58                    | 78.07, 98.26  |
| AUC <sub>∞</sub> (ng·h/mL)   | 14.11 (39.65)  | 11.40 (25.39)        | 84.55                    | 76.73, 93.15  |

Colstat was generally well tolerated in healthy volunteers at the 0.6 mg – 1.8 dose. In study (1002), Colstat was administered to healthy volunteers at 4.8 mg dose as two 0.6 mg over-encapsulated tablets followed by one 0.6 mg over-encapsulated tablet every hour for six additional doses. The most common adverse events were diarrhea, nausea and vomiting (see table below). All of the subjects experienced diarrhea, while 6 out of 15 subjects vomited between 12.5 to 19.25 hours after the start of dosing (6.5 to 13.25 hours after ingestion of the last dose). It is noteworthy that in study # 1003, only two out of the 13 subjects receiving low dose colchicine (1.8 mg over 2 hours) reported diarrhea. ECG measurements were included in safety evaluation to coincide with the PK measurements. The study was not powered to detect changes in ECG following colchicine treatment. As such there were no treatment-emergent values in QT >450 msec or change from baseline > 30 msec.

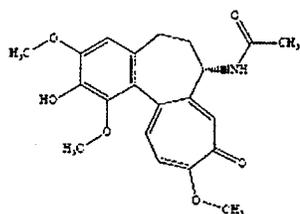
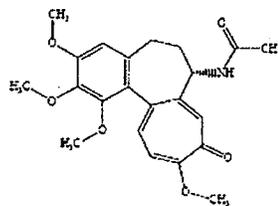
*Colchicine serum protein binding and distribution in blood components*

In vitro, colchicine binding to serum protein is low, 39 ± 5%, primarily to albumin (37 ± 4%), regardless of concentration (Sabouraud et al., Int. J. Clin. Pharmacol. Ther. 32(8)-429, 1994). Equilibrium dialysis was performed using <sup>3</sup>H-colchicine in a wide range of concentrations, from 10<sup>-10</sup>-5 to 10<sup>-6</sup> M (0.04 to 4000 ng/mL). Colchicine also binds to all serum components. Serum colchicine concentrations decline in a bi-exponential mode, reflecting a rapid clearance from the plasma and its redistribution to leukocytes (Chappey et al., Clin Pharmacol Ther 1993; 54:360-367) and red blood cells (Sabouraud et al., Int. J. Clin. Pharmacol. Ther. 32(8)- 429, 1994).

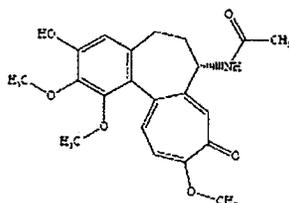
*Metabolism of colchicine*

Tateishi et. al reported (Biochem Pharmacol 1997;10:111-116) that following incubation of 5 nM (2 ng/mL) of <sup>3</sup>H-colchicine with 200 pmol of CYP450 protein for 60 minutes, two peaks were noted, 2-desmethylcolchicine (DMC) and 3-DMC. These accounted for 5.5% and 9.8% of the substrate, respectively.

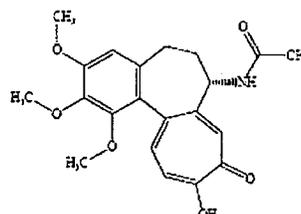
**Chemical Structure of Colchicine and Its Human Metabolites  
(% Formed in Human Microsomal Preparations)**



**2-O-Demethylcolchicine**  
(5.5%)



**3-O-Demethylcolchicine**  
(9.8%)



**10-O-Demethylcolchicine**  
(Not Detected)

In vitro drug metabolism studies by the sponsor showed that Colchicine was metabolized by NADPH-dependent CYP450s with approximately less than 5% disappearance at the conditions examined. Reaction phenotyping with recombinant CYP450s revealed some turnover by specific P450s including CYP3A4, CYP2A6, CYP2B6, CYP2C8, CYP2C9, and CYP2C19. With regard to colchicine metabolites identified in publications, plasma samples were analyzed for 2-O-demethylcolchicine (2-DMC) and 3-O-demethylcolchicine (3-DMC). In the clinical PK studies, plasma samples were analyzed for colchicine metabolites. The systemic exposure to each of these metabolites was <5% when compared to colchicine plasma levels. Often the plasma levels of these metabolites were below the limit of quantitation in many subjects.

*Colchicine is primarily eliminated in the urine*

Following administration of a 1 mg dose to 12 healthy volunteers about 40 to 65% of the absorbed dose was recovered unchanged in the urine (Achtert *et al.*, 1989 Eur. J. Drug Metab. Pharmacokinet. 14(4):317-322). Colchicine is not significantly removed by hemodialysis such that a dose adjustment is required (Bennett *et al.*, 1988).

*Colchicine is a P-gp substrate.*

The kinetics of colchicine uptake and efflux at a clinically relevant concentration (6.25 nM; 2.5 ng/mL) has been studied in two different lines of cultured human cells (HL-60 and HL-60/DNR). HL60DNR cells contain 25 times more P-gp than do HL60 cells. In the HL60/DNR cells, the accumulation of colchicine was 24 times lower than in the HL60 cells (Declèves *et al.*, Toxicol Appl Pharmacol 2006;217:153-60).

*Colchicine is not a P-gp inhibitor*

The potential for colchicine (10 µM; 4 µg/mL and 100 µM; 40 µg/mL) to inhibit the basal-to-apical (BL-AP) apparent permeability ( $P_{app}$ ) of [<sup>3</sup>H]-paclitaxel, [<sup>3</sup>H]-vinblastine, and [<sup>3</sup>H]-

digoxin was tested in MDCK/MDR1, and MDCK-wildtype (WT) cell monolayers. At the highest test concentration, colchicine significantly ( $P < 0.05$ ) inhibited the BL-AP  $P_{app}$  of only one of the three substrates tested, [ $^3$ H]-vinblastine. Typical plasma levels of colchicine are around 10 ng/mL or 0.03  $\mu$ M. (Taub ME et. al, Drug Metab Dispos 2005;33:1679-87)

### Intrinsic Factors

*Pharmacokinetic studies were not conducted by the sponsor to address the influence of age, race, bodyweight, pregnancy and organ dysfunction. However, several publications addressing these intrinsic factors have been discussed in the NDA.*

With regard to gender, pharmacokinetic parameters from different studies conducted by Mutual were comparable between male and female subjects (see table below).

### Mean (%CV) Colchicine Pharmacokinetic Parameter Values for Male and Female Healthy Volunteers in Mutual-Sponsored Studies

| Study No.              | Dose                        | Sex (N)      | $C_{max}$ (ng/mL) | $AUC_{0-4}$ (ng·h/mL) | $AUC_{\infty}$ (ng·h/mL) | $t_{1/2}$ (h) | CL/F (L/h)  | Weight Adjusted CL/F (L/hr/kg) |
|------------------------|-----------------------------|--------------|-------------------|-----------------------|--------------------------|---------------|-------------|--------------------------------|
| <u>MPC-004-07-1001</u> | 0.6 mg single-dose          | Males (9)    | 2.3 (24.8)        | 9.5 (25.3)            | 11.0 (18.9)              | --            | 56.6 (21.4) | 0.71 (32.5)                    |
|                        |                             | Females (16) | 2.6 (29.7)        | 14.3 (48.4)           | 16.0 (39.0)              | --            | 44.1 (44.3) | 0.68 (48.2)                    |
| <u>MPC-004-07-1003</u> | 1.2 mg over 2 hours         | Males (6)    | 5.1 (34.8)        | 40.7 (27.8)           | 49.2 (29.4)              | 24.9 (50.9)   | 39.0 (26.1) | 0.51 (18.6)                    |
|                        |                             | Females (7)  | 7.2 (36.8)        | 46.4 (25.2)           | 54.5 (25.0)              | 22.6 (26.1)   | 35.2 (29.1) | 0.53 (31.1)                    |
| <u>MPC-004-07-1002</u> | 4.8 mg over 6 hours         | Males (8)    | 6.8 (22.5)        | 102.5 (26.3)          | 116.0 (24.2)             | 31.2 (33.9)   | 44.4 (33.6) | 0.58 (44.0)                    |
|                        |                             | Females (7)  | 6.9 (15.8)        | 107.7 (21.8)          | 120.8 (21.1)             | 31.6 (18.1)   | 41.7 (26.5) | 0.66 (24.9)                    |
| <u>MPC-004-07-1006</u> | Day 1<br>0.6 mg single-dose | Males (11)   | 2.9 (31.7)        | 11.2 (38.1)           | 13.2 (39.7)              | --            | 53.1 (43.6) | 0.70 (42.1)                    |
|                        |                             | Females (12) | 2.8 (31.5)        | 13.5 (36.5)           | 17.7 (51.5)              | --            | 41.1 (40.4) | 0.62 (44.8)                    |

*Colchicine PK in elderly: Pharmacokinetics of colchicine was not evaluated in elderly. However, considering decrease in renal and hepatic function in elderly caution is necessary when using Colstat.*

*Colchicine PK in pediatric patients: The sponsor did not conduct any PK studies in pediatric patients. Search of public databases did not reveal any publications on PK of colchicine in pediatric patients.*

*Colchicine PK in renal impairment patients: Colchicine is significantly excreted in the urine. Four-fold decrease in colchicine clearance is noted in severe renal impaired subjects undergoing hemodialysis compared to healthy volunteers. Dosage reduction should be considered in subjects with mild and moderate renal impairment. In patients with severe renal impairment and undergoing dialysis the total daily dosage should receive 0.3 to 0.6 mg colchicine per day.*

Following administration of a 1 mg dose to 12 healthy volunteers about 40 to 65% of the absorbed dose was recovered unchanged in the urine (Achtert et al., 1989 Eur. J. Drug Metab. Pharmacokinet. 14(4):317-322).

Ben-Chitrit et. al. 1994 (J. Rheumatology, 21(4): 710) published a report on pharmacokinetics of colchicine in patients with FMF without and with severe renal impairment. As tabulated below, nine patients with FMF, 5 women and 4 men, 19-42 years old (mean 30 years) were given 1.0 mg of colchicine, with the exception of the patient who also had cirrhosis, who received 0.5 mg of colchicine. The extent of renal impairment was not characterized in the study, however, all 5 patients had biopsy-proven amyloidosis; 4 were on routine hemodialysis and 1 had a serum creatinine clearance of 15 mL/min. Subjects received two 0.5 mg colchicine tablets and blood samples were collected until 48 hours after dosing.

A significant decrease (4-fold) in clearance of colchicine was noted in FMF patients ( $0.168 \pm 0.063$ ) with severe renal impairment (see table below) compared to those with normal renal function ( $0.727 \pm 0.11$ ), where the plasma half-life of colchicine was 4 times shorter.

**Pharmacokinetics of colchicine in FMF patients with or without renal impairment.**

| Subject No.    | C <sub>max</sub><br>(ng/ml) | T <sub>max</sub><br>(h) | t <sub>1/2</sub><br>(h) | Cl/F<br>(l/h/kg) | Vd/F<br>(l/kg) |      |
|----------------|-----------------------------|-------------------------|-------------------------|------------------|----------------|------|
| <b>Group A</b> |                             |                         |                         |                  |                |      |
| 1              | 3.9                         | 1.0                     | 3.6                     | 0.746            | 3.86           |      |
| 2              | 5.1                         | 2.0                     | 4.2                     | 0.765            | 4.65           |      |
| 3              | 5.2                         | 1.0                     | 3.9                     | 0.570            | 3.16           |      |
| 4              | 8.8                         | 2.0                     | 5.8                     | 0.825            | 7.80           |      |
| Mean ± SD      | 5.8                         | 1.5                     | 4.4                     | 0.727            | 4.87           |      |
|                | 2.1                         | 0.6                     | 1.0                     | 0.110            | 2.05           |      |
| <b>Group B</b> |                             |                         |                         |                  |                |      |
| 5              | 7.3                         | 2.0                     | 18.0                    | 0.113            | 2.96           |      |
| 6              | 11.0                        | 2.0                     | 19.2                    | 0.125            | 3.45           |      |
| 7              | 8.3                         | 1.0                     | 20.3                    | 0.183            | 5.39           |      |
| 8              | 4.6                         | 2.0                     | 17.7                    | 0.250            | 6.45           |      |
| Mean ± SD      | 7.8                         | 1.8                     | 18.8                    | 0.168            | 4.56           |      |
|                | 2.6                         | 0.5                     | 1.2                     | 0.063            | 1.64           |      |
| p<             | NS                          | NS                      | 0.001                   | 0.001            | NS             |      |
| Cirrhosis      | 9*                          | 4.5                     | 1.0                     | 50.0             | 0.078          | 5.77 |

\* Patient 9 received 0.5 mg colchicine.

One patient with cirrhosis had a 10-fold lower clearance compared to the average clearance noted in healthy volunteers in this study.

*Colchicine PK in patients with hepatic impairment: PK studies of Colstat were not conducted patients with hepatic impairment. However, published studies with IV colchicine and oral colchicine in patients with severe hepatic impairment are available. Caution is warranted when Colstat is considered for patients with mild hepatic*

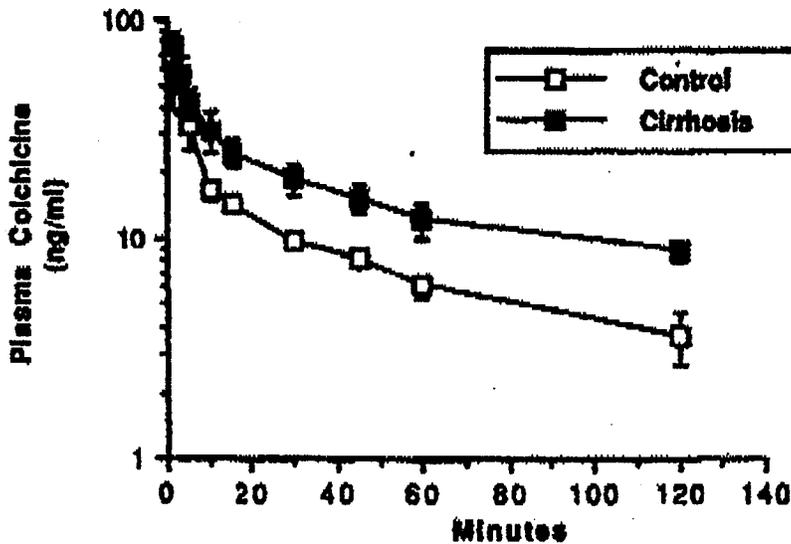
impairment. Dose reduction should be considered in patients with moderate and severe hepatic impairment.

Pharmacokinetics of 1 mg colchicine administered intravenously in 9 patients with alcoholic cirrhosis and compared to 6 healthy men. Of these patients, three had liver biopsy specimens showing cirrhosis and five patients had ascites. Following administration of colchicine 1 mg as an intravenous bolus, blood samples were only collected over 2 hours post-dose and measured using an HPLC assay with inadequate sensitivity to characterize the pharmacokinetic profile (limit of detection was 6 ng/mL).

PK parameters of colchicine in healthy volunteers and alcoholic cirrhosis patients

| Subjects         | Pugh score | Bilirubin | Albumin | Prothrombin time | Half-life (minutes)  | Volume of distribution (L/kg) | Clearance (ml/min/kg) |
|------------------|------------|-----------|---------|------------------|----------------------|-------------------------------|-----------------------|
| <b>Normal</b>    |            |           |         |                  |                      |                               |                       |
| 1                |            | 0.4       | 4.1     | 12.3             | 111.53               | 1.06                          | 6.586                 |
| 2                |            | 0.4       | 4.4     | n.a.             | 59.62                | 0.593                         | 0.898                 |
| 3                |            | 0.6       | 4.3     | 12.8             | 76.8                 | 0.795                         | 7.17                  |
| 4                |            | n.a.      | n.a.    | n.a.             | 54.66                | 0.918                         | 11.687                |
| 5                |            | 0.6       | 4.6     | 11.5             | 25.39                | 0.542                         | 14.788                |
| 6                |            | 0.3       | 4.1     | 13               | 16.47                | 0.4                           | 16.835                |
| <b>Average</b>   |            |           |         |                  | <b>57.4 ± 14.2</b>   | <b>0.718 ± 0.102</b>          | <b>10.65 ± 1.82</b>   |
| <b>Cirrhotic</b> |            |           |         |                  |                      |                               |                       |
| 1                | 6          | 2.4       | 3.6     | 14.6             | 175.56               | 0.783                         | 3.091                 |
| 2                | 5          | 1         | 4.9     | 12.8             | 64.09                | 0.292                         | 3.155                 |
| 3                | 5          | 0.9       | 3.7     | 12.9             | 69.24                | 0.327                         | 3.27                  |
| 4                | 7          | 1.2       | 2.8     | 16.6             | 145.5                | 0.747                         | 3.558                 |
| 5                | 9          | 7.2       | 3       | 16.5             | 70.68                | 0.388                         | 3.807                 |
| 6                | 6          | 1.5       | 3.4     | 13.5             | 82.46                | 0.356                         | 3.954                 |
| 7                | 8          | 9.5       | 3       | 12.9             | 220.46               | 1.438                         | 4.521                 |
| 8                | 9          | 2         | 2.2     | 16.1             | 74.52                | 0.671                         | 5.311                 |
| 9                | 6          | 2         | 3       | 14.6             | 147.27               | 1.546                         | 7.275                 |
| <b>Average</b>   |            |           |         |                  | <b>114.4 ± 19.7*</b> | <b>0.716 ± 0.158</b>          | <b>4.22 ± 0.45*</b>   |

PK profile of colchicine in healthy volunteers and alcoholic cirrhosis patients



In spite of the noted deficiencies with regard to study design (short duration of plasma sample collection, and IV use of colchicine), the observed 2.5-fold decrease in clearance of colchicine in patients with hepatic impairment ( $4.2 \pm 0.45$  ml/min/kg) compared to healthy volunteers ( $10.65 \pm 1.85$  ml/min/kg) is noteworthy. As explained above in the renal impairment section, one patient with cirrhosis had a 10-fold lower clearance and 10-fold higher  $T_{1/2}$  compared to the average clearance noted in healthy volunteers (Ben-Chitrit et. al. 1994 J. Rheumatology, 21(4): 710).

Rudi et al (1994 Scand J Gastroenterol 1994;29:346-51) studied the single dose pharmacokinetics of single-dose colchicine in 8 patients with chronic liver disease without ascites. Six patients with chronic liver disease received a single 1-mg dose of colchicine after an overnight fast.

#### Pharmacokinetics of colchicine in patients with hepatic impairment

| Patient | $C_{max}$<br>(ng/ml) | $C_{24h}$<br>(ng/ml) | $t_{max}$ (h) | $t_{1\alpha}$ (h) | $t_{1\beta}$ (h) | $AUC_{0-24}$<br>(ng · h/ml) | $AUC_{0-\infty}$<br>(ng · h/ml) | Cl/F (l/h) | Vd/F (l) |
|---------|----------------------|----------------------|---------------|-------------------|------------------|-----------------------------|---------------------------------|------------|----------|
| 1 A.S.  | 10.0                 | 0.8                  | 3.0           | 1.18              | 15.9             | 46.6                        | 65.4                            | 30.6       | 701      |
| 2 K.S.  | 4.3                  | 0.6                  | 3.0           | 2.02              | 13.5             | 28.1                        | 39.8                            | 50.3       | 978      |
| 3 E.H.  | 4.8                  | 0.7                  | 1.5           | 5.61              | 18.2             | 33.8                        | 52.2                            | 38.3       | 1010     |
| 4 M.F.  | 2.9                  | 0.0                  | 2.0           | 1.30              | 4.3              | 8.1                         | 8.4                             | 236.4      | 375      |
| 5 K.Y.  | 2.1                  | 0.2                  | 0.8           | 3.88              | 10.7             | 13.4                        | 13.6                            | 120.0      | 1850     |
| 6 H.P.  | 2.8                  | 0.0                  | 1.0           | 1.33              | 1.5              | 5.0                         | 5.5                             | 330.6      | 731      |
| 7 G.P.  | 1.5                  | 0.0                  | 3.0           | 1.93              | 6.2              | 5.4                         | 8.9                             | 223.8      | 2010     |
| 8 M.S.  | 0.4                  | 0.0                  | 3.0           | NC                | 8.2              | 2.5                         | 5.4                             | 370.8      | 3930     |

\*  $C_{max}$  = peak colchicine concentration;  $C_{24h}$  = plasma colchicine concentration after 24 h before next dosing;  $t_{max}$  = peak time;  $t_{1\alpha}$  = distribution half-life;  $t_{1\beta}$  = terminal plasma elimination half-life;  $AUC_{0-24}$  = area under the plasma concentration time curve, time 0-24 h;  $AUC_{0-\infty}$  = area under the plasma concentration time curve, extrapolated from time 0 to infinity; Cl/F = total plasma colchicine clearance; Vd/F = apparent volume of distribution; NC = not calculated because plasma concentrations were too low.

It is noteworthy that subjects 2, 3, 6, 8, 9 and 10 are elderly patients with hepatic impairment. Colchicine levels in plasma and bile were analyzed by radioimmunoassay method. The method has a detection limit of 0.15 ng/ml, and the calibration curve is linear in the range of 0.15 to 10 ng/ml, with inter- and intra-assay variations of 8.2% and 9.0%, respectively. The PK parameters ( $C_{max}$  and  $AUC_{0-\infty}$ ) from the above table are presented after normalizing to a 0.6 mg colchicine dose. In comparison, healthy volunteers receiving 0.6 mg Colstat tablets under fasting condition had an average  $C_{max}$  of  $2.5 \pm 2$  ng/mL and AUC of  $14.11 \pm 7$  ng·h/mL. Although some hepatic impaired subjects have a 2- to 3.5 fold higher  $AUC_{0-\infty}$ , some have a lower exposure when compared to the AUC data from study # 1004.

#### Colchicine $C_{max}$ and AUC normalized to 0.6 mg dose

| Patient | Age<br>(years) | Body<br>weight<br>(kg) | Child<br>Pugh<br>Class | $C_{max}$               | AUC                     | Fold increase<br>with respect<br>average healthy<br>volunteer data |
|---------|----------------|------------------------|------------------------|-------------------------|-------------------------|--------------------------------------------------------------------|
|         |                |                        |                        | Normalized<br>to 0.6 mg | normalized<br>to 0.6 mg |                                                                    |
| 3 E.H.  | 73             | 73                     | B                      | 2.88                    | 31.32                   | 2.2                                                                |
| 1 A.S.  | 36             | 52                     | B                      | 6                       | 39.24                   | 2.8                                                                |
| 8 M.S.  | 60             | 75                     | B                      | 0.24                    | 3.24                    | 0.2                                                                |
| 7 G.P.  | 25             | 71                     | B                      | 0.9                     | 5.34                    | 0.4                                                                |

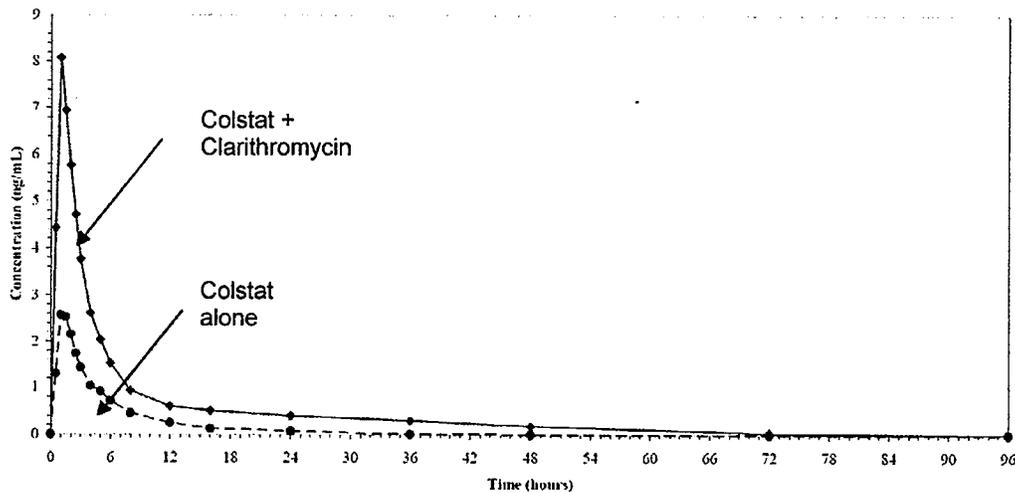
|        |    |    |   |      |       |     |
|--------|----|----|---|------|-------|-----|
| 5 K.Y. | 21 | 72 | B | 1.26 | 8.16  | 0.6 |
| 4 M.F. | 24 | 66 | B | 1.74 | 5.04  | 0.4 |
| 6 H.P. | 51 | 69 | C | 1.68 | 3.3   | 0.2 |
| 2 K.S. | 55 | 63 | C | 2.58 | 23.88 | 1.7 |

### Extrinsic Factors: Drug Interaction Studies

*Colchicine - clarithromycin drug interaction (Study # 1006): A three fold increase in colchicine C<sub>max</sub> and AUC is noted when Colstat is coadministered with clarithromycin. The drug interaction appears mainly due to P-gp inhibition rather than CYP3A4 inhibition, but additional ongoing studies could clarify the role of CYP3A4. Hence, dosage adjustment is necessary with other strong CYP3A4 and P-gp inhibitors. Colstat dose should be reduced 2/3<sup>rd</sup> to 0.3 mg twice daily when patients with FMF are also on strong CYP3A4 and P-gp inhibitors such as clarithromycin, ketoconazole, ritonavir, atazanavir, saquinavir, nefazodone, etc. Sponsor has several ongoing drug interaction studies with ritonavir, ketoconazole, grapefruit juice, cyclosporine. These studies may provide insight into Colstat dose adjustment when coadministered with other CYP3A4 and P-gp inhibitors.*

Study # 1006 was conducted to follow up on in vitro study results showing that colchicine is a substrate of CYP3A4 and P-gp. Clarithromycin, a strong inhibitor of P-gp and CYP3A4 according to the FDA guidance document, was given in a 7-day regimen (250 mg b.i.d.). There was a significant increase in exposure (3-fold in C<sub>max</sub>, 3.4-fold in AUC) to colchicine when a single dose was administered with steady-state clarithromycin, respectively (See Figure and Table below). One individual had 9.5-fold increase, two with a 5-fold increase in colchicine AUC following clarithromycin coadministration with Colstat. Colchicine plasma half-life increased from 8 hours to 30 hours on an average.

Plasma colchicine profile following administration of Colstat alone or with clarithromycin



| Parameter (units)                 | Arithmetic Mean (%CV)      |                                          |
|-----------------------------------|----------------------------|------------------------------------------|
|                                   | Median (Range) for Tmax    |                                          |
|                                   | Colchicine Alone<br>(N=23) | Colchicine +<br>Clarithromycin<br>(N=23) |
| AUC0-t (ng·hr/mL)                 | 12.37 (37.64)              | 41.95 (23.31)                            |
| AUC0-inf (ng·hr/mL)               | 15.53 (49.6)               | 52.62 (22.84)                            |
| Cmax (ng/mL)                      | 2.84 (30.97)               | 8.44 (17.63)                             |
| Tmax (hr)                         | 1.50 (0.50-2.00)           | 1.00 (0.50-2.00)                         |
| Kel (1/hr)                        | 0.1324 (46.87)             | 0.0296 (87.99)                           |
| t1/2 (hr)                         | 8.89 (126.38)              | 30.31 (41.31)                            |
| CL/F (L/hr)                       | 46.8 (43.68)               | 12.0 (23.75)                             |
| Weight-Adjusted CL/F<br>(L/hr/kg) | 0.66 (42.86)               | 0.17 (24.07)                             |
| Vd (L)                            | 431.89 (56.11)             | 493.43 (33.59)                           |

Colchicine clearance (CL/F) was decreased by 75% when co-administered with clarithromycin as compared to the reported total apparent oral clearance when colchicine was administered alone (12.0 L/hr versus 46.8 L/hr). As such the metabolites of colchicine were very low in plasma following Colstat alone or with clarithromycin. Since only about 5 to 20% of the metabolism of colchicine is via hepatic metabolism, these results suggest that factors such as P-gp inhibition may affect the disposition of colchicine to a far greater extent than the inhibition of CYP3A4 enzyme.

Fourteen of 24 subjects experienced a total of 37 AEs over the course of the study, 7 subjects (29.2%) with 21 AEs following the administration of colchicine only, 5 subjects (20.8%) with 7 AEs while receiving clarithromycin on an outpatient basis, and 4 subjects (17.4%) with 8 AEs following co-administration of colchicine with clarithromycin. Headache and gastrointestinal disorders were the most common treatment-emergent adverse events following administration of colchicine, occurring in 5 subjects (20.8%)

and 4 subjects (16.6%), respectively. With respect to GI events, there were 3 subjects (12.5%) with nausea and 2 subjects (8.3%) with diarrhea, the latter occurring only when subjects received colchicine in combination with clarithromycin.

Electrocardiogram measurements for the determination of QTcF interval were made at predose and 1 hour post dose for Period I and Period II. Categorical tabulations (counts and percentages) were provided for all time points after baseline combined. The categorical parameters are based on the following:

- QTcF outliers (in categories of >450 to ≤480, >480 to ≤500, >500 msec),
- Change in QTcF outliers (in categories of >30 to ≤60, >60 to ≤90, >90 msec)

There were no QTcF outliers based on the above definition.

*Drug Interaction Study with Oral Contraceptives: Multiple dose administration of colchicine does not affect plasma levels of ethinyl estradiol and norethindrone.*

A single-center, double-blind, randomized, placebo-controlled, two-cycle crossover study (# 1005) in healthy women was conducted to determine the effect of colchicine on the steady-state pharmacokinetic profile of ethinyl estradiol and norethindrone. Subjects were required to be on an ethinyl estradiol (0.35 mg) and norethindrone (1 mg) oral regimen (1 tablet every day for 21 days, followed by one placebo tablet every day for 7 days) for at least one-cycle prior to study start date. An open-label, one-cycle contraceptive Run-in Period was required for subjects whose prior oral contraceptive regimen was not Ortho-Novum® 1/35 or its generic equivalent given in the recommended regimen. No pharmacokinetic assessments were done during this period. In the double-blind pharmacokinetic Period, subjects continued to receive Ortho-Novum® 1/35 for two additional cycles, administered in the morning. They were randomly assigned to one of two sequences of colchicine and placebo coadministration, given during the final 14 days of “active” Ortho-Novum® 1/35 tablets (Days 8 to 21). When randomized to colchicine, subjects received over-encapsulated colchicine tablets 0.6 mg b.i.d. for 14 days; when randomized to placebo, they received matching placebo capsules for 14 days.

Geometric Mean Ethinyl Estradiol and Norethindrone Pharmacokinetic Parameter Values When Administered with Placebo versus Ethinyl Estradiol and Norethindrone Pharmacokinetic Parameter Values When Administered with Steady-State Colchicine (0.6 mg b.i.d. × 14 days) in Healthy Adult Females (MPC-004-07-1005)

| Parameter                       | Ethinyl Estradiol (EE) (n=27) |                              | Statistical Comparison<br>EE + colchicine vs. EE + placebo   |               |
|---------------------------------|-------------------------------|------------------------------|--------------------------------------------------------------|---------------|
|                                 | EE + colchicine<br>(test)     | EE + placebo<br>(reference)  | Ratio of Geometric<br>Means*                                 | 90% CI        |
| C <sub>max</sub> (ng/mL)        | 0.13                          | 0.14                         | 91.20                                                        | 85.93, 96.79  |
| AUC <sub>(0-∞)</sub> (ng·hr/mL) | 1.18                          | 1.24                         | 95.71                                                        | 91.23, 100.41 |
| Parameter                       | Norethindrone (NTD) (n=27)    |                              | Statistical Comparison<br>NTD + colchicine vs. NTD + placebo |               |
|                                 | NTD + colchicine<br>(test)    | NTD + placebo<br>(reference) | Ratio of Geometric<br>Means*                                 | 90% CI        |
| C <sub>max</sub> (ng/mL)        | 23.40                         | 23.71                        | 98.67                                                        | 91.9, 105.95  |
| AUC <sub>(0-∞)</sub> (ng·hr/mL) | 167.52                        | 171.95                       | 97.43                                                        | 92.82, 102.26 |

\* Geometric Mean Ratio = 100 exp (test-reference)  
CI = confidence interval

As shown in the table above, when ethinyl estradiol and norethindrone are co-administered with steady-state colchicine (0.6 mg b.i.d. × 14 days) hormone concentrations were not affected as compared to hormone concentrations observed when ethinyl estradiol and norethindrone were administered with placebo. This is based on the 90% confidence intervals for geometric mean ratio of ethinyl estradiol and norethindrone being within the 80 – 125% confidence interval limits.

#### *Colchicine may not inhibit major CYPs*

In vitro study results indicate that the potential for CYP inhibition type drug interactions with colchicine is “remote”. In vitro studies in human liver microsomes were conducted to evaluate the potential of colchicine to inhibit the activities of cytochrome P450 (CYP) isoforms CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 in human liver microsomes. Colchicine inhibited activities of CYP2A6 and CYP2C8 in human liver microsomes without dose-dependent pattern. The inhibitory concentration 50% (IC<sub>50</sub>) values were greater than 45.82 μM. Peak plasma levels of colchicine are in the range of 12 ng/mL or 0.03 microM. Hence, the Agency suggested I/Ki or I/IC<sub>50</sub> for CYP2A6 or CYP2C8 inhibition is <0.01, indicating the remote likelihood of CYP inhibition by colchicine. Colchicine did not inhibit activities of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 in human liver microsomes.

#### *Colchicine may not induce major CYP enzymes*

Colchicine at the tested concentrations (0.25 – 25 microM) did not induce activities of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 in hepatocytes from any of the three donors. Colchicine decreased the activity of the CYPs studied. The reasons for this decrease in activity levels are unknown and the results should be interpreted with caution since this observation may have resulted due to loss in hepatocyte viability. Further studies assessing cell viability may provide more insight into this observation.

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## 2 QBR

### 2.1 General Attributes

Mutual Pharmaceutical Company submitted a 505(b)(2) NDA 22-352 for Colstat, colchicine tablets, for the \_\_\_\_\_ familial Mediterranean fever (FMF) and \_\_\_\_\_ in adults and children up to 4 years of age. The sponsor is relying on literature for clinical pharmacology in special populations, safety and efficacy data in pediatric and adult FMF patients. Because of rare incidence of this disease, its treatment is an orphan indication.

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Mechanism of action: Several publications suggest that colchicine has anti-inflammatory properties. However, the exact mechanism of the anti-inflammatory actions is unknown and it is an active area of research.

### 2.2 General Clinical Pharmacology

Familial Mediterranean fever (FMF) is a rare hereditary disease characterized by recurrent fever, abdominal and chest pain, arthralgia, and erysipeloid erythema. Some affected individuals may also experience secondary amyloidosis, a potentially life-threatening complication that could result in renal failure. Publications of three randomized double-blind, placebo-controlled, crossover studies involving oral colchicine as prophylaxis of acute attacks of FMF support clinical safety and efficacy in FMF patients. Colchicine formulation, strength indicated in the publications is mentioned below.

| Publication                                            | Formulation, strength and Dosing           |
|--------------------------------------------------------|--------------------------------------------|
| Zemer et al., N Engl J Med 1974, 291:932-34.           | 0.5 mg colchicine tablet twice daily       |
| Dinareello et al., N Engl J Med 1974;291:934-937.      | 0.6 mg colchicine tablet three times a day |
| Goldstein and Schwabe, Ann Intern Med 1974;81:792-794. | 0.6 mg colchicine tablet three times a day |

Mutual has conducted four clinical PK studies in healthy volunteers to describe single dose and multiple dose pharmacokinetics of colchicine and its metabolites, two drug interaction studies with clarithromycin and oral contraceptives.

| Study Number    | Duration of Treatment     | Objective(s) of the Study                                                                                                                                                       | Study Design and Type of Control       | Test Product(s); Dosage Regimen; Route of Administration | Number of Subjects (Completed) | Healthy Subjects or Diagnosis of Patients |
|-----------------|---------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------|----------------------------------------------------------|--------------------------------|-------------------------------------------|
| MPC-004-07-1001 | Single dose               | Bioavailability of colchicine tablets, 0.6 mg, when administered under standard fasting conditions and compared to Col-Probenecid                                               | Crossover                              | 1 tablet, 0.6 mg, oral                                   | 28 (25)                        | Healthy Subjects                          |
| MPC-004-07-1002 | Single dose               | Determine the pharmacokinetic profile of colchicine and its metabolites, 2-, 3-, and 10-demethylcolchicine                                                                      | Double-Blind, Double-Dummy             | 1 tablet, 0.6 mg, Oral                                   | 18 (18)                        | Healthy Subjects                          |
| MPC-004-07-1003 | Single dose               | Determine the pharmacokinetic profile of colchicine and its metabolites                                                                                                         | Open-Label                             | 2 tablets, .06 mg, Oral                                  | 13 (13)                        | Healthy Subjects                          |
| MPC-004-07-1004 | Single dose multiple dose | Determine the single and multiple dose pharmacokinetics of colchicine                                                                                                           | Open-Label                             | 1 tablet, 0.6 mg, Oral                                   | 13 (13)                        | Healthy Subjects                          |
| MPC-004-07-1005 | Multiple dose             | Determine whether steady-state dosing of colchicine tablets USP, 0.6 mg influences the steady-state Pharmacokinetic profile of ethinyl estradiol or norethindrone               | Double-blind, randomized, two sequence | 0.6 mg b.i.d., Oral                                      | 30 (27)                        | Healthy Subjects                          |
| MPC-004-07-1006 | Single dose               | Confirm the extent to which multiple oral doses of clarithromycin influence the single dose pharmacokinetic profile of colchicine and its metabolites in healthy adult subjects | Open-Label                             | 1 tablet, 0.6 mg, Oral                                   | 24 (23)                        | Healthy Subjects                          |

In addition, four in vitro metabolism and drug interaction studies were also conducted.

| Study Number          | Purpose                                                                                                                 |
|-----------------------|-------------------------------------------------------------------------------------------------------------------------|
| <u>2400-0491-1800</u> | Determination of the cytochrome P450 isoforms involved in the metabolic pathway of colchicine in human liver microsomes |
| <u>305-1217</u>       | Determination of the inhibitory potential of colchicine on cytochrome P450 isoforms in human liver microsomes           |
| <u>305-1218</u>       | Determination of the induction potential of colchicine on cytochrome P450 isoforms in human liver hepatocytes           |
| <u>3210-0490-1800</u> | In Vitro Assessment of Induction/Suppression Potential of a Colchicine and Vinblastine in Primary Human Hepatocytes     |

## 2.2.1 What are the pharmacokinetic characteristics of colchicine?

### What are the characteristics of drug absorption?

#### Data from publications

Absolute bioavailability of Colstat was not studied. Two published studies reported that colchicine bioavailability is low, whether administered as an oral solution ( $47 \pm 14\%$ ) or tablets ( $37 \pm 12\%$  and  $44 \pm 17\%$ ;  $2 \times 0.5$  mg tablets manufactured by Houdé-Ish, France). Blood samples were collected up to 48 hours post-dose for healthy subjects (Ferron et al., J. Clin Pharmacol 36: 874, 1996) and 72 hours post-dose for elderly subjects (Rochdi et al., E. J. Clin Pharmacol 46: 351, 1994). Both of these publications and Mutual's data indicate that colchicine may exhibit enterohepatic recirculation as evidenced by second plasma peak around 6 hours of dosing Colstat.

#### Data from sponsor's studies

Study #1001 was a single-dose, three-way crossover comparative bioavailability and food effect study. The pharmacokinetic profile of colchicine was compared following administration of Mutual's 0.6 mg Colstat tablets with Col-Probenecid (Watson Laboratories), containing colchicine 0.5 mg (in addition to probenecid 500 mg) under fasting condition. In addition, 0.6 mg Colstat tablet was also administered after a standard high-fat breakfast to evaluate the effect of food on the rate and extent of absorption. A total of 28 healthy non-smoking, non-obese volunteers were enrolled into the study with 25 subjects (9 men and 16 women) completing the study. Subjects were mostly Caucasian and had a mean age of 24 years (range 18 to 39 years).

Peak plasma levels of colchicine were noted around 1.5 hours following administration of Colstat 0.6 mg. After dosage adjustment, Colstat tablets produced 25% higher C<sub>max</sub> and 37% higher AUC compared to Col-Probenecid. Dose-adjusted C<sub>max</sub> and AUC for colchicine after Colstat administration and the 90% confidence intervals (CI) were not within the bioequivalence interval of 80 to 125% (See table below).

#### **PK parameters and Dose-Adjusted Statistical Comparison of Mutual's Colchicine Tablets USP, 0.6 mg (Dose Adjusted to 0.5 mg) and Col-Probenecid, Containing Colchicine 0.5 mg**

| Parameter (units)            | Colchicine 0.6 mg [Mutual] (N=25) | Colchicine 0.5 mg [Col-Probenecid] (N=25) | Ratio of Dose-adjusted Geometric Means* | 90% CI         |
|------------------------------|-----------------------------------|-------------------------------------------|-----------------------------------------|----------------|
| C <sub>max</sub> (ng/mL)     | 2.50 (28.85)                      | 1.71 (32.87)                              | 125.10                                  | 111.97, 139.76 |
| AUC <sub>0-t</sub> (ng·h/mL) | 12.59 (48.62)                     | 8.09 (75.55)                              | 137.43                                  | 122.50, 154.18 |
| AUC <sub>∞</sub> (ng·h/mL)   | 14.11 (39.65)                     | 8.48 (33.36)                              | 137.13                                  | 124.46, 151.09 |
| T <sub>max</sub> (hr)        | 1.50 (0.5-2.0)                    | 1.02 (1.0-2.0)                            |                                         |                |
| t <sub>1/2</sub> (hr)        | 6.36 (73.85)                      | 3.83 (33.13)                              |                                         |                |

\*T<sub>max</sub> is presented as Median

Following coadministration of Colstat with high fat breakfast, the peak plasma colchicine levels were similar but AUC was 15% lower compared to fasting condition.

| Parameter (units)            | Arithmetic Mean (%CV) |                      | Statistical Comparison Fed vs. Fasted (n=25) |               |
|------------------------------|-----------------------|----------------------|----------------------------------------------|---------------|
|                              | Fasted (N=25)         | High-Fat Meal (N=25) | Ratio of Geometric Means                     | 90% CI        |
| C <sub>max</sub> (ng/mL)     | 2.50 (28.85)          | 2.50 (27.84)         | 100.37                                       | 89.84, 112.14 |
| T <sub>max</sub> (hr)        | 1.50 (0.5-2.0)        | 1.50 (0.5-4.0)       | --                                           | --            |
| AUC <sub>0-t</sub> (ng·h/mL) | 12.59 (48.62)         | 10.49 (48.62)        | 87.58                                        | 78.07, 98.26  |
| AUC <sub>∞</sub> (ng·h/mL)   | 14.11 (39.65)         | 11.40 (25.39)        | 84.55                                        | 76.73, 93.15  |

Study # 1004 was conducted to evaluate the pharmacokinetic profile of colchicine following single and multiple oral doses of Mutual's colchicine tablets, 0.6 mg in healthy volunteers. In Period 1, study subjects received a 0.6-mg dose of colchicine after an overnight fast of at least 13 hours. In Period 2, subjects received a 0.6-mg dose of colchicine in the morning after an overnight fast and in the evening (approximately 12 hours later) for 10 days.

In healthy adults receiving oral colchicine tablets, mean maximum plasma colchicine concentration were noted at 1.5 hours after a single dose. The elimination half-life as calculated following a single oral dose is approximately 5 hours. With regard to colchicine metabolites identified in publication, in all samples 2-O-demethylcolchicine (2-DMC) concentrations were below the level of quantitation (LOQ, 0.2 ng/mL) and only two samples from 1 subject (of 13 subjects) had a detectable 3-O-demethylcolchicine (3-DMC) concentration. These samples were collected at 1.0 and 1.5 hours post-dose (at steady state) and the 3-DMC concentrations were ——— (near the LOQ).

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Review of trough plasma concentrations indicates that steady state was attained by approximately the tenth day of dosing for most subjects. Ratio of C<sub>max</sub> at steady-state was nearly 1.5-fold higher than following the initial dose. With the higher plasma levels that occur with repeated dosing, a longer terminal elimination half life was noted (26.6 hours).

**Mean Pre-Dose Colchicine Concentrations (ng/mL) (MPC-004-07-1004)**

|         | Day of Dosing with 0.6 mg every 12 hours |                              |                               |                               |
|---------|------------------------------------------|------------------------------|-------------------------------|-------------------------------|
|         | 8 <sup>th</sup> Day (Day 22)             | 9 <sup>th</sup> Day (Day 23) | 10 <sup>th</sup> Day (Day 24) | 11 <sup>th</sup> Day (Day 25) |
| Morning | 0.83                                     | 0.89                         | 1.08                          | 0.94                          |
| Evening | 0.78                                     | 0.79                         | 0.87                          | --                            |

**Mean (%CV) Colchicine Pharmacokinetic Parameter Values Following Administration of Single and Multiple (b.i.d.) Oral Doses of Colchicine 0.6 mg in Healthy Adults (MPC-004-07-1004)**

|                                             | $C_{max}$<br>(ng/mL) | $T_{max}$ <sup>1</sup><br>(h) | $AUC_{0-t}$<br>(ng-h/mL) | $AUC_{\infty}$<br>(ng-h/mL) | $AUC_{0-t}$<br>(ng-h/mL) | Vd/F (L)           | CL/F<br>(L/hr)   | $K_e$<br>(h <sup>-1</sup> ) | $t_{1/2}$<br>(h) |
|---------------------------------------------|----------------------|-------------------------------|--------------------------|-----------------------------|--------------------------|--------------------|------------------|-----------------------------|------------------|
| <b>Colchicine 0.6-mg Single Dose (N=13)</b> |                      |                               |                          |                             |                          |                    |                  |                             |                  |
| Day 1                                       | 2.45<br>(28.66)      | 1.50<br>(1.0 – 3.0)           | 10.49<br>(33.77)         | 12.27<br>(36.05)            | --                       | 341.54<br>(54.35)  | 54.05<br>(30.98) | 0.183<br>(32.38)            | 4.95<br>(89.54)  |
| <b>Colchicine 0.6 mg b.i.d. × 10 days</b>   |                      |                               |                          |                             |                          |                    |                  |                             |                  |
| Day 25                                      | 3.55<br>(23.74)      | 1.31<br>(0.5 – 3.0)           | --                       | --                          | 20.37<br>(16.31)         | 1150.95<br>(18.73) | 30.31<br>(18.95) | 0.027<br>(16.34)            | 26.60<br>(16.26) |

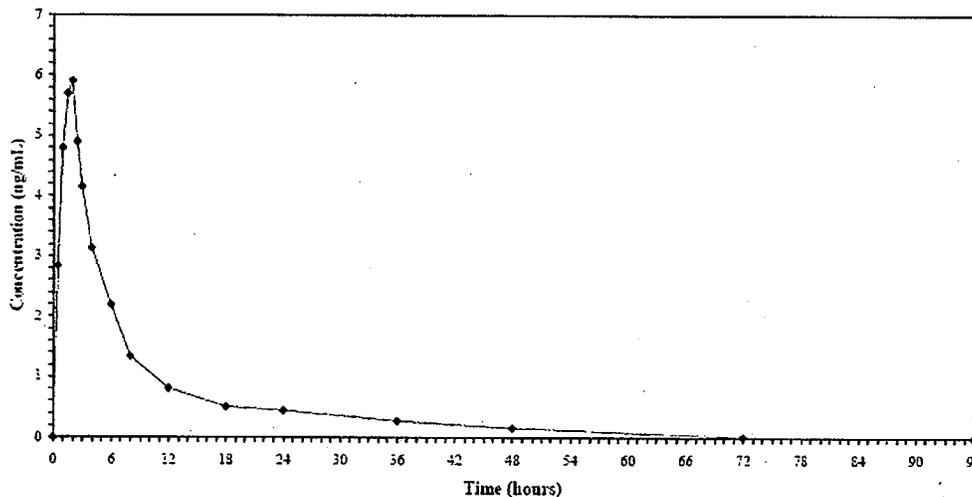
<sup>1</sup> $T_{max}$  mean (range)

CL = Dose/ $AUC_{0-t}$  (Calculated from mean values)

Vd = CL/ $K_e$  (Calculated from mean values)

In another PK study # 1003, pharmacokinetic profile of a low- dose regimen of colchicine (1.8 mg over 2 hours) was determined in healthy volunteers. Subjects received two 0.6 mg colchicine tablets initially, followed by one 0.6 mg colchicine tablet 1 hour later.

**Plasma Concentration Profile after Low-Dose Colchicine (1.8 mg over 2 hours) Administration in Healthy Adults, N = 13 (MPC-004-07-1003)**



**Mean (%CV) Colchicine Pharmacokinetic Parameter Values after Low-Dose Colchicine (1.8 mg over 2 hours) Administration in Healthy Adults (MPC-004-07-1003)**

|                          | $C_{max}$<br>(ng/mL) | $T_{max}$ <sup>1</sup><br>(h) | $AUC_{0-t}$<br>(ng-h/mL) | $AUC_{\infty}$<br>(ng-h/mL) | Vd/F<br>(L)        | CL/F<br>(L/hr)   | $K_e$<br>(h <sup>-1</sup> ) | $t_{1/2}$<br>(h) |
|--------------------------|----------------------|-------------------------------|--------------------------|-----------------------------|--------------------|------------------|-----------------------------|------------------|
| <b>Low Dose<br/>N=13</b> | 6.19<br>(39.30)      | 1.81<br>(1.0-2.5)             | 43.79<br>(26.12)         | 52.07<br>(26.29)            | 1188.72<br>(26.88) | 36.95<br>(27.04) | 0.0326<br>(30.80)           | 23.63<br>(39.10) |

<sup>1</sup> $T_{max}$  reported mean (range), relative to first dose

When colchicine is administered in this low-dose regimen, peak plasma colchicine concentrations are noted around 1.81 hours after the initial dose.

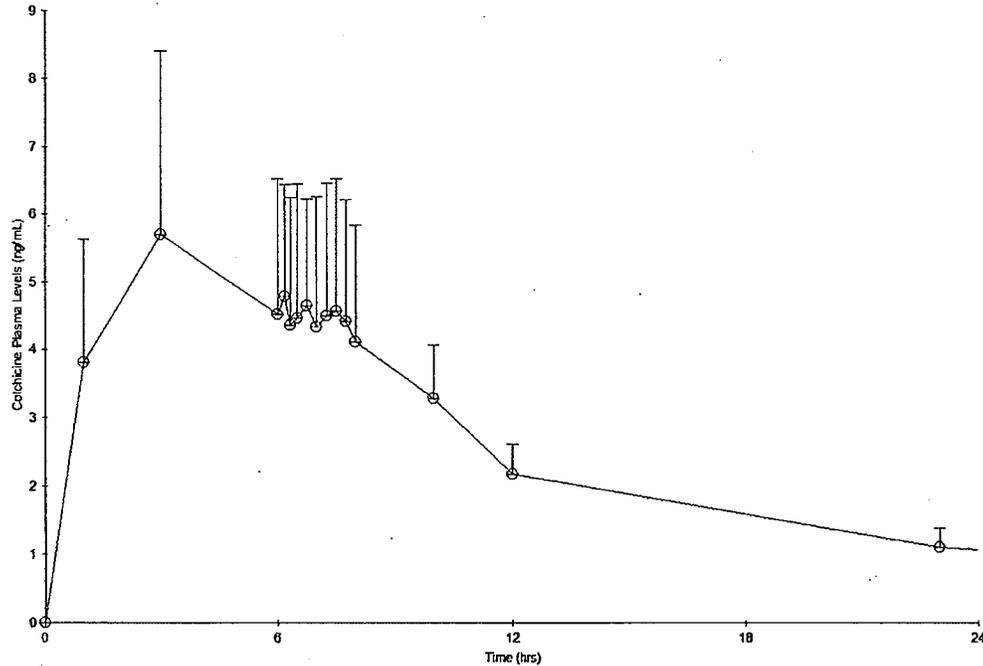
In study # 1002, pharmacokinetics of oral colchicine tablets were investigated at 4.8 mg dose in healthy volunteers in a single center, randomized, double-blind, double-dummy pharmacokinetic and safety study. Subjects were randomized to receive the following treatments:

- a) 4.8 mg colchicine administered as two 0.6 mg over-encapsulated tablets followed by one 0.6 mg over-encapsulated tablet every hour for six additional doses.
- b) over-encapsulated 400 mg moxifloxacin tablet

Eighteen healthy, non-smoking subjects (10 men and 8 women) with a mean age of 28.7 years (range 18 to 50 years) and within 15% of ideal body weight were enrolled in this study. Fifteen subjects were randomized to receive colchicine and 3 subjects were randomized to receive moxifloxacin.

Peak plasma levels of colchicine were noted around 4.5 hours after initial colchicine dose administered. Plasma levels declined thereafter with a “shoulder” or secondary peak in concentrations noted around 7.75 hours following first dose. This secondary peak is probably a result of repeated administration of colchicine tablets every hour (See figure below).

**Plasma Concentration Profile after Standard-Dose Colchicine (4.8 mg over 6 hours) Administration in Healthy Adults, N = 15 (MPC-004-07-1002)**



2-DMC concentrations were below LOQ for all subjects. All 15 subjects had at least one measurable 3-DMC concentration; the 3-DMC concentrations that ranged from \_\_\_\_\_ and were observed 3.12 to 8.12 hours post-dose. Summary mean 3-DMC pharmacokinetic parameter values can be found in Table below. The observed mean 3-DMC C<sub>max</sub>, AUC<sub>0-t</sub>, and AUC<sub>∞</sub> concentrations were approximately 4.7%, 2%, and 4.1% of the observed mean colchicine C<sub>max</sub>, AUC<sub>0-t</sub>, and AUC<sub>∞</sub> concentrations, respectively.

**b(4)**

**Mean (%CV) 3-DMC Pharmacokinetic Parameter Values after Standard-Dose Colchicine (4.8 mg over 6 hours) Administration in Healthy Adults (MPC-004-07-1002)**

|                         | $C_{max}$ (ng/mL)<br>N=15 | $T_{max}$ (h)<br>N=14 | $AUC_{0-4}$ (ng-h/mL)<br>N=13 | $AUC_{0-8}$ (ng-h/mL)<br>N=8 | $K_e$ (h <sup>-1</sup> )<br>N=8 | $t_{1/2}$ (h)<br>N=8 |
|-------------------------|---------------------------|-----------------------|-------------------------------|------------------------------|---------------------------------|----------------------|
| Standard Dose<br>N = 15 | 0.32<br>(16.35)           | 5.06<br>(3.12-8.12)   | 2.09<br>(40.29)               | 4.84<br>(42.73)              | 0.1418<br>(60.15)               | 6.93<br>(64.35)      |

<sup>†</sup>  $T_{max}$  reported mean (range)

In this study (1002), most common adverse events were diarrhea, nausea and vomiting (see table below). All of the subjects experienced diarrhea, while 6 out of 15 subjects vomited between 12.5 to 19.25 hours after the start of dosing (6.5 to 13.25 hours after ingestion of the last dose). It is noteworthy that in study # 1003, only two out of the 13 subjects receiving low dose colchicine (1.8 mg over 2 hours) reported diarrhea. ECG measurements were included in safety evaluation to coincide with the PK measurements. The study was not powered to detect changes in ECG following colchicine treatment. The inclusion of three subjects in moxifloxacin group does not comprise an adequate active control for ECG changes. As such there were no treatment-emergent values in QT >450 msec or change from baseline > 30 msec.

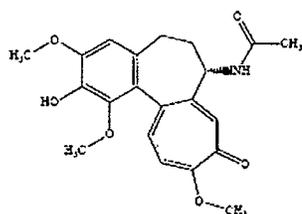
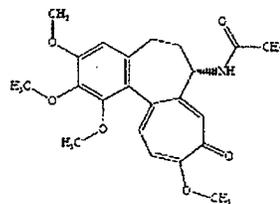
#### What are the characteristics of drug distribution?

In vitro, colchicine binding to serum protein is low,  $39 \pm 5\%$ , primarily to albumin ( $37 \pm 4\%$ ), regardless of concentration (Sabouraud et al., Int. J. Clin. Pharmacol. Ther. 32(8)-429, 1994). Equilibrium dialysis was performed using <sup>3</sup>H-colchicine in a wide range of concentrations, from  $10^{-10}$  to  $10^{-5}$  M (0.04 to 4000 ng/mL). Colchicine also binds to all serum components. Serum colchicine concentrations decline in a bi-exponential mode, reflecting a rapid clearance from the plasma and its redistribution to leukocytes (Chappey et al., Clin Pharmacol Ther 1993; 54:360-367) and red blood cells (Sabouraud et al., Int. J. Clin. Pharmacol. Ther. 32(8)- 429, 1994).

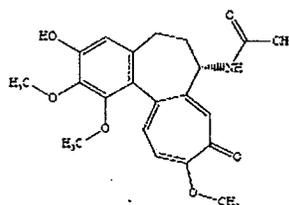
#### What are the characteristics of drug metabolism?

Tateishi et. al reported (Biochem Pharmacol 1997;10:111-116) that following incubation of 5 nM (2 ng/mL) of <sup>3</sup>H-colchicine with 200 pmol of CYP450 protein for 60 minutes, two peaks were noted, 2-desmethylcolchicine (DMC) and 3-DMC. These accounted for 5.5% and 9.8% of the substrate, respectively.

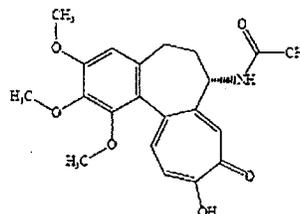
**Chemical Structure of Colchicine and Its Human Metabolites  
(% Formed in Human Microsomal Preparations)**



**2-O-Demethylcolchicine**  
(5.5%)



**3-O-Demethylcolchicine**  
(9.8%)



**10-O-Demethylcolchicine**  
(Not Detected)

In vitro drug metabolism studies by the sponsor showed that Colchicine was metabolized by NADPH-dependent CYP450s with approximately less than 5% disappearance at the conditions examined. Reaction phenotyping with recombinant CYP450s revealed some turnover by specific P450s including CYP3A4, CYP2A6, CYP2B6, CYP2C8, CYP2C9, and CYP2C19. However, the most effective CYP3A4 metabolism was noted at both 43 and 430 nM colchicine concentrations.

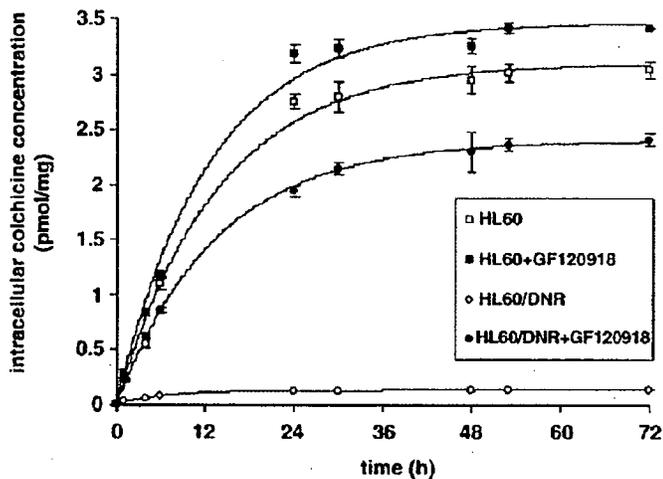
**What are the characteristics of drug elimination?**

Following administration of a 1 mg dose to 12 healthy volunteers about 40 to 65% of the absorbed dose was recovered unchanged in the urine (Achtert *et al.*, 1989 Eur. J. Drug Metab. Pharmacokinet. 14(4):317-322). Colchicine is not significantly removed by hemodialysis such that a dose adjustment is required (Bennett *et al.*, 1988).

**Is colchicine a P-gp Substrate?**

*Colchicine is a P-gp substrate.*

The kinetics of colchicine uptake and efflux at a clinically relevant concentration (6.25 nM; 2.5 ng/mL) has been studied in two different lines of cultured human cells (HL-60 and HL-60/DNR). HL60DNR cells contain 25 times more P-gp than do HL60 cells. In the HL60/DNR cells, the accumulation of colchicine was 24 times lower than in the HL60 cells (Declèves *et al.*, Toxicol Appl Pharmacol 2006:217:153-60).



Colchicine accumulation by HL-60 (□, ■) and HL60/DNR (○, ●) cells. Cells were incubated with 6.25 nM [<sup>3</sup>H]-colchicine without (○, □) or with (●, ■) 10 μM GF120918. Values are means ± SD of three independent experiments.

#### Is colchicine a P-gp Inhibitor?

*Colchicine is not a P-gp inhibitor*

The potential for colchicine (10 μM; 4 μg/mL and 100 μM; 40 μg/mL) to inhibit the basal-to-apical (BL-AP) apparent permeability ( $P_{app}$ ) of [<sup>3</sup>H]-paclitaxel, [<sup>3</sup>H]-vinblastine, and [<sup>3</sup>H]-digoxin was tested in MDCK/MDR1, and MDCK-wildtype (WT) cell monolayers. At the highest test concentration, colchicine significantly ( $P < 0.05$ ) inhibited the BL-AP  $P_{app}$  of only one of the three substrates tested, [<sup>3</sup>H]-vinblastine. Typical plasma levels of colchicine are around 10 ng/mL or 0.03 μM. (Taub ME et. al, Drug Metab Dispos 2005;33:1679-87)

### 2.3 Intrinsic Factors

I. What intrinsic factors (age, gender, race, weight, height, disease, genetic polymorphism, pregnancy, and organ dysfunction) influence exposure and/or response and what is the impact of any differences in exposure on the pharmacodynamics?

*Pharmacokinetic studies were not conducted by the sponsor to address the influence of age, race, bodyweight, pregnancy and organ dysfunction. However, several publications addressing these intrinsic factors have been discussed in the NDA.*

With regard to gender, pharmacokinetic parameters from different studies conducted by Mutual were comparable between male and female subjects (see table below).

Mean (%CV) Colchicine Pharmacokinetic Parameter Values for Male and Female Healthy Volunteers in Mutual-Sponsored Studies

| Study No.       | Dose                     | Sex (N)      | C <sub>max</sub> (ng/mL) | AUC <sub>0-1</sub> (ng·h/mL) | AUC <sub>∞</sub> (ng·h/mL) | t <sub>1/2</sub> (h) | CL/F (L/h)  | Weight Adjusted CL/F (L/hr/kg) |
|-----------------|--------------------------|--------------|--------------------------|------------------------------|----------------------------|----------------------|-------------|--------------------------------|
| MPC-004-07-1001 | 0.6 mg single-dose       | Males (9)    | 2.3 (24.8)               | 9.5 (25.3)                   | 11.0 (18.9)                | --                   | 56.6 (21.4) | 0.71 (32.5)                    |
|                 |                          | Females (16) | 2.6 (29.7)               | 14.3 (48.4)                  | 16.0 (39.0)                | --                   | 44.1 (44.3) | 0.68 (48.2)                    |
| MPC-004-07-1003 | 1.2 mg over 2 hours      | Males (6)    | 5.1 (34.8)               | 40.7 (27.8)                  | 49.2 (29.4)                | 24.9 (50.9)          | 39.0 (26.1) | 0.51 (18.6)                    |
|                 |                          | Females (7)  | 7.2 (36.8)               | 46.4 (25.2)                  | 54.5 (25.0)                | 22.6 (26.1)          | 35.2 (29.1) | 0.53 (31.1)                    |
| MPC-004-07-1002 | 4.8 mg over 6 hours      | Males (8)    | 6.8 (22.5)               | 102.5 (26.3)                 | 116.0 (24.2)               | 31.2 (33.9)          | 44.4 (33.6) | 0.58 (44.0)                    |
|                 |                          | Females (7)  | 6.9 (15.8)               | 107.7 (21.8)                 | 120.8 (21.1)               | 31.6 (18.1)          | 41.7 (26.5) | 0.66 (24.9)                    |
| MPC-004-07-1006 | Day 1 0.6 mg single-dose | Males (11)   | 2.9 (31.7)               | 11.2 (38.1)                  | 13.2 (39.7)                | --                   | 53.1 (43.6) | 0.70 (42.1)                    |
|                 |                          | Females (12) | 2.8 (31.5)               | 13.5 (36.5)                  | 17.7 (51.5)                | --                   | 41.1 (40.4) | 0.62 (44.8)                    |

2. Based upon what is known about exposure-response relationships and their variability, and the groups studied (volunteers vs. patients); what dosage regimen adjustments, if any, are recommended for each of these subgroups (examples shown below)? If dosage regimen adjustments are not based upon exposure-response relationships, describe the alternative basis for the recommendation.

**a) elderly**

*Pharmacokinetics of colchicine was not evaluated in elderly. However, considering decrease in renal and hepatic function in elderly caution is necessary when using Colstat.*

**b) pediatric patients**

*The sponsor did not conduct any PK studies in pediatric patients. Search of public databases did not reveal any publications on PK of colchicine in pediatric patients.*

**c) renal impairment**

*Colchicine is significantly excreted in the urine. Four-fold decrease in colchicine clearance is noted in severe renal impaired subjects compared to healthy volunteers. Dosage reduction should be considered in subjects with mild and moderate renal impairment. In patients with severe renal impairment and undergoing dialysis the total daily dosage should be less than 1.2 mg/day.*

Following administration of a 1 mg dose to 12 healthy volunteers about 40 to 65% of the absorbed dose was recovered unchanged in the urine (Achtert et al., 1989 Eur. J. Drug Metab. Pharmacokinet. 14(4):317-322).

Ben-Chitrit et. al. 1994 (J. Rheumatology, 21(4): 710) published a report on pharmacokinetics of colchicine in patients with FMF without and with severe renal impairment. As tabulated below, nine patients with FMF, 5 women and 4 men, 19-42 years old (mean 30 years) were given 1.0 mg of colchicine, with the exception of the patient who also had cirrhosis, who received 0.5 mg of colchicine. The extent of renal

impairment was not characterized in the study, however, all 5 patients had biopsy-proven amyloidosis; 4 were on routine hemodialysis and 1 had a serum creatinine clearance of 15 mL/min. Subjects received two 0.5 mg colchicine tablets and blood samples were collected until 48 hours after dosing.

|         | Subject No. | Sex | Age (years) | Weight (kg) | Height (cm) |
|---------|-------------|-----|-------------|-------------|-------------|
| Group A | 1*          | M   | 30          | 60          | 180         |
|         | 2*          | F   | 24          | 71          | 168         |
|         | 3*          | M   | 21          | 50          | 160         |
|         | 4*          | F   | 19          | 40          | 165         |
| Group B | 5**         | M   | 36          | 61          | 163         |
|         | 6*†         | F   | 33          | 41          | 150         |
|         | 7*†         | F   | 32          | 68          | 167         |
|         | 8*†         | F   | 37          | 48          | 155         |
|         | 9* ††       | M   | 42          | 58          | 168         |

\* Normal liver function: Alkaline phosphatase, gamma-glutamyl transpeptidase, aspartate aminotransferase, alanine aminotransferase, bilirubin.

\*\* Creatinine clearance 15 ml/min.

† Receiving hemodialysis.

†† Cirrhosis of liver.

A significant decrease (4-fold) in clearance of colchicine was noted in FMF patients ( $0.168 \pm 0.063$ ) with severe renal impairment (see table below) compared to those with normal renal function ( $0.727 \pm 0.11$ ), where the plasma half-life of colchicine was 4 times shorter.

#### Pharmacokinetics of colchicine in FMF patients with or without renal impairment.

| Subject No. | C <sub>max</sub> (ng/ml) | T <sub>max</sub> (h) | t <sub>1/2</sub> (h) | Cl/F (l/h/kg) | Vd/F (l/kg) |      |
|-------------|--------------------------|----------------------|----------------------|---------------|-------------|------|
| Group A     |                          |                      |                      |               |             |      |
| 1           | 3.9                      | 1.0                  | 3.6                  | 0.746         | 3.86        |      |
| 2           | 5.1                      | 2.0                  | 4.2                  | 0.765         | 4.65        |      |
| 3           | 5.2                      | 1.0                  | 3.9                  | 0.570         | 3.16        |      |
| 4           | 8.8                      | 2.0                  | 5.8                  | 0.825         | 7.80        |      |
| Mean ± SD   | 5.8                      | 1.5                  | 4.4                  | 0.727         | 4.87        |      |
|             | 2.1                      | 0.6                  | 1.0                  | 0.110         | 2.05        |      |
| Group B     |                          |                      |                      |               |             |      |
| 5           | 7.3                      | 2.0                  | 18.0                 | 0.113         | 2.96        |      |
| 6           | 11.0                     | 2.0                  | 19.2                 | 0.125         | 3.45        |      |
| 7           | 8.3                      | 1.0                  | 20.3                 | 0.183         | 5.39        |      |
| 8           | 4.6                      | 2.0                  | 17.7                 | 0.250         | 6.45        |      |
| Mean ± SD   | 7.8                      | 1.8                  | 18.8                 | 0.168         | 4.56        |      |
|             | 2.6                      | 0.5                  | 1.2                  | 0.063         | 1.64        |      |
| p <         | NS                       | NS                   | 0.001                | 0.001         | NS          |      |
| Cirrhosis   | 9*                       | 4.5                  | 1.0                  | 50.0          | 0.078       | 5.77 |

\* Patient 9 received 0.5 mg colchicine.

One patient with cirrhosis had a 10-fold lower clearance compared to the average clearance noted in healthy volunteers in this study.

#### d) hepatic impairment

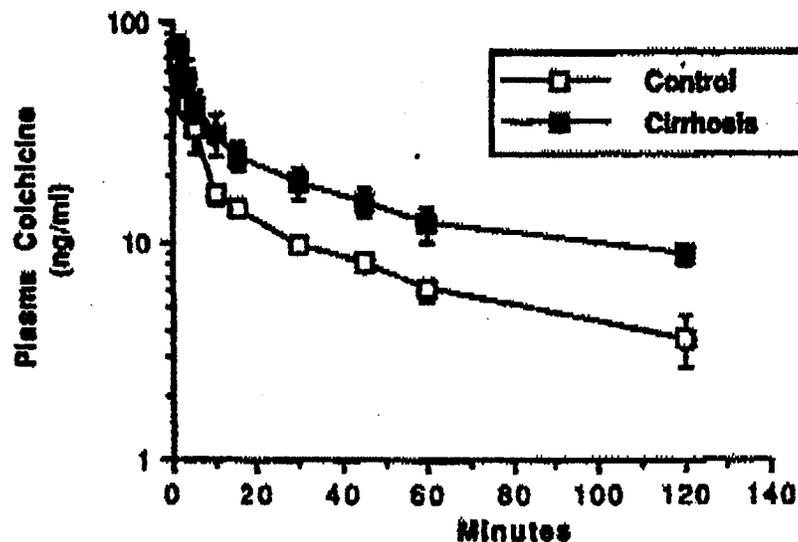
*Caution is warranted when Colstat is considered for patients with mild and moderate hepatic impairment. Dose reduction should be considered in patients with severe hepatic impairment.*

Pharmacokinetics of 1 mg colchicine administered intravenously in 9 patients with alcoholic cirrhosis and compared to 6 healthy men. Of these patients, three had liver biopsy specimens showing cirrhosis and five patients had ascites. Following administration of colchicine 1 mg as an intravenous bolus, blood samples were only collected over 2 hours post-dose and measured using an HPLC assay with inadequate sensitivity to characterize the pharmacokinetic profile (limit of detection was 6 ng/mL).

#### PK parameters of colchicine in healthy volunteers and alcoholic cirrhosis patients

| Subjects         | Pugh score | Bilirubin | Albumin | Prothrombin time | Half-life (minutes)  | Volume of distribution (L/kg) | Clearance (ml/min · kg) |
|------------------|------------|-----------|---------|------------------|----------------------|-------------------------------|-------------------------|
| <b>Normal</b>    |            |           |         |                  |                      |                               |                         |
| 1                |            | 0.4       | 4.1     | 12.3             | 111.53               | 1.06                          | 6.586                   |
| 2                |            | 0.4       | 4.4     | n.a.             | 59.62                | 0.593                         | 8.888                   |
| 3                |            | 0.6       | 4.3     | 12.8             | 76.8                 | 0.795                         | 7.17                    |
| 4                |            | n.a.      | n.a.    | n.a.             | 54.66                | 0.918                         | 11.637                  |
| 5                |            | 0.6       | 4.6     | 11.5             | 25.39                | 0.542                         | 14.788                  |
| 6                |            | 0.3       | 4.1     | 13               | 16.47                | 0.4                           | 16.835                  |
| <b>Average</b>   |            |           |         |                  | <b>57.4 ± 14.2</b>   | <b>0.718 ± 0.102</b>          | <b>10.65 ± 1.82</b>     |
| <b>Cirrhotic</b> |            |           |         |                  |                      |                               |                         |
| 1                | 6          | 2.4       | 3.6     | 14.8             | 175.58               | 0.783                         | 3.091                   |
| 2                | 5          | 1         | 4.9     | 12.8             | 64.09                | 0.292                         | 3.155                   |
| 3                | 5          | 0.9       | 3.7     | 12.9             | 69.24                | 0.327                         | 3.27                    |
| 4                | 7          | 1.2       | 2.8     | 16.6             | 145.5                | 0.747                         | 3.558                   |
| 5                | 9          | 7.2       | 3       | 16.5             | 70.68                | 0.388                         | 3.907                   |
| 6                | 6          | 1.5       | 3.4     | 13.5             | 62.46                | 0.356                         | 3.954                   |
| 7                | 8          | 9.5       | 3       | 12.9             | 220.46               | 1.438                         | 4.521                   |
| 8                | 9          | 2         | 2.2     | 16.1             | 74.52                | 0.571                         | 5.311                   |
| 9                | 6          | 2         | 3       | 14.6             | 147.27               | 1.546                         | 7.275                   |
| <b>Average</b>   |            |           |         |                  | <b>114.4 ± 19.7*</b> | <b>0.716 ± 0.158</b>          | <b>4.22 ± 0.46*</b>     |

#### PK profile of colchicine in healthy volunteers and alcoholic cirrhosis patients



In spite of the noted deficiencies with regard to study design (short duration of plasma sample collection, and IV use of colchicine), the observed 2.5-fold decrease in clearance of colchicine in patients with hepatic impairment ( $4.2 \pm 0.45$  ml/min/kg) compared to healthy volunteers ( $10.65 \pm 1.85$  ml/min/kg) is noteworthy. As explained above in the renal impairment section, one patient with cirrhosis had a 10-fold lower clearance and 10-fold higher  $T_{1/2}$  compared to the average clearance noted in healthy volunteers (Ben-Chitrit et. al. 1994 J. Rheumatology, 21(4): 710).

Rudi et al (1994 Scand J Gastroenterol 1994;29:346-51) studied the single dose pharmacokinetics of single-dose colchicine in 8 patients with chronic liver disease without ascites. Six patients with chronic liver disease received a single 1-mg dose of colchicine after an overnight fast.

#### Demographic characteristics and diagnosis of patients with hepatic impairment

| Patient | Age (years) | Body weight (kg) | Diagnosis*                     | Bilirubin (mg/dl) | Albumin (g/dl) | Prothrombin time (sec) | S-Crea* (mg/dl) | Nutritional state | Encephalopathy |
|---------|-------------|------------------|--------------------------------|-------------------|----------------|------------------------|-----------------|-------------------|----------------|
| 1 A.S.  | 36          | 52               | PBC stage I                    | 0.9               | 4.4            | 12.0                   | 0.8             | Normal            | 0              |
| 2 K.S.  | 55          | 63               | Alcoholic cirrhosis            | 19.0              | 3.5            | 23.2                   | 0.8             | Reduced           | 1              |
| 3 E.H.  | 73          | 73               | Posthepatic cirrhosis (hep. B) | 1.0               | 3.6            | 13.0                   | 1.0             | Normal            | 0              |
| 4 M.F.  | 24          | 66               | Alcoholic cirrhosis            | 2.2               | 3.0            | 17.8                   | 0.8             | Normal            | 0              |
| 5 K.Y.  | 21          | 72               | Posthepatic cirrhosis (hep. B) | 1.7               | 3.5            | 15.8                   | 0.9             | Normal            | 0              |
| 6 H.P.  | 51          | 69               | Alcoholic cirrhosis            | 5.0               | 2.9            | 19.1                   | 0.9             | Normal            | 0-1            |
| 7 G.P.  | 25          | 71               | Chronic active hepatitis C     | 1.5               | 4.5            | 12.8                   | 0.9             | Normal            | 0              |
| 8 M.S.  | 60          | 75               | Chronic active hepatitis-B     | 0.4               | 3.8            | 12.0                   | 0.7             | Normal            | 0              |
| 9 I.R.  | 59          | 98               | Choledocholithiasis            | 14.2              | 3.7            | 14.0                   | 1.4             | Normal            | 0              |
| 10 E.E. | 65          | 68               | Choledocholithiasis            | 7.5               | 3.8            | 14.6                   | 1.2             | Normal            | 0              |

\* PBC = primary biliary cirrhosis; S-crea = serum creatinine.

#### Pharmacokinetics of colchicine in patients with hepatic impairment

| Patient | C <sub>max</sub><br>(ng/ml) | C <sub>24h</sub><br>(ng/ml) | t <sub>max</sub> (h) | t <sub>1/2α</sub> (h) | t <sub>1/2β</sub> (h) | AUC <sub>0-24</sub><br>(ng · h/ml) | AUC <sub>0-∞</sub><br>(ng · h/ml) | Cl/F (l/h) | Vd/F (l) |
|---------|-----------------------------|-----------------------------|----------------------|-----------------------|-----------------------|------------------------------------|-----------------------------------|------------|----------|
| 1 A.S.  | 10.0                        | 0.8                         | 3.0                  | 1.18                  | 15.9                  | 46.6                               | 65.4                              | 30.6       | 701      |
| 2 K.S.  | 4.3                         | 0.6                         | 3.0                  | 2.62                  | 13.5                  | 28.1                               | 39.8                              | 50.3       | 978      |
| 3 E.H.  | 4.8                         | 0.7                         | 1.5                  | 3.61                  | 18.2                  | 33.8                               | 52.2                              | 38.3       | 1010     |
| 4 M.F.  | 2.9                         | 0.0                         | 2.0                  | 1.30                  | 4.3                   | 8.1                                | 8.4                               | 236.4      | 375      |
| 5 K.Y.  | 2.1                         | 0.2                         | 0.8                  | 3.88                  | 10.7                  | 13.4                               | 13.6                              | 120.0      | 1850     |
| 6 H.P.  | 2.8                         | 0.0                         | 1.0                  | 1.33                  | 1.5                   | 5.0                                | 5.5                               | 330.6      | 731      |
| 7 G.P.  | 1.5                         | 0.0                         | 3.0                  | 1.93                  | 6.2                   | 5.4                                | 8.9                               | 223.8      | 2010     |
| 8 M.S.  | 0.4                         | 0.0                         | 3.0                  | NC                    | 8.2                   | 2.5                                | 5.4                               | 370.8      | 3930     |

\* C<sub>max</sub> = peak colchicine concentration; C<sub>24h</sub> = plasma colchicine concentration after 24 h before next dosing; t<sub>max</sub> = peak time; t<sub>1/2α</sub> = distribution half-life; t<sub>1/2β</sub> = terminal plasma elimination half-life; AUC<sub>0-24</sub> = area under the plasma concentration time curve, time 0-24 h; AUC<sub>0-∞</sub> = area under the plasma concentration time curve, extrapolated from time 0 to infinity; Cl/F = total plasma colchicine clearance; Vd/F = apparent volume of distribution; NC = not calculated because plasma concentrations were too low.

It is noteworthy that subjects 2, 3, 6, 8, 9 and 10 are elderly patients with hepatic impairment. Colchicine levels in plasma and bile were analyzed by radioimmunoassay method. The method has a detection limit of 0.15 ng/ml, and the calibration curve is linear in the range of 0.15 to 10.0 ng/ml, with inter- and intra-assay variations of 8.2% and 9.0%, respectively. The PK parameters (C<sub>max</sub> and AUC<sub>0-∞</sub>) from the above table are presented after normalizing to a 0.6 mg colchicine dose. In comparison, healthy volunteers receiving 0.6 mg Colstat tablets under fasting condition had an average C<sub>max</sub> of 2.5 ± 2 ng/mL and AUC of 14.11 ± 7 ng·h/mL. Although some hepatic impaired subjects have a 2- to 2.5 fold higher AUC<sub>0-∞</sub>, some have a lower exposure when compared to the AUC data from study # 1004.

#### Colchicine C<sub>max</sub> and AUC normalized to 0.6 mg dose

| Patient | Age (years) | Body weight (kg) | Child Pugh Class | C <sub>max</sub> Normalized to 0.6 mg | AUC normalized to 0.6 mg | Fold increase with respect average healthy volunteer data |
|---------|-------------|------------------|------------------|---------------------------------------|--------------------------|-----------------------------------------------------------|
| 3 E.H.  | 73          | 73               | B                | 2.88                                  | 31.32                    | 2.2                                                       |
| 1 A.S.  | 36          | 52               | B                | 6                                     | 39.24                    | 2.8                                                       |
| 8 M.S.  | 60          | 75               | B                | 0.24                                  | 3.24                     | 0.2                                                       |
| 7 G.P.  | 25          | 71               | B                | 0.9                                   | 5.34                     | 0.4                                                       |
| 5 K.Y.  | 21          | 72               | B                | 1.26                                  | 8.16                     | 0.6                                                       |
| 4 M.F.  | 24          | 66               | B                | 1.74                                  | 5.04                     | 0.4                                                       |
| 6 H.P.  | 51          | 69               | C                | 1.68                                  | 3.3                      | 0.2                                                       |
| 2 K.S.  | 55          | 63               | C                | 2.58                                  | 23.88                    | 1.7                                                       |

## 2.4 Extrinsic Factors

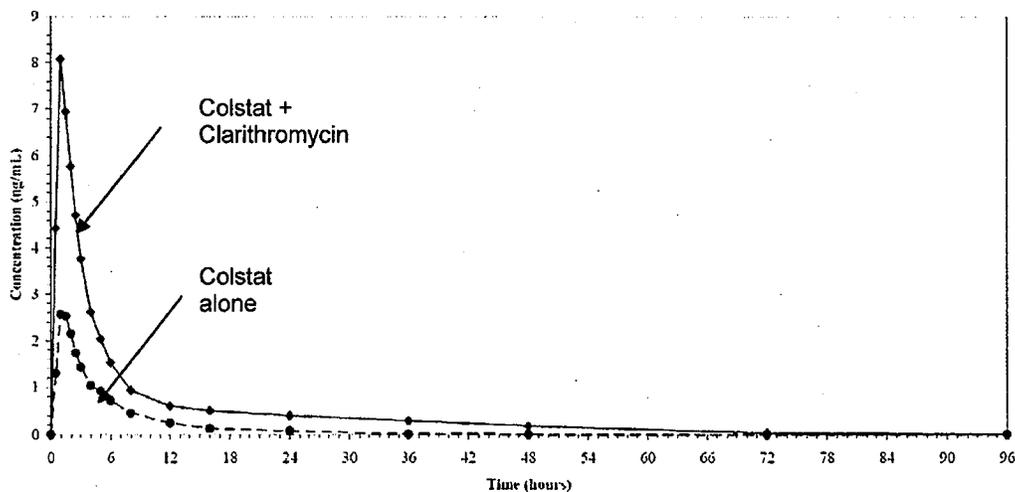
In vitro studies show that colchicine is a substrate of P-glycoprotein and it is metabolized by CYP3A4. Hence, sponsor evaluated the effect of a strong CYP3A4 and P-gp inhibitor on the pharmacokinetic of colchicine.

### Drug Interaction Study with clarithromycin (Study # 1006):

*A three fold increase in colchicine C<sub>max</sub> and AUC is noted when Colstat is coadministered with clarithromycin. The drug interaction appears mainly due to P-gp inhibition rather than CYP3A4 inhibition, but the data is not clear. Hence, dosage adjustment is necessary with other strong CYP3A4 and P-gp inhibitors. Colstat dose should be reduced to 0.6 mg per day when patients with FMF are also on strong CYP3A4 and P-gp inhibitors such as clarithromycin, ketoconazole, ritonavir, atazanavir, saquinavir, nefazodone, etc. Sponsor has several ongoing drug interaction studies with ritonavir, ketoconazole, grapefruit juice and cyclosporine. These studies may provide insight into Colstat dose adjustment when coadministered with other CYP3A4 and P-gp inhibitors.*

Study # 1006 was conducted to follow up on in vitro study results showing that colchicine is a substrate of CYP3A4 and P-gp. Clarithromycin, a strong inhibitor of P-gp and CYP3A4 according to the FDA guidance document, was given in a 7-day regimen (250 mg b.i.d.). There was a significant increase in exposure (3-fold in C<sub>max</sub>, 3.4-fold in AUC) to colchicine when a single dose was administered with steady-state clarithromycin, respectively (See Figure and Table below). One individual had 9.5-fold increase, two with a 5-fold increase in colchicine AUC following clarithromycin coadministration with Colstat. Colchicine plasma half-life increased from 8 hours to 30 hours on an average.

### Plasma colchicine profile following administration of Colstat alone or with clarithromycin



| Parameter (units)                 | Arithmetic Mean (%CV)      |                                          |
|-----------------------------------|----------------------------|------------------------------------------|
|                                   | Median (Range) for Tmax    |                                          |
|                                   | Colchicine Alone<br>(N=23) | Colchicine +<br>Clarithromycin<br>(N=23) |
| AUC0-t (ng·hr/mL)                 | 12.37 (37.64)              | 41.95 (23.31)                            |
| AUC0-inf (ng·hr/mL)               | 15.53 (49.6)               | 52.62 (22.84)                            |
| Cmax (ng/mL)                      | 2.84 (30.97)               | 8.44 (17.63)                             |
| Tmax (hr)                         | 1.50 (0.50-2.00)           | 1.00 (0.50-2.00)                         |
| Kel (1/hr)                        | 0.1324 (46.87)             | 0.0296 (87.99)                           |
| t1/2 (hr)                         | 8.89 (126.38)              | 30.31 (41.31)                            |
| CL/F (L/hr)                       | 46.8 (43.68)               | 12.0 (23.75)                             |
| Weight-Adjusted CL/F<br>(L/hr/kg) | 0.66 (42.86)               | 0.17 (24.07)                             |
| Vd (L)                            | 431.89 (56.11)             | 493.43 (33.59)                           |

Colchicine clearance (CL/F) was decreased by 75% when co-administered with clarithromycin as compared to the reported total apparent oral clearance when colchicine was administered alone (12.0 L/hr versus 46.8 L/hr). As such the metabolites of colchicine were very low in plasma following Colstat alone or with clarithromycin. Since only about 5 to 20% of the metabolism of colchicine is via hepatic metabolism, these results suggest that factors such as P-gp inhibition may affect the disposition of colchicine to a far greater extent than the inhibition of CYP3A4 enzyme.

Fourteen of 24 subjects experienced a total of 37 AEs over the course of the study, 7 subjects (29.2%) with 21 AEs following the administration of colchicine only, 5 subjects (20.8%) with 7 AEs while receiving clarithromycin on an outpatient basis, and 4 subjects (17.4%) with 8 AEs following co-administration of colchicine with clarithromycin. Headache and gastrointestinal disorders were the most common treatment-emergent adverse events following administration of colchicine, occurring in 5 subjects (20.8%) and 4 subjects (16.6%), respectively. With respect to GI events, there were 3 subjects (12.5%) with nausea and 2 subjects (8.3%) with diarrhea, the latter occurring only when subjects received colchicine in combination with clarithromycin.

Electrocardiogram measurements for the determination of QTcF interval were made at predose and 1hour post dose for Period I and Period II. Categorical tabulations (counts and percentages) were provided for all time points after baseline combined. The categorical parameters are based on the following:

- QTcF outliers (in categories of >450 to ≤480, >480 to ≤500, >500 msec),
- Change in QTcF outliers (in categories of >30 to ≤60, >60 to ≤90, >90 msec)

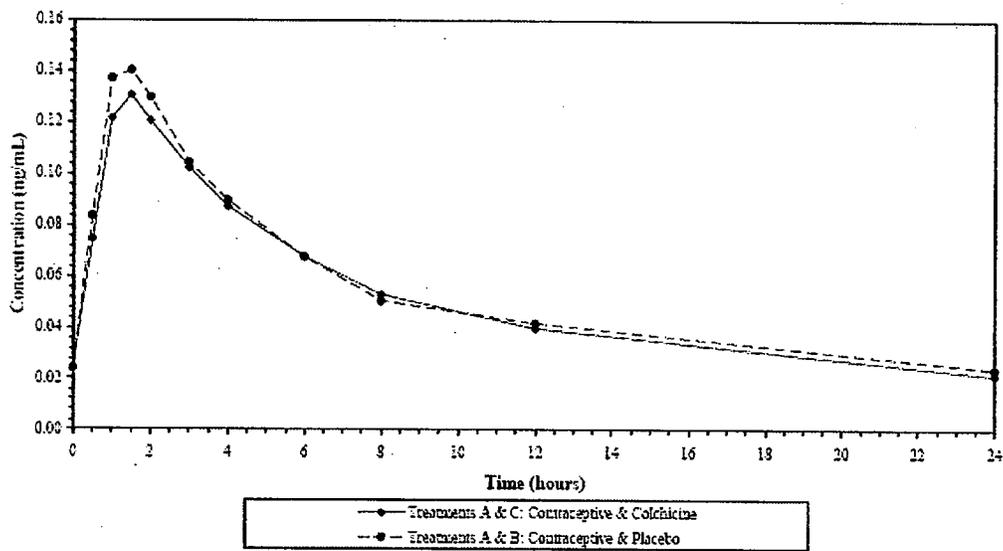
There were no QTcF outliers based on the above definition.

#### **Drug Interaction Study with Oral Contraceptives:**

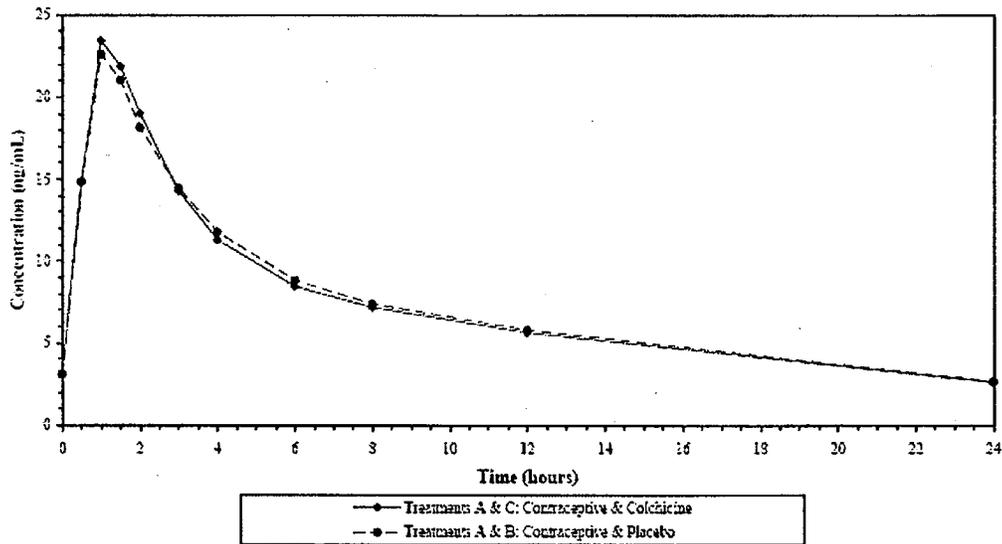
*Multiple dose administration of colchicine does not affect plasma levels of ethinyl estradiol and norethindrone.*

A single-center, double-blind, randomized, placebo-controlled, two-cycle crossover study (# 1005) in healthy women was conducted to determine the effect of colchicine on the steady-state pharmacokinetic profile of ethinyl estradiol and norethindrone. Subjects were required to be on an ethinyl estradiol (0.35 mg) and norethindrone (1 mg) oral regimen (1 tablet every day for 21 days, followed by one placebo tablet every day for 7 days) for at least one-cycle prior to study start date. An open-label, one-cycle contraceptive Run-in Period was required for subjects whose prior oral contraceptive regimen was not Ortho-Novum® 1/35 or its generic equivalent given in the recommended regimen. No pharmacokinetic assessments were done during this period. In the double-blind pharmacokinetic Period, subjects continued to receive Ortho-Novum® 1/35 for two additional cycles, administered in the morning. They were randomly assigned to one of two sequences of colchicine and placebo coadministration, given during the final 14 days of “active” Ortho-Novum® 1/35 tablets (Days 8 to 21). When randomized to colchicine, subjects received over-encapsulated colchicine tablets 0.6 mg b.i.d. for 14 days; when randomized to placebo, they received matching placebo capsules for 14 days.

Plasma Concentration Profiles of Ethinyl Estradiol + Placebo versus Ethinyl Estradiol + Colchicine Administered to 27 Healthy Female Adults (MPC-004-07-1005)



Plasma Concentration Profiles of Norethindrone + Placebo versus Norethindrone + Colchicine Administered to 27 Healthy Female Adults (MPC-004-07-1005)



Geometric Mean Ethinyl Estradiol and Norethindrone Pharmacokinetic Parameter Values When Administered with Placebo versus Ethinyl Estradiol and Norethindrone Pharmacokinetic Parameter Values When Administered with Steady-State Colchicine (0.6 mg b.i.d. × 14 days) in Healthy Adult Females (MPC-004-07-1005)

| Parameter                | Ethinyl Estradiol (EE) (n=27) |                              | Statistical Comparison<br>EE + colchicine vs. EE + placebo   |               |
|--------------------------|-------------------------------|------------------------------|--------------------------------------------------------------|---------------|
|                          | EE + colchicine<br>(test)     | EE + placebo<br>(reference)  | Ratio of Geometric<br>Means*                                 | 90% CI        |
| $C_{max}$ (ng/mL)        | 0.13                          | 0.14                         | 91.20                                                        | 85.93, 96.79  |
| $AUC_{(0-2)}$ (ng-hr/mL) | 1.18                          | 1.24                         | 95.71                                                        | 91.23, 100.41 |
| Parameter                | Norethindrone (NTD) (n=27)    |                              | Statistical Comparison<br>NTD + colchicine vs. NTD + placebo |               |
|                          | NTD + colchicine<br>(test)    | NTD + placebo<br>(reference) | Ratio of Geometric<br>Means*                                 | 90% CI        |
| $C_{max}$ (ng/mL)        | 23.40                         | 23.71                        | 98.67                                                        | 91.9, 105.95  |
| $AUC_{(0-2)}$ (ng-hr/mL) | 167.52                        | 171.95                       | 97.43                                                        | 92.82, 102.26 |

\* Geometric Mean Ratio =  $100 \exp(\text{test-reference})$   
 CI = confidence interval

As shown in the table above, when ethinyl estradiol and norethindrone are co-administered with steady-state colchicine (0.6 mg b.i.d. × 14 days) hormone concentrations were not affected as compared to hormone concentrations observed when ethinyl estradiol and norethindrone were administered with placebo. This is based on the 90% confidence intervals for geometric mean ratio of ethinyl estradiol and norethindrone being within the 80 – 125% confidence interval limits.

**What is the potential for colchicine to cause CYP inhibition type drug interactions?**

In vitro study results indicate that the potential for CYP inhibition type drug interactions with colchicine is “remote”.

In vitro studies in human liver microsomes were conducted to evaluate the potential of colchicine to inhibit the activities of cytochrome P450 (CYP) isoforms CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 in human liver microsomes. Colchicine inhibited activities of CYP2A6 and CYP2C8 in human liver microsomes without dose-dependent pattern. The inhibitory concentration 50% (IC50) values were greater than 45.82  $\mu$ M. Peak plasma levels of colchicine are in the range of 12 ng/mL or 0.03 microM. Hence, the Agency suggested I/Ki or I/IC50 for CYP2A6 or CYP2C8 inhibition is <0.1, indicating the remote likelihood of CYP inhibition by colchicine. Colchicine did not inhibit activities of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 in human liver microsomes.

**What is the potential for colchicine to cause CYP induction type drug interaction?**

*Colchicine at the tested concentrations did not induce activities of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 in hepatocytes from any of the three donors. Colchicine decreased the activity of the CYPs studied. The reasons for this decrease in activity levels are unknown and the results should be interpreted with caution since this observation may have resulted due to loss in hepatocyte viability. Further studies assessing cell viability may provide more insight into this observation.*

In vitro studies were conducted to evaluate the potential of colchicine to induce the activities of cytochrome P450 (CYP) isoforms CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 in three different batches of cryopreserved human hepatocytes. Rifampicin (25 microM) which was employed as positive control for CYP3A4 induction produced a 27-fold increase in CYP3A4 activity after 48 hour exposure with plated cryopreserved hepatocytes. Omeprazole (50 microM) was employed as a positive control for CYP1A2 induction and produced 6 -7.5 fold increase in CYP1A2 activity. Phenobarbital (1mM) was employed as a positive control for CYP2B6 induction and it produced 7.6-fold, 6.4-fold increase in CYP2B6 activity in hepatocytes from donor 1 and 2, respectively. However, CYP2B6 induction was not noted with phenobarbital in hepatocyte batch obtained from donor 3.

Colchicine at the tested concentrations (0.25, 2.5, and 25 microM) did not induce CYP1A2, CYP2B6 and CYP3A4 activity in human hepatocytes prepared from all three donors. On the contrary, significant decrease in CYP1A2, CYP2B6, CYP3A4 (see tables below), CYP2C activity (not shown here) was noted following colchicine treatment. However, sponsor indicated that hepatocyte viability was not assessed following colchicine treatment. Hence, it is not certain if this decrease in CYP activity is due to decrease in cell viability. Even if this were not an artifact, the downregulation of CYPs is not a well known phenomenon and hence the significance of these results is not clear.

**CYP1A2 Activity in Cryopreserved Human Hepatocyte Monolayers Following Incubation with Colchicine**

| Colchicine<br>( $\mu\text{M}$ ) | Acetaminophen Formation  |                            | Specific Activity<br>( $\text{pmol}/\text{min}/\text{million cells}$ ) |                      | Percent<br>of VC |                    |
|---------------------------------|--------------------------|----------------------------|------------------------------------------------------------------------|----------------------|------------------|--------------------|
|                                 | Raw<br>( $\mu\text{M}$ ) | Adjusted ( $\mu\text{M}$ ) | Individual                                                             | Mean $\pm$ SD        |                  |                    |
| <b>Human Donor 1</b>            |                          |                            |                                                                        |                      |                  |                    |
| 0<br>(VC)                       |                          |                            | 0.181                                                                  | 0.177 $\pm$ 0.00766  | 1.61             | 1.58 $\pm$ 0.0684  |
|                                 |                          |                            | 0.167                                                                  |                      |                  |                    |
|                                 |                          |                            | 0.175                                                                  |                      |                  |                    |
|                                 |                          |                            | 0.185                                                                  |                      |                  |                    |
| 0.25                            |                          |                            | 0.0309                                                                 | 0.0406 $\pm$ 0.0121  | 0.276            | 0.362 $\pm$ 0.108  |
|                                 |                          |                            | 0.0367                                                                 |                      |                  |                    |
|                                 |                          |                            | 0.0541                                                                 |                      |                  |                    |
| 2.5                             |                          |                            | 0.0439                                                                 | 0.0459 $\pm$ 0.00183 | 0.392            | 0.410 $\pm$ 0.0163 |
|                                 |                          |                            | 0.0474                                                                 |                      |                  |                    |
|                                 |                          |                            | 0.0464                                                                 |                      |                  |                    |
| 25                              |                          |                            | 0.0407                                                                 | 0.0415 $\pm$ 0.00248 | 0.363            | 0.371 $\pm$ 0.0222 |
|                                 |                          |                            | 0.0443                                                                 |                      |                  |                    |
|                                 |                          |                            | 0.0396                                                                 |                      |                  |                    |
| <b>Human Donor 2</b>            |                          |                            |                                                                        |                      |                  |                    |
| 0<br>(VC)                       |                          |                            | 0.225                                                                  | 0.236 $\pm$ 0.01034  | 2.01             | 2.11 $\pm$ 0.0923  |
|                                 |                          |                            | N/A                                                                    |                      |                  |                    |
|                                 |                          |                            | 0.240                                                                  |                      |                  |                    |
|                                 |                          |                            | 0.244                                                                  |                      |                  |                    |
| 0.25                            |                          |                            | 0.0467                                                                 | 0.0462 $\pm$ 0.00145 | 0.417            | 0.413 $\pm$ 0.0130 |
|                                 |                          |                            | 0.0473                                                                 |                      |                  |                    |
|                                 |                          |                            | 0.0446                                                                 |                      |                  |                    |
| 2.5                             |                          |                            | 0.0470                                                                 | 0.0488 $\pm$ 0.00234 | 0.419            | 0.436 $\pm$ 0.0209 |
|                                 |                          |                            | 0.0481                                                                 |                      |                  |                    |
|                                 |                          |                            | 0.0515                                                                 |                      |                  |                    |
| 25                              |                          |                            | 0.0400                                                                 | 0.0423 $\pm$ 0.00207 | 0.357            | 0.378 $\pm$ 0.0185 |
|                                 |                          |                            | 0.0438                                                                 |                      |                  |                    |
|                                 |                          |                            | 0.0432                                                                 |                      |                  |                    |
| <b>Human Donor 3</b>            |                          |                            |                                                                        |                      |                  |                    |
| 0<br>(VC)                       |                          |                            | 0.721                                                                  | 0.757 $\pm$ 0.0439   | 6.43             | 6.76 $\pm$ 0.392   |
|                                 |                          |                            | 0.718                                                                  |                      |                  |                    |
|                                 |                          |                            | 0.799                                                                  |                      |                  |                    |
|                                 |                          |                            | 0.791                                                                  |                      |                  |                    |
| 0.25                            |                          |                            | 0.153                                                                  | 0.180 $\pm$ 0.0236   | 1.36             | 1.61 $\pm$ 0.211   |
|                                 |                          |                            | 0.192                                                                  |                      |                  |                    |
|                                 |                          |                            | 0.195                                                                  |                      |                  |                    |
| 2.5                             |                          |                            | 0.164                                                                  | 0.180 $\pm$ 0.0173   | 1.46             | 1.61 $\pm$ 0.155   |
|                                 |                          |                            | 0.179                                                                  |                      |                  |                    |
|                                 |                          |                            | 0.198                                                                  |                      |                  |                    |
| 25                              |                          |                            | 0.153                                                                  | 0.157 $\pm$ 0.00699  | 1.36             | 1.40 $\pm$ 0.0625  |
|                                 |                          |                            | 0.165                                                                  |                      |                  |                    |
|                                 |                          |                            | 0.153                                                                  |                      |                  |                    |

**b(4)**

**CYP2B6 Activity in Cryopreserved Human Hepatocyte Monolayers Following Incubation with Colchicine**

| Colchicine<br>( $\mu\text{M}$ ) | Raw<br>( $\mu\text{M}$ ) | Nirvanol Formation |                        | Specific Activity<br>(pmol/min/million cells) |                      | Percent<br>of VC |
|---------------------------------|--------------------------|--------------------|------------------------|-----------------------------------------------|----------------------|------------------|
|                                 |                          | Individual         | Mean $\pm$ SD          | Individual                                    | Mean $\pm$ SD        |                  |
| <b>Human Donor 1</b>            |                          |                    |                        |                                               |                      |                  |
| 0<br>(VC)                       |                          | 0.0711             | 0.0737 $\pm$ 0.00204   | 0.635                                         | 0.658 $\pm$ 0.0183   | 100              |
|                                 |                          | 0.0730             |                        | 0.652                                         |                      |                  |
|                                 |                          | 0.0758             |                        | 0.676                                         |                      |                  |
|                                 |                          | 0.0749             |                        | 0.668                                         |                      |                  |
| 0.25                            |                          | 0.0363             | 0.0362 $\pm$ 0.0000781 | 0.324                                         | 0.323 $\pm$ 0.000697 | 49.2             |
|                                 |                          | 0.0363             |                        | 0.324                                         |                      |                  |
|                                 |                          | 0.0361             |                        | 0.323                                         |                      |                  |
| 2.5                             |                          | 0.0309             | 0.0357 $\pm$ 0.00414   | 0.276                                         | 0.318 $\pm$ 0.0370   | 48.4             |
|                                 |                          | 0.0383             |                        | 0.342                                         |                      |                  |
|                                 |                          | 0.0378             |                        | 0.337                                         |                      |                  |
| 25                              |                          | 0.0348             | 0.0356 $\pm$ 0.00260   | 0.310                                         | 0.318 $\pm$ 0.0232   | 48.3             |
|                                 |                          | 0.0335             |                        | 0.299                                         |                      |                  |
|                                 |                          | 0.0385             |                        | 0.344                                         |                      |                  |
| <b>Human Donor 2</b>            |                          |                    |                        |                                               |                      |                  |
| 0<br>(VC)                       |                          | 0.0694             | 0.0850 $\pm$ 0.0105    | 0.620                                         | 0.759 $\pm$ 0.0941   | 100              |
|                                 |                          | 0.0924             |                        | 0.825                                         |                      |                  |
|                                 |                          | 0.0898             |                        | 0.802                                         |                      |                  |
|                                 |                          | 0.0885             |                        | 0.790                                         |                      |                  |
| 0.25                            |                          | 0.0445             | 0.0420 $\pm$ 0.00424   | 0.397                                         | 0.375 $\pm$ 0.0378   | 49.3             |
|                                 |                          | 0.0371             |                        | 0.331                                         |                      |                  |
|                                 |                          | 0.0443             |                        | 0.396                                         |                      |                  |
| 2.5                             |                          | 0.0463             | 0.0426 $\pm$ 0.00385   | 0.413                                         | 0.380 $\pm$ 0.0343   | 50.1             |
|                                 |                          | 0.0386             |                        | 0.345                                         |                      |                  |
|                                 |                          | 0.0428             |                        | 0.382                                         |                      |                  |
| 25                              |                          | 0.0520             | 0.0510 $\pm$ 0.00314   | 0.464                                         | 0.455 $\pm$ 0.0281   | 60.0             |
|                                 |                          | 0.0475             |                        | 0.424                                         |                      |                  |
|                                 |                          | 0.0535             |                        | 0.478                                         |                      |                  |
| <b>Human Donor 3</b>            |                          |                    |                        |                                               |                      |                  |
| 0<br>(VC)                       |                          | <0.0250            | <0.0250 $\pm$ 0.000    | <0.223                                        | <0.223 $\pm$ 0.000   | 100              |
|                                 |                          | <0.0250            |                        | <0.223                                        |                      |                  |
|                                 |                          | <0.0250            |                        | <0.223                                        |                      |                  |
|                                 |                          | <0.0250            |                        | <0.223                                        |                      |                  |
| 0.25                            |                          | <0.0250            | <0.0250 $\pm$ 0.000    | <0.223                                        | <0.223 $\pm$ 0.000   | 100              |
|                                 |                          | <0.0250            |                        | <0.223                                        |                      |                  |
|                                 |                          | <0.0250            |                        | <0.223                                        |                      |                  |
| 2.5                             |                          | <0.0250            | <0.0250 $\pm$ 0.000    | <0.223                                        | <0.223 $\pm$ 0.000   | 100              |
|                                 |                          | <0.0250            |                        | <0.223                                        |                      |                  |
|                                 |                          | <0.0250            |                        | <0.223                                        |                      |                  |
| 25                              |                          | <0.0250            | <0.0250 $\pm$ 0.000    | <0.223                                        | <0.223 $\pm$ 0.000   | 100              |
|                                 |                          | <0.0250            |                        | <0.223                                        |                      |                  |
|                                 |                          | <0.0250            |                        | <0.223                                        |                      |                  |

Abbreviations: SD, standard deviation; VC, vehicle control (1% water/1% acetone/nitrile)

<sup>a</sup> The observed analyzed value ( $\mu\text{M}$ ) was below the lowest concentration on the standard curve (0.025  $\mu\text{M}$ ).

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

**b(4)**

| Colchicine<br>( $\mu\text{M}$ ) | 6 $\beta$ -Hydroxytestosterone Formation |                            |                     | Specific Activity<br>(pmol/min/million cells) |                    | Percent<br>of VC |
|---------------------------------|------------------------------------------|----------------------------|---------------------|-----------------------------------------------|--------------------|------------------|
|                                 | Raw<br>( $\mu\text{M}$ )                 | Adjusted ( $\mu\text{M}$ ) |                     | Individual                                    | Mean $\pm$ SD      |                  |
|                                 |                                          | Individual                 | Mean $\pm$ SD       |                                               |                    |                  |
| Human Donor 1                   |                                          |                            |                     |                                               |                    |                  |
| 0<br>(VC)                       |                                          | 0.419                      | 0.379 $\pm$ 0.0415  | 3.74                                          | 3.38 $\pm$ 0.370   | 100              |
|                                 |                                          | 0.376                      |                     | 3.35                                          |                    |                  |
|                                 |                                          | 0.398                      |                     | 3.56                                          |                    |                  |
|                                 |                                          | 0.323                      |                     | 2.88                                          |                    |                  |
| 0.25                            |                                          | 0.111                      | 0.166 $\pm$ 0.0510  | 0.993                                         | 1.48 $\pm$ 0.456   | 43.8             |
|                                 |                                          | 0.212                      |                     | 1.89                                          |                    |                  |
|                                 |                                          | 0.175                      |                     | 1.56                                          |                    |                  |
| 2.5                             |                                          | 0.146                      | 0.152 $\pm$ 0.00674 | 1.30                                          | 1.36 $\pm$ 0.0602  | 40.2             |
|                                 |                                          | 0.152                      |                     | 1.35                                          |                    |                  |
|                                 |                                          | 0.159                      |                     | 1.42                                          |                    |                  |
| 25 <sup>a</sup>                 |                                          | <0.100                     | <0.100 $\pm$ 0.000  | <0.893                                        | <0.893 $\pm$ 0.000 | <26.4            |
|                                 |                                          | <0.100                     |                     | <0.893                                        |                    |                  |
|                                 |                                          | <0.100                     |                     | <0.893                                        |                    |                  |
| Human Donor 2                   |                                          |                            |                     |                                               |                    |                  |
| 0<br>(VC)                       |                                          | <0.100                     | <0.125 $\pm$ 0.0172 | <0.893                                        | <1.12 $\pm$ 0.153  | 100              |
|                                 |                                          | 0.136                      |                     | 1.22                                          |                    |                  |
|                                 |                                          | 0.131                      |                     | 1.17                                          |                    |                  |
|                                 |                                          | 0.135                      |                     | 1.21                                          |                    |                  |
| 0.25                            |                                          | <0.100                     | <0.100 $\pm$ 0.000  | <0.893                                        | <0.893 $\pm$ 0.000 | 79.7             |
|                                 |                                          | <0.100                     |                     | <0.893                                        |                    |                  |
|                                 |                                          | <0.100                     |                     | <0.893                                        |                    |                  |
| 2.5                             |                                          | <0.100                     | <0.100 $\pm$ 0.000  | <0.893                                        | <0.893 $\pm$ 0.000 | 79.7             |
|                                 |                                          | <0.100                     |                     | <0.893                                        |                    |                  |
|                                 |                                          | <0.100                     |                     | <0.893                                        |                    |                  |
| 25 <sup>a</sup>                 |                                          | <0.100                     | <0.100 $\pm$ 0.000  | <0.893                                        | <0.893 $\pm$ 0.000 | 79.7             |
|                                 |                                          | <0.100                     |                     | <0.893                                        |                    |                  |
|                                 |                                          | <0.100                     |                     | <0.893                                        |                    |                  |
| Human Donor 3                   |                                          |                            |                     |                                               |                    |                  |
| 0<br>(VC)                       |                                          | 0.504                      | 0.518 $\pm$ 0.0127  | 4.50                                          | 4.63 $\pm$ 0.113   | 100              |
|                                 |                                          | 0.534                      |                     | 4.77                                          |                    |                  |
|                                 |                                          | 0.516                      |                     | 4.60                                          |                    |                  |
|                                 |                                          | 0.519                      |                     | 4.64                                          |                    |                  |
| 0.25                            |                                          | 0.221                      | 0.201 $\pm$ 0.0380  | 1.97                                          | 1.80 $\pm$ 0.339   | 38.9             |
|                                 |                                          | 0.226                      |                     | 2.02                                          |                    |                  |
|                                 |                                          | 0.158                      |                     | 1.41                                          |                    |                  |
| 2.5                             |                                          | 0.249                      | 0.244 $\pm$ 0.00500 | 2.23                                          | 2.18 $\pm$ 0.0446  | 47.0             |
|                                 |                                          | 0.241                      |                     | 2.15                                          |                    |                  |
|                                 |                                          | 0.241                      |                     | 2.15                                          |                    |                  |
| 25 <sup>a</sup>                 |                                          | <0.100                     | <0.118 $\pm$ 0.0157 | <0.893                                        | <1.05 $\pm$ 0.141  | <22.7            |
|                                 |                                          | 0.130                      |                     | 1.16                                          |                    |                  |
|                                 |                                          | 0.123                      |                     | 1.10                                          |                    |                  |

Abbreviations: SD, standard deviation; VC, vehicle control (1% water/1% acetonitrile)

<sup>a</sup> Test Article interference was observed in each of these samples.

<sup>b</sup> The observed analyzed value ( $\mu\text{M}$ ) was below the lowest concentration on the standard curve (0.1  $\mu\text{M}$ ).

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

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**CYP3A4 Activity in Cryopreserved Human Hepatocyte Monolayers Following Incubation with Colchicine**

| Colchicine<br>( $\mu\text{M}$ ) | 6 $\beta$ -Hydroxytestosterone Formation |                            | Specific Activity<br>(pmol/min/million cells) |                     | Percent<br>of VC |                    |       |
|---------------------------------|------------------------------------------|----------------------------|-----------------------------------------------|---------------------|------------------|--------------------|-------|
|                                 | Raw<br>( $\mu\text{M}$ )                 | Adjusted ( $\mu\text{M}$ ) | Individual                                    | Mean $\pm$ SD       |                  |                    |       |
| <b>Human Donor 1</b>            |                                          |                            |                                               |                     |                  |                    |       |
| 0<br>(VC)                       |                                          |                            | 0.419                                         | 0.379 $\pm$ 0.0415  | 3.74             | 3.38 $\pm$ 0.370   | 100   |
|                                 |                                          |                            | 0.376                                         |                     | 3.35             |                    |       |
|                                 |                                          |                            | 0.398                                         |                     | 3.56             |                    |       |
|                                 |                                          |                            | 0.323                                         |                     | 2.88             |                    |       |
| 0.25                            |                                          |                            | 0.111                                         | 0.166 $\pm$ 0.0510  | 0.993            | 1.48 $\pm$ 0.456   | 43.8  |
|                                 |                                          |                            | 0.212                                         |                     | 1.89             |                    |       |
|                                 |                                          |                            | 0.175                                         |                     | 1.56             |                    |       |
| 2.5                             |                                          |                            | 0.146                                         | 0.152 $\pm$ 0.00674 | 1.30             | 1.36 $\pm$ 0.0602  | 40.2  |
|                                 |                                          |                            | 0.152                                         |                     | 1.35             |                    |       |
|                                 |                                          |                            | 0.159                                         |                     | 1.42             |                    |       |
| 25 <sup>a</sup>                 |                                          |                            | <0.100                                        | <0.100 $\pm$ 0.000  | <0.893           | <0.893 $\pm$ 0.000 | <26.4 |
|                                 |                                          |                            | <0.100                                        |                     | <0.893           |                    |       |
|                                 |                                          |                            | <0.100                                        |                     | <0.893           |                    |       |
| <b>Human Donor 2</b>            |                                          |                            |                                               |                     |                  |                    |       |
| 0<br>(VC)                       |                                          |                            | <0.100                                        | <0.125 $\pm$ 0.0172 | <0.893           | <1.12 $\pm$ 0.153  | 100   |
|                                 |                                          |                            | 0.136                                         |                     | 1.22             |                    |       |
|                                 |                                          |                            | 0.131                                         |                     | 1.17             |                    |       |
|                                 |                                          |                            | 0.135                                         |                     | 1.21             |                    |       |
| 0.25                            |                                          |                            | <0.100                                        | <0.100 $\pm$ 0.000  | <0.893           | <0.893 $\pm$ 0.000 | 79.7  |
|                                 |                                          |                            | <0.100                                        |                     | <0.893           |                    |       |
|                                 |                                          |                            | <0.100                                        |                     | <0.893           |                    |       |
| 2.5                             |                                          |                            | <0.100                                        | <0.100 $\pm$ 0.000  | <0.893           | <0.893 $\pm$ 0.000 | 79.7  |
|                                 |                                          |                            | <0.100                                        |                     | <0.893           |                    |       |
|                                 |                                          |                            | <0.100                                        |                     | <0.893           |                    |       |
| 25 <sup>a</sup>                 |                                          |                            | <0.100                                        | <0.100 $\pm$ 0.000  | <0.893           | <0.893 $\pm$ 0.000 | 79.7  |
|                                 |                                          |                            | <0.100                                        |                     | <0.893           |                    |       |
|                                 |                                          |                            | <0.100                                        |                     | <0.893           |                    |       |
| <b>Human Donor 3</b>            |                                          |                            |                                               |                     |                  |                    |       |
| 0<br>(VC)                       |                                          |                            | 0.504                                         | 0.518 $\pm$ 0.0127  | 4.50             | 4.63 $\pm$ 0.113   | 100   |
|                                 |                                          |                            | 0.534                                         |                     | 4.77             |                    |       |
|                                 |                                          |                            | 0.516                                         |                     | 4.60             |                    |       |
|                                 |                                          |                            | 0.519                                         |                     | 4.64             |                    |       |
| 0.25                            |                                          |                            | 0.221                                         | 0.201 $\pm$ 0.0380  | 1.97             | 1.80 $\pm$ 0.339   | 38.9  |
|                                 |                                          |                            | 0.226                                         |                     | 2.02             |                    |       |
|                                 |                                          |                            | 0.158                                         |                     | 1.41             |                    |       |
| 2.5                             |                                          |                            | 0.249                                         | 0.244 $\pm$ 0.00500 | 2.23             | 2.18 $\pm$ 0.0446  | 47.0  |
|                                 |                                          |                            | 0.241                                         |                     | 2.15             |                    |       |
|                                 |                                          |                            | 0.241                                         |                     | 2.15             |                    |       |
| 25 <sup>a</sup>                 |                                          |                            | <0.100                                        | <0.118 $\pm$ 0.0157 | <0.893           | <1.05 $\pm$ 0.141  | <22.7 |
|                                 |                                          |                            | 0.130                                         |                     | 1.16             |                    |       |
|                                 |                                          |                            | 0.123                                         |                     | 1.10             |                    |       |

Abbreviations: SD, standard deviation; VC, vehicle control (1% water/1% acetonitrile)

<sup>a</sup> Test Article interference was observed in each of these samples.

<sup>b</sup> The observed analyzed value ( $\mu\text{M}$ ) was below the lowest concentration on the standard curve (0.1  $\mu\text{M}$ ).

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

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### Additional reports on drug interactions with colchicine

Several published reports/case studies of colchicine toxicity have been documented when colchicine is coadministered with CYP3A4 and P-gp inhibitors. Some of these publications are tabulated below. Although the basis for the drug interaction is a speculation, the consequences do warrant caution when concomitant administration of these agents is anticipated.

#### Published Interactions Involving Colchicine<sup>3</sup>

| Drug / Compound                               | Reference(s)                                                                               | Speculated Basis for Interaction                                                   | Effect                                                                |
|-----------------------------------------------|--------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|-----------------------------------------------------------------------|
| Cyclosporine                                  | <u>Minetti and Minetti (2003)</u>                                                          | P-gp inhibition by cyclosporine; both are CYP3A4 substrates                        | Increased colchicine plasma levels and toxicity                       |
| Grapefruit juice                              | <u>Goldbart et al. (2000)</u>                                                              | CYP3A4 (moderate) inhibition by grapefruit juice; OATP & P-gp inhibition           | Colchicine toxicity                                                   |
| <b>Macrolide Antibiotics</b>                  |                                                                                            |                                                                                    |                                                                       |
| Clarithromycin                                | <u>Cheng et al. (2005); Dogukan et al. (2001); Hung et al. (2005); Akdag et al. (2006)</u> | CYP3A4 and P-gp inhibition (strong) by clarithromycin                              | Colchicine toxicities, including fatalities                           |
| Erythromycin                                  | <u>Caraco et al. (1992)</u>                                                                | CYP3A4 (moderate) and P-gp inhibition by erythromycin                              | Colchicine toxicity                                                   |
| <b>Lipid Lowering Agents</b>                  |                                                                                            |                                                                                    |                                                                       |
| HMG-CoA Reductase Inhibitors                  | Simvastatin: <u>Baker et al. (2004); Hsu et al. (2002)</u>                                 | Both are CYP3A4 and P-gp substrates; P-gp inhibition by simvastatin                | Acute myopathy or rhabdomyolysis (could be attributed to either drug) |
|                                               | Fluvastatin: <u>Atasovu et al. (2005)</u>                                                  | Synergistic myotoxicity via PK & PD mechanism; fluvastatin is not a P-gp inhibitor |                                                                       |
|                                               | Pravastatin: <u>Alavli et al. (2005)</u>                                                   | Synergistic myotoxicity via PK & PD mechanism; pravastatin is not a P-gp inhibitor |                                                                       |
|                                               | Atorvastatin: <u>Tufan et al. (2006)</u>                                                   | Both are CYP3A4 substrates; P-gp inhibition by atorvastatin                        |                                                                       |
| Fibrates                                      | Gemfibrozil: <u>Atmaca et al., 2002</u>                                                    | Synergistic toxic effect of both drugs                                             |                                                                       |
|                                               | Fenofibrate & Diltiazem: <u>Sinsawaiwong et al., 1997</u>                                  | Mechanism-based inhibition of CYP3A4 by diltiazem.                                 |                                                                       |
| Verapamil                                     | <u>Tröger et al. (2005)</u>                                                                | CYP3A4 (moderate) and P-gp inhibition by verapamil                                 | Colchicine toxicity                                                   |
| Verapamil & Digoxin                           | <u>Dawson et al., 1997</u> Da                                                              | CYP3A4 (moderate) and P-gp inhibition by verapamil                                 | Rhabdomyolysis                                                        |
| Fludione <sup>+</sup> & Digoxin or Furosemide | <u>Gras-Champel et al. (2005)</u>                                                          | Digoxin and furosemide are transporter substrates or inhibitors                    | Increased INR upon addition of colchicine                             |
| Digoxin                                       | <u>Debie et al., 2003; Chattopadhyay et al., 2001</u>                                      | Digoxin is a P-gp substrate; possibly due to P-gp interaction                      | Rhabdomyolysis                                                        |
| Vinblastine & Tolbutamide                     | <u>Besana et al. 1987</u>                                                                  | Vinblastine is a P-gp substrate; possibly due to P-gp interaction                  | Neuromyopathy                                                         |

## 2.5 General Biopharmaceutics

Colstat is an immediate release scored tablet formulation containing 0.6 mg colchicine (See table below).

Composition of Colchicine Tablets USP, 0.6 mg

| Ingredient                     | 0.6 mg Tablets         |       |
|--------------------------------|------------------------|-------|
|                                | Amount per Tablet (mg) | % w/w |
| Colchicine (Active)            | 0.60 <sup>1</sup>      |       |
| Lactose monohydrate, NF        |                        |       |
| Pregelatinized starch, NF      |                        |       |
| Microcrystalline cellulose, NF |                        |       |
| Sodium starch glycolate, NF    |                        |       |
| Magnesium stearate, NF         |                        |       |
| Carnauba Wax                   |                        |       |
| <b>TOTAL</b>                   |                        | 100   |

<sup>1</sup> Registered trademark of \_\_\_\_\_ contains FD&C Blue #2 \_\_\_\_\_ FD&C Red #40 \_\_\_\_\_ hypromellose, polydextrose, polyethylene glycol, titanium dioxide, and triacetin.

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## 2.6 Analytical

An HPLC with tandem mass spectrometry (LC MS/MS) method was developed and validated for the determination of colchicine in human plasma. Colchicine and internal standard \_\_\_\_\_ are extracted from human plasma matrix (EDTA as the anticoagulant) using a solid phase extraction. The concentrations of quality control (QC) standards are 0.20 ng/mL for the lower level QC, 0.60 ng/mL for the Low QC, 3.20 ng/mL for the Mid-QC, 32.00 ng/mL for the High-QC, and 40.00 ng/mL for the upper level QC (PRACS Bioanalytical method 181.1).

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A validated LC-MS/MS method was employed for the analysis of metabolites, 2- and 3-O-demethylcolchicine (2-DMC and 3-DMC). Absolute recovery of the metabolite was greater than 95% at all concentrations. The method has been shown to be linear over the range 0.20 to 40.00 ng/mL for both metabolites (PRACS Bioanalytical method 181.3).

### 3 Labeling

Sponsor proposed labeling in the clinical pharmacology relevant sections is presented in regular text. Initial draft of labeling additions and deletions are indicated as bold text and strikethrough text.

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32 Page(s) Withheld

       § 552(b)(4) Trade Secret / Confidential

  √   § 552(b)(4) Draft Labeling

       § 552(b)(5) Deliberative Process

## 4.2 Individual Study Reviews

### 4.2.1 Analytical Method Validation Summary

Colchicine in Human Plasma  
PRACS Bioanalytical Method #181.1

#### 1 COLCHICINE VALIDATION

Validation demonstrates that the analytical method can accurately and precisely quantitate Colchicine in human plasma over the concentration range stated. Validation tests are also used to establish the parameters under which Colchicine is stable while being assayed with the analytical method. Two different instruments and more than one column were used in the validation.

##### 1.1 REFERENCE STANDARDS

Colchicine, (Lot TRC-0207072005), purity 98%, and the internal standard (IS),  
\_\_\_\_\_ (Lot 4-JWA-47-1), purity 98%, were obtained from \_\_\_\_\_  
\_\_\_\_\_. The certificates of analysis are attached in Appendix 1.

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##### 1.2 STANDARD CURVE AND QUALITY CONTROLS

The accuracy and precision for this validation was accomplished by analyzing three standard curves prepared in human plasma and consisting of nine concentrations prepared in two replicates for three separate batches. Quality Controls (QCs) were also prepared to assess accuracy, precision, and stability. The standard curve concentrations ranged from 200 pg/mL to 40000 pg/mL. The concentration of QCs were 200 pg/mL for QC 1 (LLQC), 600 pg/mL for QC 2 (Low), 3200 pg/mL for QC 3, 16000 pg/mL for QC 4 (Mid), and 32000 pg/mL for QC 5 (High). The instrument software was used to acquire raw data, determine the chromatographic peak area of Colchicine and \_\_\_\_\_ internal standard (IS). The peak area data was exported to the Watson LIMS to construct a regression equation based on the peak area ratios of Colchicine to IS. The three validation runs, showing calibration standards and QCs are shown in Table 1 through Table 3, while a summary of the three-batch validation is shown in Table 4 for standards and Table 5 for QCs. For examples of chromatograms produced during method validation, see Appendix 2.

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##### 1.3 BIOLOGICAL MATRIX

Blank human plasma for preparation of calibration standard and quality control standards (QC) contained K<sub>2</sub> EDTA as the anticoagulant and was obtained from Biological Specialties, Colmar PA.

##### 1.4 GOODNESS OF FIT

Goodness of fit is reported as the coefficient of determination ( $r^2$ ) and was obtained from least squares regression (weighted  $1/x$ ) of the standard curve fitted to a linear model. All validation standard curves showed  $r^2$  values of 0.9994 or greater. The mean value for the slope and intercept were 0.00058 and 0.00298, respectively (Table 4).

## 1.5 INTER-BATCH AND INTRA-BATCH ACCURACY AND PRECISION

Precision is defined as the variability of replicate measurements and is reported as the coefficient of variation (CV). Accuracy is defined as the difference between the mean of a set of results and the "true" value. Both values are reported as percentages.

The inter-batch accuracy and precision of standards are shown in Table 4, while Table 5 reports the inter-batch accuracy and precision of QCs. The inter-batch accuracy for standards was between 98.57% and 101.92%, while the CV was  $\leq 5.60\%$ . The inter-batch accuracy of QCs was between 101.17% and 106.22%, while the CV was  $\leq 6.38\%$ .

The intra-batch accuracy and precision was assessed by analyzing six replicates of the QC 1 at 200 pg/mL, QC 2 at 600 pg/mL, QC 3 at 3200 pg/mL QC 4 at 16000 pg/mL, and QC 5 at 32000 pg/mL. The intra-batch accuracy ranged from 96.75% to 109.17%, while the CV was  $\leq 5.93\%$  (Table 5). This method was demonstrated to be able to accurately and precisely quantitate all standards and QC samples.

## 1.6 SELECTIVITY

Selectivity is defined as the ability of the analytical method to measure a response from the analyte without interference from the biological matrix. This was accomplished by evaluating seven different lots of human plasma without IS. No significant baseline interference was detected at the retention times of Colchicine or IS. Seven lots out of seven lots tested passed acceptance criteria (Table 6).

## 1.7 ABSOLUTE RECOVERY

Absolute recovery was defined as the area of extracted standard (referred to as "matrix") versus the area of an extracted double blank (no analyte and no IS) reconstituted in a solution of the analyte in reconstitution solution, at actual sample concentrations (referred to as "neat"). The area was calculated based on the average of six samples at each level, for both the matrix and neat. The absolute recovery of Colchicine was 86.78% at 600 pg/mL, 82.78% at 16000 pg/mL, and 85.30% at 32000 pg/mL with an average absolute recovery of 84.95% (Table 7). Absolute recovery of \_\_\_\_\_ was also calculated in a similar manner. The concentration of \_\_\_\_\_ that represented 100% recovery in all samples was 2.50 ng/mL. The absolute recovery of \_\_\_\_\_ was 76.28% (Table 8).

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## 1.8 SOLUTION STABILITY

Colchicine (10.0  $\mu\text{g/mL}$ ) and \_\_\_\_\_ (10.0  $\mu\text{g/mL}$ ) stock solution stabilities were determined by comparing previously prepared solutions, which were stored for several days under given conditions (for example, refrigerator and room temperature), with fresh

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solutions. Solutions were considered stable if the concentration remained within 10% of the freshly prepared solution.

Colchicine stock solution in methanol was tested at 148:47 hours–minutes at ~4 °C and room temperature. The Colchicine stock solution passed for the refrigerated stability (Table 9) but failed for the room temperatures stability at this time (Table 11).

\_\_\_\_\_ stock solution in methanol was tested for 148:31 hours–minutes at ~4 °C and at room temperature and passed for both temperatures (Table 13 and Table 15)

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Colchicine stock solution in methanol was re-tested and found to be stable for 265:02 hours–minutes at ~4 °C (Table 10) and at room temperature (Table 12) in Colchicine validation 181.2. \_\_\_\_\_ stock solution in methanol was re-tested and found to be stable for 264:43 hours–minutes at ~4 °C (Table 14) and at room temperature (Table 16) in Colchicine validation 181.2. Because solution stability is not dependent upon the biological matrix, the solution stability in validation 181.2 Colchicine in Beagle Plasma is used to extend the solution stability for this validation.

#### **1.9 FREEZE/THAW STABILITY**

Freeze/Thaw stability is a measure of the stability of Colchicine in human plasma after being frozen, then thawed over a number of cycles. Freeze/Thaw stability was performed using replicates of QC 2 and QC 5.

Replicates of QC 2 and QC 5 QCs (reference samples) that were thawed only once were analyzed along with a standard curve (prepared fresh) and all the stability samples. The concentrations were computed from the standard curve and compared. If the comparison of the mean concentrations of the stability and reference samples remains within  $\pm 15\%$ , the freeze/thaw samples are considered stable. The results show that Colchicine is stable for four freeze/thaw cycles over the concentration range of this study (Table 17).

#### **1.10 SHORT TERM STABILITY**

Short term stability is a measure of the stability of Colchicine in human plasma while being held at room temperature for a given time interval. Short term stability was performed using replicates of QC 2 and QC 5.

Replicates of QC 2 and QC 5 reference samples and standard curve were prepared and analyzed along with all the stability samples. The concentrations were computed from the standard curve and compared. If the comparison of the mean concentrations of the stability and reference samples remains within  $\pm 15\%$ , the short term samples are considered stable.

For room temperature stability, QCs were thawed in a water bath (~35 °C) and then placed on the bench at room temperature (stability samples). Colchicine was found to be stable for 20:38 hours–minutes at room temperature (Table 18).

#### **1.11 LONG TERM STABILITY**

Long term stability is a measure of the stability of Colchicine in human plasma samples while stored at -20 °C. A standard curve is prepared and analyzed along with quality control standards that have been frozen for a period of time. Studies show that Colchicine is stable in human plasma at -20 °C for up to eight days (Table 19). This study is ongoing and will not be concluded for several months.

#### **1.12 PROCESSED SAMPLE STABILITY**

Processed sample stability is tested by preparing six replicates of QC 2 and QC 5. Once processed, the QCs (stability samples) are allowed to sit at -4 °C. Replicates of QC 2 and QC 5 reference samples were prepared and analyzed along with the stability samples using a freshly prepared standard curve. The concentrations were computed from the standard curve and compared. If the comparison of the mean concentrations of the reference and stability samples remains within  $\pm 15\%$ , the samples are considered stable. Colchicine processed samples initially tested for 95:37 hours–minutes failed acceptance criteria (Table 20). Colchicine processed samples were re-tested and were demonstrated to be stable for 47:43 hours–minutes at -4 °C (Table 21)

#### **1.13 DILUTION INTEGRITY TEST**

During analysis of samples with unknown concentrations, there are occasions when a dilution of a sample must be made. The effect of diluting a sample with blank matrix was tested by preparing a Dilution QC in human plasma at a Colchicine concentration 20% greater than the highest calibration standard, in this case 48000 pg/mL. Six replicates each of 1:1 (v:v sample–blank), 1:2 (v:v sample–blank), 1:3 (v:v sample–blank), and 1:4 (v:v sample–blank) dilutions prepared from the human plasma sample and blank human plasma were processed and analyzed along with a calibration curve. The reported values represent the concentration of each sample after correction for the dilution. Correcting for the dilution factors resulted in an accuracy of 95.63%, 97.08%, 97.92%, and 99.38% for 1:1, 1:2, 1:3, and 1:4 dilutions, respectively (Table 22).

#### **1.14 MATRIX EFFECT TEST**

Interference from the matrix was tested by preparing an LLOQ and ULOQ (upper limit of quantitation) in six different lots of human plasma and analyzing them. The CV of the LLOQ was 3.39% and the ULOQ was 1.40% (Table 23). Discernable matrix effects were not observed in the human plasma lots tested.

### 1.15 POTENTIAL INTERFERENCES

Interference from common over-the-counter medications is always a concern in bioanalytical work. The method validated for Colchicine was also tested with several over-the-counter drugs including acetylsalicylic acid, naproxen, ibuprofen, caffeine, pseudoephedrine, and acetaminophen. These drugs neither eluted with nor interfered with the analysis of Colchicine or —————. See Table 24 for the results of this test.

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### 1.16 RE-INJECTION STABILITY

Re-injection stability is tested by preparing an intra-batch accuracy and precision run. Once processed and injected, the intra-batch accuracy and precision run is allowed to sit at ~4 °C. The intra-batch accuracy and precision run is then re-injected. The reference quality controls (QC) must meet established acceptance criteria (four of six of the QCs at each level tested must be within  $\pm 15\%$  of the nominal value. The CV of all of the QC replicates tested should be  $\leq 15\%$ . The mean of all the QC replicates tested should be  $\pm 15\%$  of the nominal value). The drug is considered stable for the time duration tested (the time of the first QC 1-1 injection to the time of the final QC 1-1 injection). The test was acceptable for 28:32 hours–minutes (Table 25).

## 2 SOP DEVIATIONS DURING VALIDATION

During the performance of this study, all results were generated using a LIMS system (Thermo Watson LIMS version 7.1.0.01). The LIMS system has been successfully validated with a full IQ/OQ/PQ performed in-house using the vendor supplied ——— package and a custom ——— developed by PRACS Institute, Ltd. to validate the system according to regulatory guidance as it is used in-house. At the time of this report writing, the LIMS system has not been fully certified by a final report as the final review and analysis of the data from the PQ validation is still under its final QA and Statistical review. A Gap Analysis was performed, reviewed and approved regarding this issue by Senior Bioanalytical and QA management. No issues were found to affect the data integrity generated by the LIMS system prior to the final report being completed.

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## 1 METHOD SUMMARY

An automated solid phase extraction procedure with analysis of the extract by LC-MS/MS was developed and validated for the determination of Colchicine, 2-Demethyl Colchicine, and 3-Demethyl Colchicine in human plasma containing K<sub>2</sub>-EDTA as the anticoagulant. The standard curve ranged from 0.2000 ng/mL to 40.00 ng/mL. Standards and QCs were extracted according to the extraction procedure illustrated in the analytical procedure. The multiple reaction monitoring (MRM) mode was used to monitor the precursor and product ions for the analytes, Colchicine, 2-Demethyl Colchicine, and 3-Demethyl Colchicine, and the internal standard, \_\_\_\_\_ Peak areas were exported to Watson LIMS to create a calibration curve using weighted (1/x<sup>2</sup>) least squares regression fit to a linear model and to calculate concentrations. The values are reported in ng/mL.

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## 2 GENERAL VALIDATION

Validation demonstrates that the analytical method can accurately and precisely quantitate Colchicine, 2-Demethyl Colchicine, and 3-Demethyl Colchicine in human plasma over the concentration range stated. Validation tests are also used to establish the parameters under which Colchicine, 2-Demethyl Colchicine, and 3-Demethyl Colchicine are stable while being assayed with the analytical method. One instrument and multiple columns were used in the validation.

### 2.1 BIOLOGICAL MATRIX

Blank human plasma for preparation of calibration standard and quality control standards (QC) contained K<sub>2</sub>-EDTA as the anticoagulant and was obtained from \_\_\_\_\_

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## 3 COLCHICINE VALIDATION

### 3.1 REFERENCE STANDARDS

Colchicine, Lot TRC-0207072005, purity 98%, was obtained from \_\_\_\_\_  
\_\_\_\_\_ The internal standard (IS), \_\_\_\_\_ Lot 4-JWA-47-1, purity 98%, was  
obtained from \_\_\_\_\_ The certificates of analysis are attached in  
Appendix 1.

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### 3.2 STANDARD CURVE AND QUALITY CONTROLS

The accuracy and precision for this validation was accomplished by analyzing four standard curves prepared in human plasma and consisting of nine concentrations prepared in singlet in four separate batches. Quality Controls (QCs) were also prepared to assess accuracy, precision, and stability. The standard curve concentrations ranged from 0.2000 ng/mL to 40.00 ng/mL. The concentration of QCs were 0.2000 ng/mL for the

LLQC, 0.6000 ng/mL for the Low QC, 3.200 ng/mL for the Mid QC, 32.00 ng/mL for the High QC, and 40.00 ng/mL for the ULQC.

The instrument software was used to acquire raw data, and determine the chromatographic peak area of Colchicine and ————— internal standard (IS). The peak area data was exported to Watson LIMS to construct a regression equation based on the peak area ratios of Colchicine to IS. The results of the four validation runs containing calibration standards and QCs are reported in Table 1 through Table 8, while a summary of the inter-batch validation data are reported in Table 9 for standards and Table 10 for QCs. The first accuracy and precision run for Colchicine failed because the blanks (extracted samples without drug or internal standard) and zeros (extracted samples without drug with internal standard) contained a peak in the Colchicine channel >20% of the LLOQ. The inter-batch data in Table 10 contains data for all four accuracy and precision runs. For examples of chromatograms produced during method validation, see Appendix 2.

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### 3.3 LINEARITY

Linearity is reported as the coefficient of determination ( $r^2$ ) and was obtained from least squares regression (weighted  $1/x^2$ ) of the standard curve fit to a linear model. All Colchicine standard curves showed  $r^2$  values of 0.9953 or greater. The mean value for the slope and intercept were 0.3886 and 0.02067, respectively.

### 3.4 INTER-BATCH AND INTRA-BATCH ACCURACY AND PRECISION

Accuracy is defined as the closeness of an observation or mean to the true value, and is expressed as %Bias. Precision is defined as the coefficient of variation (CV) of individual replicates from the calculated values.

The inter-batch accuracy and precision of standards are shown in Table 9, while Table 10 reports the inter-batch accuracy and precision of QCs. The inter-batch %Bias for standards was between -7.9% and 6.3%, while the CV was  $\leq 3.3\%$ . The inter-batch %Bias of QCs was between -5.4% and 8.0%, while the CV was  $\leq 4.6\%$ .

The intra-batch accuracy and precision was assessed by analyzing six replicates at each QC level: 0.2000 ng/mL for the LLQC, 0.6000 ng/mL for the Low QC, 3.200 ng/mL for the Mid QC, 32.00 ng/mL for the High QC, 40.00 ng/mL for the ULQC. The intra-batch %Bias ranged from -7.0% to 13.3%, while the CV was  $\leq 4.3\%$  (Table 10). This method was demonstrated to be able to accurately and precisely quantitate all standards and QC samples.

### 3.5 SELECTIVITY

Selectivity is defined as the ability of the analytical method to measure a response from the analyte without interference from the biological matrix. This was accomplished by evaluating seven different lots of human plasma with and without IS. No significant

baseline interference was detected at the retention times of Colchicine or IS. Seven out of seven lots tested passed acceptance criteria (Table 11).

### 3.6 ABSOLUTE RECOVERY

Absolute recovery was defined as the area of extracted standard (referred to as "matrix") versus the area of an extracted double blank (no analyte and no IS) reconstituted using reconstitution solution containing analyte at actual sample concentrations (referred to as "neat"). The area was calculated based on the average of six replicates at each level, for both the matrix and neat. The absolute recovery of Colchicine was 98.63% at 0.6000 ng/mL, 93.36% at 3.200 ng/mL, and 92.69% at 32.00 ng/mL with an average absolute recovery of 94.90% (Table 12). Absolute recovery of \_\_\_\_\_ was also calculated in a similar manner. The concentration of \_\_\_\_\_ that represented 100% recovery in all samples was 10.00 ng/mL. The absolute recovery of \_\_\_\_\_ was 87.89% (Table 13).

b(4)

### 3.7 SOLUTION STABILITY

The Colchicine stock solution stability in Methanol was evaluated at room temperature (Table 14) and in the refrigerator at  $4\text{ }^{\circ}\text{C} \pm 6\text{ }^{\circ}\text{C}$  (Table 15) and was determined to be stable for 265:02 hours–minutes in validation 181.2.

The stock solution stability of \_\_\_\_\_ was evaluated at room temperature and in the refrigerator at  $4\text{ }^{\circ}\text{C} \pm 6\text{ }^{\circ}\text{C}$ . \_\_\_\_\_ stock solution in Methanol was determined to be stable for 264:43 hours–minutes at  $4\text{ }^{\circ}\text{C} \pm 6\text{ }^{\circ}\text{C}$  (Table 17) and room temperature (Table 16).

b(4)

The 10.00 ng/mL \_\_\_\_\_ working solution was prepared in Water and stored at room temperature. The \_\_\_\_\_ working solution did not meet acceptance criteria (Table 18 and Table 19) and will be prepared fresh daily.

b(4)

### 3.8 FREEZE/THAW STABILITY

Stability of Colchicine in human plasma at three different concentrations (Low, Mid, and High) was evaluated over 6 freeze/thaw cycles. Each cycle consisted of taking QC samples that were frozen at  $-20\text{ }^{\circ}\text{C} \pm 10\text{ }^{\circ}\text{C}$ , thawing them in a room temperature water bath for 2 hours and freezing them again. Low, Mid, and High QCs that underwent 1, 2, 3, 4 and 6 cycles were analyzed against a freshly prepared standard curve. Freeze/Thaw stability was assessed over three separate runs with the first one containing data for cycles 1, 2 and 3 (Table 20), the second one containing data for cycle 4 (Table 21) and the third containing data for cycle 6 (Table 22). The %Bias was between 0.4% and 9.7% for the Low QCs, 0.0% and 8.7% for the Mid QCs, and -9.2% and -1.1% for the High QCs. The results show that Colchicine is stable for six freeze/thaw cycles (Table 22). A batch run assessing stability for cycles 4 and 6 failed due to the Low freeze/thaw QCs and the Low and Mid qualifying QCs not meeting acceptance criteria (Table 23). Cycle 4 and 6 were repeated as two separate tests resulting in the acceptable data mentioned above.

### **3.9 STABILITY OF COLCHICINE IN HUMAN PLASMA AT ROOM TEMPERATURE**

The stability of Colchicine in human plasma was determined at room temperature by thawing Low, Mid, and High QC samples and storing them at room temperature for a minimum of 24 hours before extracting 6 sets of each along with a freshly prepared standard curve. The %Bias ranged from -6.6% to 7.7%. The results show that Colchicine in human plasma at room temperature is stable for 47:46 hours–minutes (Table 24).

### **3.10 BENCH TOP STABILITY OF EXTRACTED SAMPLES**

The stability of Colchicine in extracted samples stored on the bench top at room temperature was determined. After six sets of Low, Mid, and High QCs were extracted and reconstituted they were stored at room temperature for a minimum of 24 hours before they were analyzed along with a freshly prepared standard curve. The %Bias ranged from -9.2% to 4.3%. The results show that extracted Colchicine samples that are stored at room temperature are stable for 95:59 hours–minutes (Table 28). This test was also performed and met acceptance criteria for the shorter durations of 23:08 hours–minutes (Table 25) and 48:29 hours–minutes (Table 27). One run failed to meet acceptance criteria for the Low and Mid stability and qualifying QCs (Table 26).

### **3.11 REFRIGERATOR STABILITY OF EXTRACTED SAMPLES**

The stability of Colchicine in extracted samples stored in a refrigerator at  $4\text{ }^{\circ}\text{C} \pm 6\text{ }^{\circ}\text{C}$  was determined. After six sets of Low, Mid, and High QCs were extracted and reconstituted they were stored in a refrigerator for a minimum of 24 hours before they were analyzed along with a freshly prepared standard curve. The %Bias ranged from -7.9% to 3.5%. The results show that extracted Colchicine samples that are stored at  $4\text{ }^{\circ}\text{C} \pm 6\text{ }^{\circ}\text{C}$  are stable for 108:02 hours–minutes (Table 33). This test was also performed and met acceptance criteria for 58:01 hours–minutes (Table 32). Additional earlier attempts failed due to stability QCs or qualifying QCs not meeting acceptance criteria (Tables 29 – 31).

### **3.12 LONG TERM STABILITY**

Long term stability is a measure of the stability of Colchicine in human plasma samples while stored at  $-20\text{ }^{\circ}\text{C} \pm 10\text{ }^{\circ}\text{C}$ . A standard curve is prepared and analyzed along with quality control standards that have been frozen for a period of time. Studies show that Colchicine is stable in human plasma at  $-20\text{ }^{\circ}\text{C} \pm 10\text{ }^{\circ}\text{C}$  for up to nineteen days (Table 36). Long term stability of Colchicine was also established for 1 day (Table 34) and 8 days (Table 35) before being extended to 19 days. This study is ongoing and will not be concluded for several months.

### 3.13 DILUTION INTEGRITY

During analysis of samples with unknown concentrations, there are occasions when a dilution of a sample must be made. The effect of diluting a sample with blank matrix was tested by preparing a dilution integrity sample (DI) in human plasma at a concentration five times greater than the highest calibration standard, in this case 200 ng/mL. In addition to the DI samples, the High QC sample (DHQC) was also diluted in the same manner for this test.

Six replicates each of the DI and the DHQC were diluted 1:9 (v:v sample-blank) with human plasma, processed, and analyzed along with a freshly prepared standard curve. Correcting for the dilution factors resulted in a %Bias of -8.2% for the DI samples and -8.2% for the DHQC samples (Table 37).

### 3.14 MATRIX EFFECT

Interference from the matrix was tested by preparing a Low QC and High QC in six different lots of human plasma and analyzing four sets of each QC along with a standard curve freshly prepared in a unique seventh lot. The CVs of the Lows ranged from 0.9% to 3.1% and the CVs of the Highs ranged from 0.6% to 2.3% (Table 38). Discernable matrix effects were not observed in the human plasma lots tested.

### 3.15 INTERFERING COMPOUNDS TEST

Interference from common over-the-counter medications is always a concern in bioanalytical work. The method validated for Colchicine was also tested with several over-the-counter drugs including acetylsalicylic acid, naproxen, ibuprofen, caffeine, pseudoephedrine, and acetaminophen. These drugs neither eluted with nor interfered with the analysis of Colchicine or \_\_\_\_\_ See Table 39 for the results of this test.

b(4)

## 4 2-DEMETHYL COLCHICINE VALIDATION

### 4.1 REFERENCE STANDARDS

2-Demethyl Colchicine, Batch RD/CCE/IMP/01, purity 96.0%, was obtained from \_\_\_\_\_ The certificate of analysis is attached in Appendix 1.

b(4)

### 4.2 STANDARD CURVE AND QUALITY CONTROLS

The accuracy and precision for this validation was accomplished by analyzing four standard curves prepared in human plasma and consisting of nine concentrations prepared in singlet in four separate batches. Quality Controls (QCs) were also prepared to assess accuracy, precision, and stability. The standard curve concentrations ranged from 0.2000 ng/mL to 40.00 ng/mL. The concentration of QCs were 0.2000 ng/mL for the

LLQC, 0.6000 ng/mL for the Low QC, 3.200 ng/mL for the Mid QC, 32.00 ng/mL for the High QC, and 40.00 ng/mL for the ULQC.

The instrument software was used to acquire raw data, and determine the chromatographic peak area of 2-Demethyl Colchicine and \_\_\_\_\_ internal standard (IS). The peak area data was exported to Watson LIMS to construct a regression equation based on the peak area ratios of 2-Demethyl Colchicine to IS. The results of the four validation runs, containing calibration standards and QCs are reported in Table 40 through Table 47, while a summary of the inter-batch validation data are reported in Table 48 for standards and Table 49 for QCs. Data for a fourth accuracy and precision run are included in the inter-batch data (Table 49) even though the QCs failed to meet acceptance criteria. For examples of chromatograms produced during method validation, see Appendix 2.

b(4)

#### 4.3 LINEARITY

Linearity is reported as the coefficient of determination ( $r^2$ ) and was obtained from least squares regression (weighted  $1/x^2$ ) of the standard curve fit to a linear model. All 2-Demethyl Colchicine standard curves showed  $r^2$  values of 0.9920 or greater. The mean value for the slope and intercept were 0.2800 and -0.0004545, respectively.

#### 4.4 INTER-BATCH AND INTRA-BATCH ACCURACY AND PRECISION

Accuracy is defined as the closeness of an observation or mean to the true value, and is expressed as %Bias. Precision is defined as the coefficient of variation (CV) of individual replicates from the calculated values.

The inter-batch accuracy and precision of standards are shown in Table 48, while Table 49 reports the inter-batch accuracy and precision of QCs. The inter-batch %Bias for standards was between -5.0% and 2.9%, while the CV was  $\leq$  5.8%. The inter-batch %Bias of QCs was between 1.9% and 10.1%, while the CV was  $\leq$  13.0%.

The intra-batch accuracy and precision was assessed by analyzing six replicates at each QC level: 0.2000 ng/mL for the LLQC, 0.6000 ng/mL for the Low QC, 3.200 ng/mL for the Mid QC, 32.00 ng/mL for the High QC, 40.00 ng/mL for the ULQC. The intra-batch %Bias ranged from -13.4% to 20.3%, while the CV was  $\leq$  8.4% (Table 49). This method was demonstrated to be able to accurately and precisely quantitate all standards and QC samples.

#### 4.5 SELECTIVITY

Selectivity is defined as the ability of the analytical method to measure a response from the analyte without interference from the biological matrix. This was accomplished by evaluating seven different lots of human plasma with and without IS. No significant baseline interference was detected at the retention times of 2-Demethyl Colchicine or IS. Seven out of seven lots tested passed acceptance criteria (Table 50).

#### 4.6 ABSOLUTE RECOVERY

Absolute recovery was defined as the area of extracted standard (referred to as “matrix”) versus the area of an extracted double blank (no analyte and no IS) reconstituted using reconstitution solution containing analyte at actual sample concentrations (referred to as “neat”). The area was calculated based on the average of six replicates at each level, for both the matrix and neat. The absolute recovery of 2-Demethyl Colchicine was 95.79% at 0.6000 ng/mL, 96.52% at 3.200 ng/mL, and 99.34% at 32.00 ng/mL with an average absolute recovery of 97.22% (Table 51).

#### 4.7 SOLUTION STABILITY

The 2-Demethyl Colchicine stock solution stability in Methanol was evaluated at room temperature and in the refrigerator at  $4\text{ }^{\circ}\text{C} \pm 6\text{ }^{\circ}\text{C}$ . It was determined to be stable for 340:43 hours–minutes at room temperature (Table 54) and at  $4\text{ }^{\circ}\text{C} \pm 6\text{ }^{\circ}\text{C}$  (Table 58). The test was performed and met acceptance criteria for the shorter durations of 99:10 hours–minutes (Tables 55 and 59) and 146:42 hours–minutes (Tables 52 and 56). The test did not meet acceptance criteria for 241:33 hours–minutes at room temperature or at  $4\text{ }^{\circ}\text{C} \pm 6\text{ }^{\circ}\text{C}$  due to the difference between the means being  $> 10\%$  (Tables 53 and 57).

#### 4.8 FREEZE/THAW STABILITY

Stability of 2-Demethyl Colchicine in human plasma at three different concentrations (Low, Mid, and High) was evaluated over 6 freeze/thaw cycles. Each cycle consisted of taking QC samples that were frozen at  $-20\text{ }^{\circ}\text{C} \pm 10\text{ }^{\circ}\text{C}$ , thawing them in a room temperature water bath for 2 hours and freezing them again. Low, Mid, and High QCs that underwent 1, 2, 3, 4 and 6 cycles were analyzed against a freshly prepared standard curve. Freeze/Thaw stability was assessed over three separate runs with the first one containing data for cycles 1, 2 and 3 (Table 60), the second one containing data for cycle 4 (Table 61) and the third containing data for cycle 6 (Table 62). The %Bias was between -14.1% and 2.9% for the Low QCs, -7.0% and 7.8% for the Mid QCs, and -9.0% and 3.4% for the High QCs. The results show that 2-Demethyl Colchicine is stable for six freeze/thaw cycles (Table 62). Two batch runs assessing stability for cycles 4 and 6 failed due to the Mid qualifying QCs not meeting acceptance criteria (Tables 63 and 64). Cycle 4 and 6 were repeated as two separate tests resulting in the acceptable data mentioned above.

#### 4.9 STABILITY OF 2-DEMETHYL COLCHICINE IN HUMAN PLASMA AT ROOM TEMPERATURE

The stability of 2-Demethyl Colchicine in human plasma was determined at room temperature by thawing Low, Mid, and High QC samples and storing them at room temperature for a minimum of 24 hours before extracting 6 sets of each along with a freshly prepared standard curve. The %Bias ranged from -4.7% to 10.8%. The results show that 2-Demethyl Colchicine in human plasma at room temperature is stable for 47:46 hours–minutes (Table 65).

#### **4.10 BENCH TOP STABILITY OF EXTRACTED SAMPLES**

The stability of 2-Demethyl Colchicine in extracted samples stored on the bench top at room temperature was determined. After six sets of Low, Mid, and High QCs were extracted and reconstituted they were stored at room temperature for a minimum of 24 hours before they were analyzed along with a freshly prepared standard curve. The %Bias ranged from -3.0% to 0.0%. The results show that extracted 2-Demethyl Colchicine samples that are stored at room temperature are stable for 95:59 hours–minutes (Table 69). This test was also performed and met acceptance criteria for 23:08 hours–minutes (Table 66). A test for 48:29 hours–minutes (Table 68) did not meet acceptance criteria due to the %Bias values being >15%. A test for 47:40 hours–minutes (Table 67) failed because the Mid qualifying QCs did not meet acceptance criteria.

#### **4.11 REFRIGERATOR STABILITY OF EXTRACTED SAMPLES**

The stability of 2-Demethyl Colchicine in extracted samples stored in a refrigerator at  $4\text{ }^{\circ}\text{C} \pm 6\text{ }^{\circ}\text{C}$  was determined. After six sets of Low, Mid, and High QCs were extracted and reconstituted they were stored in a refrigerator for a minimum of 24 hours before they were analyzed along with a freshly prepared standard curve. The %Bias ranged from -3.3% to 2.5%. The results show that extracted 2-Demethyl Colchicine samples that are stored at  $4\text{ }^{\circ}\text{C} \pm 6\text{ }^{\circ}\text{C}$  are stable for 108:02 hours–minutes (Table 74). Additional earlier attempts failed due to stability QCs or qualifying QCs not meeting acceptance criteria (Tables 70 – 73).

#### **4.12 LONG TERM STABILITY**

Long term stability is a measure of the stability of 2-Demethyl Colchicine in human plasma samples while stored at  $-20\text{ }^{\circ}\text{C} \pm 10\text{ }^{\circ}\text{C}$ . A standard curve is prepared and analyzed along with quality control standards that have been frozen for a period of time. Studies show that 2-Demethyl Colchicine is stable in human plasma at  $-20\text{ }^{\circ}\text{C} \pm 10\text{ }^{\circ}\text{C}$  for up to one day (Table 75). Long term stability of 2-Demethyl Colchicine was also tested for 8 days (Table 76) and 19 days (Table 77) but failed due to qualifying QCs not meeting acceptance criteria. This study is ongoing and will not be concluded for several months.

#### **4.13 DILUTION INTEGRITY**

During analysis of samples with unknown concentrations, there are occasions when a dilution of a sample must be made. The effect of diluting a sample with blank matrix was tested by preparing a dilution integrity sample (DI) in human plasma at a concentration five times greater than the highest calibration standard, in this case 200.0 ng/mL. In addition to the DI samples, the High QC sample (DHQC) was also diluted in the same manner for this test.

Six replicates each of the DI and the DHQC were diluted 1:9 (v:v sample–blank) with human plasma, processed, and analyzed along with a freshly prepared standard curve.

Correcting for the dilution factors resulted in a %Bias of 3.2% for the DI samples and 3.1% for the DHQC samples (Table 78). The initial attempt at the dilution integrity test failed due to the %Bias values not meeting acceptance criteria (Table 79).

#### 4.14 MATRIX EFFECT

Interference from the matrix was tested by preparing a Low QC and High QC in six different lots of human plasma and analyzing four sets of each QC along with a standard curve freshly prepared in a unique seventh lot. The CVs of the Lows ranged from 1.2% to 8.8% and the CVs of the Highs ranged from 1.7% to 7.0% (Table 80). Discernable matrix effects were not observed in the human plasma lots tested.

#### 4.15 INTERFERING COMPOUNDS TEST

Interference from common over-the-counter medications is always a concern in bioanalytical work. The method validated for 2-Demethyl Colchicine was also tested with several over-the-counter drugs including acetylsalicylic acid, naproxen, ibuprofen, caffeine, pseudoephedrine, and acetaminophen. These drugs neither eluted with nor interfered with the analysis of 2-Demethyl Colchicine or \_\_\_\_\_ See Table 81 for the results of this test.

b(4)

### 5 3-DEMETHYL COLCHICINE VALIDATION

#### 5.1 REFERENCE STANDARDS

3-Demethyl Colchicine, Lot Batch RD/CCE/IMP/04, purity 99.8%, was obtained from \_\_\_\_\_ The certificate of analysis is attached in Appendix 1.

b(4)

#### 5.2 STANDARD CURVE AND QUALITY CONTROLS

The accuracy and precision for this validation was accomplished by analyzing four standard curves prepared in human plasma and consisting of nine concentrations prepared in singlet in four separate batches. Quality Controls (QCs) were also prepared to assess accuracy, precision, and stability. The standard curve concentrations ranged from 0.2000 ng/mL to 40.00 ng/mL. The concentration of QCs were 0.2000 ng/mL for the LLQC, 0.6000 ng/mL for the Low QC, 3.200 ng/mL for the Mid QC, 32.00 ng/mL for the High QC, and 40.00 ng/mL for the ULQC.

The instrument software was used to acquire raw data, and determine the chromatographic peak area of 3-Demethyl Colchicine and \_\_\_\_\_ internal standard (IS). The peak area data was exported to Watson LIMS to construct a regression equation based on the peak area ratios of 3-Demethyl Colchicine to IS. The results of the four validation runs, containing calibration standards and QCs are reported in Table 82 through Table 90, while a summary of the inter-batch validation data are reported in Table 91 for standards and Table 92 for QCs without the Grubbs' outlier and Table 93 for

b(4)

QCs with the Grubbs' outlier. Data for the fourth accuracy and precision run are included in the inter-batch data even though the Mid QCs failed to meet acceptance criteria (Tables 92 and 93). For examples of chromatograms produced during method validation, see Appendix 2.

### 5.3 LINEARITY

Linearity is reported as the coefficient of determination ( $r^2$ ) and was obtained from least squares regression (weighted  $1/x^2$ ) of the standard curve fit to a linear model. All 3-Demethyl Colchicine standard curves showed  $r^2$  values of 0.9934 or greater. The mean value for the slope and intercept were 0.3772 and 0.009688, respectively.

### 5.4 INTER-BATCH AND INTRA-BATCH ACCURACY AND PRECISION

Accuracy is defined as the closeness of an observation or mean to the true value, and is expressed as %Bias. Precision is defined as the coefficient of variation (CV) of individual replicates from the calculated values.

The inter-batch accuracy and precision of standards are shown in Table 91, while Table 92 reports the inter-batch accuracy and precision of QCs. The inter-batch %Bias for standards was between -4.6% and 4.5%, while the CV was  $\leq 7.0\%$ . The inter-batch %Bias of QCs was between -6.5% and 6.0%, while the CV was  $\leq 10.4\%$ .

The intra-batch accuracy and precision was assessed by analyzing six replicates at each QC level: 0.2000 ng/mL for the LLQC, 0.6000 ng/mL for the Low QC, 3.200 ng/mL for the Mid QC, 32.00 ng/mL for the High QC, 40.00 ng/mL for the ULQC. The intra-batch %Bias for the QCs ranged from -14.5% to 15.3%, while the CV of the QCs was  $\leq 5.9\%$  (Table 92). This method was demonstrated to be able to accurately and precisely quantitate all standards and QC samples.

### 5.5 SELECTIVITY

Selectivity is defined as the ability of the analytical method to measure a response from the analyte without interference from the biological matrix. This was accomplished by evaluating seven different lots of human plasma with and without IS. No significant baseline interference was detected at the retention times of 3-Demethyl Colchicine or IS. Seven out of seven lots tested passed acceptance criteria (Table 94).

### 5.6 ABSOLUTE RECOVERY

Absolute recovery was defined as the area of extracted standard (referred to as "matrix") versus the area of an extracted double blank (no analyte and no IS) reconstituted using reconstitution solution containing analyte at actual sample concentrations (referred to as "neat"). The area was calculated based on the average of six replicates at each level, for both the matrix and neat. The absolute recovery of 3-Demethyl Colchicine was 98.56%

at 0.6000 ng/mL, 97.93% at 3.200 ng/mL, and 97.75% at 32.00 ng/mL with an average absolute recovery of 98.08% (Table 95).

### 5.7 SOLUTION STABILITY

The 3-Demethyl Colchicine stock solution stability in Methanol was evaluated at room temperature and in the refrigerator at  $4\text{ }^{\circ}\text{C} \pm 6\text{ }^{\circ}\text{C}$ . It was determined to be stable for 340:47 hours–minutes at room temperature (Table 98) and at  $4\text{ }^{\circ}\text{C} \pm 6\text{ }^{\circ}\text{C}$  (Table 102). The test was performed but did not meet acceptance criteria at  $4\text{ }^{\circ}\text{C} \pm 6\text{ }^{\circ}\text{C}$  or at room temperature for 99:26 hours–minutes (Tables 99 and 103), 150:34 hours–minutes (Table 96 and 100) and 241:21 hours–minutes (Tables 97 and 101) due to the difference between the means being  $> 10\%$ .

### 5.8 FREEZE/THAW STABILITY

Stability of 3-Demethyl Colchicine in human plasma at three different concentrations (Low, Mid, and High) was evaluated over 6 freeze/thaw cycles. Each cycle consisted of taking QC samples that were frozen at  $-20\text{ }^{\circ}\text{C} \pm 10\text{ }^{\circ}\text{C}$ , thawing them in a room temperature water bath for 2 hours and freezing them again. Low, Mid, and High QCs that underwent 1, 2, 3, 4 and 6 cycles were analyzed against a freshly prepared standard curve. Freeze/Thaw stability was assessed over three separate runs with the first one containing data for cycles 1, 2 and 3 (Table 104), the second one containing data for cycle 4 with the Grubbs' Outlier value (Table 106) and without the Grubbs' Outlier value (Table 105), and the third containing data for cycle 6 (Table 107). The %Bias was between -7.7% and -3.3% for the Low QCs, -4.9% and -0.4% for the Mid QCs, and -14.6% and 0.4% for the High QCs. The results show that 3-Demethyl Colchicine is stable for six freeze/thaw cycles (Tables 107). A batch run assessing stability for cycles 4 and 6 failed due to the Low and Mid freeze/thaw QCs, Mid qualifying QCs and Standard 1 not meeting acceptance criteria (Table 108). Cycle 4 and 6 were repeated as two separate tests resulting in the acceptable data mentioned above.

### 5.9 STABILITY OF 3-DEMETHYL COLCHICINE IN HUMAN PLASMA AT ROOM TEMPERATURE

The stability of 3-Demethyl Colchicine in human plasma was determined at room temperature by thawing Low, Mid, and High QC samples and storing them at room temperature for a minimum of 24 hours before extracting 6 sets of each along with a freshly prepared standard curve. The %Bias ranged from -13.0% to -3.3%. The results show that 3-Demethyl Colchicine in human plasma at room temperature is stable for 40:39 hours–minutes (Table 109). Short term stability that was initially assessed for 47:46 hour–minutes failed due to the Mid qualifying QCs not meeting acceptance criteria (Table 110).

### **5.10 BENCH TOP STABILITY OF EXTRACTED SAMPLES**

The stability of 3-Demethyl Colchicine in extracted samples stored on the bench top at room temperature was determined. After six sets of Low, Mid, and High QCs were extracted and reconstituted they were stored at room temperature for a minimum of 24 hours before they were analyzed along with a freshly prepared standard curve. The %Bias ranged from -13.4% to -1.2%. The results show that extracted 3-Demethyl Colchicine samples that are stored at room temperature are stable for 95:59 hours–minutes (Table 114). This test was also performed and met acceptance criteria for 23:08 hours–minutes (Table 111). A test for 48:29 hours–minutes (Table 113) failed due to the zeros (extracted sample without drug) not meeting acceptance criteria. A test for 47:40 hours–minutes (Table 112) failed due to the low bench top stability QCs, Mid qualifying QCs and Standard 1 not meeting acceptance criteria.

### **5.11 REFRIGERATOR STABILITY OF EXTRACTED SAMPLES**

The stability of 3-Demethyl Colchicine in extracted samples stored in a refrigerator at  $4\text{ }^{\circ}\text{C} \pm 6\text{ }^{\circ}\text{C}$  was determined. After six sets of Low, Mid, and High QCs were extracted and reconstituted they were stored in a refrigerator for a minimum of 24 hours before they were analyzed along with a freshly prepared standard curve. The %Bias ranged from -6.1% to 4.8%. The results show that extracted 3-Demethyl Colchicine samples that are stored at  $4\text{ }^{\circ}\text{C} \pm 6\text{ }^{\circ}\text{C}$  are stable for 31:11 hours–minutes (Table 117). Additional attempts failed due to stability QCs, qualifying QCs or zeros (extracted sample without drug) not meeting acceptance criteria (Tables 115 – 116 and Tables 118 – 119).

### **5.12 LONG TERM STABILITY**

Long term stability is a measure of the stability of 3-Demethyl Colchicine in human plasma samples while stored at  $-20\text{ }^{\circ}\text{C} \pm 10\text{ }^{\circ}\text{C}$ . A standard curve is prepared and analyzed along with quality control standards that have been frozen for a period of time. Studies show that 3-Demethyl Colchicine is stable in human plasma at  $-20\text{ }^{\circ}\text{C} \pm 10\text{ }^{\circ}\text{C}$  for up to eight days (Table 121). Long term stability of 3-Demethyl Colchicine was also established for 1 day (Table 120). Long term was tested for 19 days but failed due to qualifying QCs not meeting acceptance criteria (Table 122). This study is ongoing and will not be concluded for several months.

### **5.13 DILUTION INTEGRITY**

During analysis of samples with unknown concentrations, there are occasions when a dilution of a sample must be made. The effect of diluting a sample with blank matrix was tested by preparing a dilution integrity sample (DI) in human plasma at a concentration five times greater than the highest calibration standard, in this case 200.0 ng/mL. In addition to the DI samples, the High QC sample (DHQC) was also diluted in the same manner for this test.

Six replicates each of the DI and the DHQC were diluted 1:9 (v:v sample-blank) with human plasma, processed, and analyzed along with a freshly prepared standard curve. Correcting for the dilution factors resulted in a %Bias of 3.3% for the DI samples and -1.2% for the DHQC samples (Table 123). The initial attempt at the dilution integrity failed due to the %Bias value for the Diluted High QCs not meeting acceptance criteria (Table 124).

#### 5.14 MATRIX EFFECT

Interference from the matrix was tested by preparing a Low QC and High QC in six different lots of human plasma and analyzing four sets of each QC along with a standard curve freshly prepared in a unique seventh lot. The CVs of the Lows ranged from 0.1% to 6.0% and the CVs of the Highs ranged from 0.8% to 3.3% (Table 125). Discernable matrix effects were not observed in the human plasma lots tested.

#### 5.15 INTERFERING COMPOUNDS TEST

Interference from common over-the-counter medications is always a concern in bioanalytical work. The method validated for 3-Demethyl Colchicine was also tested with several over-the-counter drugs including acetylsalicylic acid, naproxen, ibuprofen, caffeine, pseudoephedrine, and acetaminophen. These drugs neither eluted with nor interfered with the analysis of 3-Demethyl Colchicine or \_\_\_\_\_ See Table 126 for the results of this test.

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### 6 SOP DEVIATIONS DURING VALIDATION

None

### 7 COMPUTER SOFTWARE VALIDATION

\_\_\_\_\_ was used to acquire the data. The \_\_\_\_\_ algorithm was used to determine the peak area of the analytes and internal standard. The area data was exported to Watson LIMS for regression analysis. \_\_\_\_\_ software used in this validation has undergone further qualification in our laboratory after installation and prior to use in this validation.

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During the performance of this validation, all results were generated using a LIMS system (Thermo Watson LIMS version 7.1.0.01). The LIMS system has been successfully validated with a full IQ/OQ/PQ performed in-house using the vendor supplied \_\_\_\_\_ and a custom \_\_\_\_\_ developed by PRACS Institute, Ltd. to validate the system according to regulatory guidance as it is used in-house. Wherever possible Watson LIMS was used to generate the spreadsheets contained in this report. Watson LIMS could not be utilized for some of the tests for validation. Spreadsheets for those tests were created using Excel™.

b(4)

#### 4.2.2 Synopsis of Study MPC-004-07-1001

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| <b>SPONSOR:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | Mutual Pharmaceutical Company, Inc.<br>1100 Orthodox Street<br>Philadelphia, PA 19124-3131            |
| <b>NAME OF TEST PRODUCT:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | Colchicine Tablets USP, 0.6 mg                                                                        |
| <b>ACTIVE INGREDIENT:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Colchicine                                                                                            |
| <b>STUDY TITLE:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Randomized, 3-Way Crossover Bioequivalence Study of Single-Dose Colchicine Tablets and Effect of Food |
| <b>PRINCIPAL INVESTIGATOR AND STUDY SITE:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Anthony R. Godfrey, Pharm.D.<br>4801 Amber Valley Parkway<br>Fargo, ND 58104, USA                     |
| <b>STUDY DURATION:</b> 15 September to 17 October 2007                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                                                       |
| <b>STUDY TYPE:</b> Phase 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                                                                                       |
| <p><b>OBJECTIVES:</b> The objectives of this study were:</p> <ul style="list-style-type: none"> <li>• To determine the bioequivalence (dose-normalized) of colchicine tablets USP, 0.6 mg, when administered under standard fasting conditions and Col-Probenecid® (assays for probenecid were not performed).</li> <li>• To determine the effect of food on colchicine tablets USP, 0.6 mg.</li> <li>• To evaluate the safety and tolerability of colchicine tablets USP, 0.6 mg.</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                       |
| <p><b>METHODOLOGY:</b> This was a randomized, single-dose, 3-way crossover study in design. Subjects were dosed as a single cohort and remained housed for 24 hours post-dose. They received single doses of either colchicine 0.6 mg in a fed or fasting condition, or Col-Probenecid® (0.5 mg colchicine / 500 mg probenecid) in a fasting condition, in a randomly assigned sequence. There was a 14-day washout between dosing periods.</p> <p>On Days 1 – 5 (Period I), 15 – 19 (Period II), and 29 – 33 (Period III), blood samples were collected for analysis of colchicine, 2-demethylcolchicine (2DMC), and 3-demethylcolchicine (3DMC) plasma concentrations prior to dosing and for 96 hours post-dose (with blood samples after 24 hours collected on an outpatient basis). Plasma samples were sent to the bioanalytical laboratory of PRACS Institute, Ltd. - Cetero Research, for determination of colchicine, 2DMC and 3DMC plasma concentrations.</p> |                                                                                                       |

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                                                               |
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| Subjects underwent a medical review prior to final discharge from the study.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                               |
| <b>NUMBER OF SUBJECTS:</b> A total of 28 healthy adult subjects participated in this study. The sample size was based on inter-subject %CVs for colchicine pharmacokinetic (PK) parameters reported in the published literature (25% – 50%). Based on this information, 28 subjects were projected to provide a sufficient power to assess relative bioequivalence.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                                                                                                                               |
| <b>MAIN DIAGNOSIS FOR ENTRY:</b> All subjects were asymptomatic, healthy, non-smoking adult subjects between the ages of 18 and 45 years who met the inclusion/exclusion criteria for this study.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                                                               |
| <b>TEST PRODUCT:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Colchicine Tablets USP, 0.6 mg<br>Mutual Pharmaceutical Co., Inc.<br>Lot No.: BB 374 0215; Expiration: N/A                                                    |
| <b>REFERENCE PRODUCT:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Probenecid and Colchicine Tablets USP<br>(Col-Probenecid <sup>®</sup> ), 500 mg–0.5 mg<br>Watson Laboratories, Inc.<br>Lot No.: L6M1440; Expiration: DEC 2008 |
| <b>ROUTE OF ADMINISTRATION:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Oral                                                                                                                                                          |
| <b>DURATION OF TREATMENT:</b> The subjects received 1 tablet of either the test or reference product in each of the treatment conditions over a 5-week period with a 14-day washout period between dosing time points.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                                                                                                                               |
| <b>PRIMARY EFFICACY VARIABLE:</b> Not applicable.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                                                               |
| <b>SECONDARY EFFICACY VARIABLE:</b> Not applicable.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                                                                                                                               |
| <b>CRITERIA FOR EVALUATION:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                                                               |
| <p><b>Pharmacokinetics:</b> Blood for pharmacokinetic sampling was obtained from all subjects within 1 hour prior to dosing (0 hour) and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, and 96 hours post-dose. Analytical data from the blood samples collected during the study conduct were used to calculate values for the following pharmacokinetic parameters: <math>AUC_{0-t}</math>, <math>AUC_{0-\infty}</math>, <math>C_{max}</math>, <math>T_{max}</math>, <math>k_e</math>, <math>V_d</math>, <math>CL/F</math>, and <math>t_{1/2}</math>. Samples were also separately calculated for men and women.</p> <p>Analyses of variance (ANOVA) were performed on the ln-transformed <math>AUC_{0-t}</math>, <math>AUC_{0-\infty}</math>, and <math>C_{max}</math> as appropriate. The ANOVA model included factors accounting for the following sources of variation: sequence, subjects within sequence, period, and treatment. The statistical analyses were performed using the SAS<sup>®</sup> procedure.</p> <p><b>Safety:</b> All subjects were monitored throughout the confinement portion of the study. Sitting</p> |                                                                                                                                                               |

(5 minutes) blood pressure and pulse were measured prior to dosing and at 1, 2, 4, and 24 hours after each dose; 12-lead ECGs were obtained in triplicate prior to dosing and at 1, 2, and 4 hours after dosing. Subjects were also queried for adverse events at screening, check-in, during the confinement portion of the study, and at study exit (or early termination). All subjects underwent clinical laboratory testing including hematology, biochemistry, and for post-menopausal women at screening only, follicle stimulating hormone; and, for women of childbearing potential, pregnancy tests. Clinically laboratory testing (hematology and serum chemistry) was repeated prior to check-in for each period and 24 hours after the last dose of study drug. Additionally, vital signs were taken and physical examinations were performed at screening and at study exit (or early termination).

#### **SUMMARY OF RESULTS:**

**Demographic Summary:** The mean age of the subjects was approximately 24 years (Mean = 23.7 yrs  $\pm$  5.7 yrs). Ages ranged from 18 to 39 years. However, sex was not evenly distributed (18 females; 10 males) and a majority of the subjects were white (approximately 85.7%).

**Safety Summary:** No serious adverse events (SAEs) were reported over the course of this study. No subject was discontinued due to an AE. Nine (9) subjects experienced a total of eighteen (18) adverse events (AEs) over the course of the study. Seven (7) of the 18 AEs were reported by 5 subjects following the administration of colchicine under fasting conditions. Five (5) of the 18 AEs were reported by 3 subjects following the administration of colchicine under fed conditions. Six (6) of the 18 AEs were reported by 4 subjects following the administration of Col-Probenecid<sup>®</sup> under fasting conditions. There was no apparent difference between the dosing conditions with respect to adverse events. Overall, the most common AEs were dizziness and headache, occurring on at least 1 occasion in 4 subjects (14.1%) and 3 subjects (10.7%), respectively. Generally, these were considered by the Investigator to be related to treatment. Gastrointestinal AEs occurred in 2 subjects (7.1%) and manifested as stomach discomfort, nausea, and vomiting in 1 subject (treatment-related), and nausea in another subject (not judged treatment-related by the medical investigator). Events were mild or moderate in intensity.

There were no clinically significant findings from an assessment of the clinical laboratory test results, vital signs data, or physical examination results. No consistent or clinically relevant abnormalities or changes versus baseline in ECG intervals or morphology were observed in any subject. QTcF values remained within normal limits in all subjects and no relevant changes in QTcF versus baseline were observed.

**Pharmacokinetic Summary:** The bioanalytical laboratory of PRACS Institute, Ltd. - Cetero Research, determined the colchicine, 2DMC, and 3DMC plasma concentrations and sent the data to the Statistical Division of PRACS Institute, Ltd. - Cetero Research. There were no statistical analyses done for 2DMC and 3DMC, since all of the plasma concentration values for these metabolites were BLQ, with the exception of 3 measurements of 3DMC, single time points in 1 subject each.

For all subjects, the reported mean  $C_{max}$  following a single 0.6 mg dose of Mutual's colchicine tablets USP, administered in the fasting state was 2.503 ng/mL  $\pm$  0.722, occurring at the median of 1.5 hours post-dose (range 0.5 to 2 hours). In comparison, following a single dose of Col-Probenecid<sup>®</sup> containing 0.5 mg colchicine, the mean  $C_{max}$  was 1.706 ng/mL  $\pm$  0.560, occurring at the median of 1.0 hours post-dose (range 1 to 2 hours). Mean colchicine volume of distribution values when administered as the test colchicine tablet (Mutual) was 379 L as compared to 348 L for the reference colchicine tablet (Col-Probenecid<sup>®</sup>). Plasma levels declined with similar mean half-lives: 6.0 hours  $\pm$  4.69 (estimated in only 24 subjects) *versus* 3.8 hours  $\pm$  1.267 hours for the Mutual drug and the reference listed drug (RLD), respectively; clearance was 48.8 L/hr and 65.2 L/hr, respectively.

Summary mean (%CV) pharmacokinetic parameters (non-transformed) are presented across all subjects administered Mutual's colchicine tablets USP, 0.6 mg in the fasted and fed states for all subjects below.

| Parameter (units)                 | Arithmetic Mean (%CV)<br>Median (Range) for $T_{max}$   |                                                                                         |                                                     |
|-----------------------------------|---------------------------------------------------------|-----------------------------------------------------------------------------------------|-----------------------------------------------------|
|                                   | Colchicine Tablets<br>USP (0.6 mg)<br>Fasting<br>(N=25) | Colchicine Tablets<br>USP (0.6 mg)<br>Dose Normalized to<br>0.5 mg<br>Fasting<br>(N=25) | Colchicine Tablets USP<br>(0.6 mg)<br>Fed<br>(N=25) |
| AUC <sub>0-1</sub> (ng·hr/mL)     | 12.59 (48.62)                                           | 10.49 (48.62)                                                                           | 10.49 (38.37)                                       |
| AUC <sub>0-∞</sub> (ng·hr/mL)     | 14.11 (39.65)                                           | 11.76 (39.65)                                                                           | 11.40 (25.39)                                       |
| $C_{max}$ (ng/mL)                 | 2.50 (28.85)                                            | 2.09 (28.85)                                                                            | 2.50 (27.84)                                        |
| $T_{max}$ (hr)                    | 1.50 (0.50-2.00)                                        | 1.50 (0.50-2.00)                                                                        | 1.50 (0.50-4.00)                                    |
| $K_e$ (1/hr)                      | 0.150 (43.35)                                           | 0.150 (43.35)                                                                           | 0.189 (37.6)                                        |
| $t_{1/2}$ (hr)                    | 6.36 (73.85)                                            | 6.36 (73.85)                                                                            | 4.46 (62.95)                                        |
| CL/F (L/hr)                       | 48.82 (36.77)                                           | 48.82 (36.77)                                                                           | 55.67 (24.13)                                       |
| Weight-Adjusted CL/F<br>(L/hr/kg) | 0.69 (41.95)                                            | 0.69 (41.95)                                                                            | 0.77 (21.49)                                        |
| $V_d$ (L)                         | 379.15 (44.49)                                          | 379.15 (44.49)                                                                          | 325.31 (34.12)                                      |

Following a single-dose administration of Mutual's colchicine tablet USP, 0.6 mg, with a high-fat breakfast, a mean  $C_{max}$  of 2.5 ng/mL  $\pm$  0.695 was observed at the median of 1.5 hours post-dose (range 0.5 to 4 hours), the same as seen when dosing was in the fasted state. The extent of absorption is decreased by approximately 15%, marginally outside the 90% CI for "bioequivalence".

Similar results were seen when data were analyzed for men and women separately, both in terms of the overall pharmacokinetic profile and the effect of food.

For the assessment of relative bioavailability in all subjects,  $C_{max}$ , AUC<sub>0-1</sub>, and AUC<sub>0-∞</sub> values were dose-normalized. Administration of Mutual's colchicine tablets USP, 0.6 mg, and

Col-Probenecid<sup>®</sup>, the RLD for the combination product, under fasting conditions resulted in dose-adjusted C<sub>max</sub> and AUC for which the 90% confidence intervals (CI) were not within the bioequivalence interval of 80 to 125% (i.e., the range did not encompass 100%). These results, taken together with the observations regarding the difference in T<sub>max</sub>, indicate that the Mutual drug product, after dose normalization, results in an increased rate and extent of absorption of colchicine as compared to Col-Probenecid<sup>®</sup>.

| Geometric Means, Ratio of Means, and 90% Confidence Intervals<br>Ln-Transformed Data<br>Colchicine<br>All Subjects<br>N=25 |                                     |                             |         |                  |
|----------------------------------------------------------------------------------------------------------------------------|-------------------------------------|-----------------------------|---------|------------------|
| Parameter                                                                                                                  | Colchicine Tablets,<br>USP (0.6 mg) | Col-Probenecid <sup>®</sup> | % Ratio | 90% CI           |
| AUC <sub>0-t</sub><br>(ng·hr/mL)                                                                                           | 9.43                                | 6.86                        | 137.43  | (122.5, 154.18)  |
| AUC <sub>0-∞</sub><br>(ng·hr/mL)                                                                                           | 10.78                               | 7.86                        | 137.13  | (124.46, 151.09) |
| C <sub>max</sub><br>(ng/mL)                                                                                                | 1.99                                | 1.59                        | 125.10  | (111.97, 139.76) |

#### SUMMARY CONCLUSIONS:

**Pharmacokinetic:** These results, taken together with the observations regarding the difference in T<sub>max</sub>, indicate that the Mutual drug product, after dose normalization, results in an increased rate and extent of absorption of colchicine as compared to Col-Probenecid<sup>®</sup> and bioequivalence was not demonstrated. The results also indicate the extent of absorption is decreased by approximately 15%, marginally outside the 90% CI for "bioequivalence" and can conclude a food-effect is present; however, the clinical significance of the food-effect is unknown. There is no change in the rate of absorption when administered under fed conditions.

**Safety:** Nine (9) of 28 subjects experienced a total of 18 AEs over the course of the study. Seven (7) of the 18 AEs were reported by 5 (19.2%) subjects following the administration of colchicine under fasting conditions. Five (5) of the 18 AEs were reported by 3 subjects (11.1%) following the administration of colchicine under fed conditions. Six (6) of the 18 AEs were reported by 4 subjects (14.8%) following the administration of Col-Probenecid<sup>®</sup> under fasting conditions.

Overall, colchicine was well tolerated as a single, oral dose (1 × 0.6 mg tablet or 1 × 0.5 mg / 500 mg tablet) administered under fasting and fed conditions. The most common adverse events overall were dizziness (17.9%), headache (10.7%), and nausea (7.1%). All other adverse events occurred in single subjects. All events were mild or moderate in intensity and none resulted in discontinuation.

#### 4.2.3 Synopsis of Study MPC-004-07-1002

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                             |
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| <b>SPONSOR:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | Mutual Pharmaceutical Company, Inc.<br>1100 Orthodox Street<br>Philadelphia, PA 19124-3131                                                                  |
| <b>NAME OF TEST PRODUCTS:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Colchicine Tablets USP, 0.6 mg (over-encapsulated) or Placebo to match the over-encapsulated colchicine tablet                                              |
| <b>ACTIVE INGREDIENT:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Colchicine                                                                                                                                                  |
| <b>STUDY TITLE:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | A Randomized, Double-Blind, Double-Dummy Pharmacokinetic and Exploratory ECG Safety Study of a Standard Acute Gout Regimen (Total Dose 4.8 mg over 6 Hours) |
| <b>PRINCIPAL INVESTIGATOR AND STUDY SITE:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Anthony R. Godfrey, Pharm.D.<br>4801 Amber Valley Parkway<br>Fargo, ND 58104, USA                                                                           |
| <b>STUDY DURATION:</b> 28 November 2007 – 02 December 2007                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                             |
| <b>STUDY TYPE:</b> Phase 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                             |
| <b>OBJECTIVES:</b> The objectives of this study were to determine the following: <ul style="list-style-type: none"><li>• To determine the pharmacokinetic profile of colchicine and its metabolites, 2-, 3- and 10-demethylcolchicine (2DMC, 3DMC, and 10DMC, respectively) following a standard acute gout regimen (total dose 4.8 mg over 6 hours) of the to-be-marketed product, colchicine tablets USP, 0.6 mg.</li><li>• To determine whether or not there is a trend toward the effect of this regimen on ECG parameters, primarily the corrected QT interval and other ECG parameters.</li><li>• To study the safety and tolerability of a standard regimen used in the treatment of an acute gout flare.</li></ul> |                                                                                                                                                             |

**METHODOLOGY:** This was a single-center, randomized, double-blind, double-dummy pharmacokinetic and ECG safety study. Subjects were randomized to receive either colchicine (test product) or moxifloxacin (positive control for QTc prolongation). Subjects received double-blind, double-dummy treatments as described below on Day -1 and Day 1. Subjects were confined to the study unit beginning on the afternoon of Day -2 until 48 hours post-dose (Day 3).

Serial pharmacokinetic blood samples were collected on Days 1 - 5. Collection began prior to the first dose of colchicine and continued for 96 hours thereafter (with blood samples after 48 hours collected on an outpatient basis). Plasma samples were sent to the bioanalytical laboratory of PRACS Institute, Ltd. - Cetero Research, for determination of colchicine, 2DMC, 3DMC, and 10DMC plasma concentrations.

Subjects underwent continuous 24-hour ECG monitoring by 12-lead Holter. In order to establish a baseline for ECG analysis, monitoring was performed under identical conditions at Baseline (Day -1) and following dosing with active drug (Day 1).

**NUMBER OF SUBJECTS:** A total of 18 healthy adult subjects participated in this study. Fifteen (15) subjects were randomly assigned to receive colchicine, a sample size considered adequate to characterize the pharmacokinetic profile when given in this treatment regimen; it was also judged to be a suitable sample size with which to determine a trend indicating whether or not the drug has a propensity to prolong ventricular conduction. Three (3) subjects were randomly assigned to receive moxifloxacin, judged to be the minimum number suitable to verify a trend indicating adequate assay sensitivity.

**MAIN DIAGNOSIS FOR ENTRY:** All subjects were asymptomatic, healthy, non-smoking adult subjects between the ages of 18 and 55 years who were not receiving concomitant medications, vitamins, supplements or foods that could interfere with the pharmacokinetics. Subjects were to be documented to be generally healthy on the basis of medical history, physical examination, clinical laboratory testing, and ECG.

**TREATMENTS:**

| Product                                                                                                                                   | Dose and Mode of Administration                                 | Lot No. |
|-------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------|---------|
| <b>Test Product</b>                                                                                                                       |                                                                 |         |
| Colchicine: DB "B" Gray Opaque Capsules containing Colchicine Tablets USP, 0.6 mg (Lot # BB 374 0215) and cellulose microcrystalline, USP | 4.8 mg on Day 1 (1.2 mg, followed by 0.6 mg hourly for 6 hours) | 38061B0 |

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                                                                                                                                              |             |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|
| <b>Moxifloxacin Placebo:</b> Size 00 Blue Capsules containing cellulose microcrystalline. NF                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | Two capsules followed by one capsule hourly for 6 hours on Day -1 and Day 1                                                                                                  | P-070535F10 |
| <b>Reference Product (Positive Control for QT evaluation)</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                                                                                              |             |
| <b>Moxifloxacin:</b> Size 00 Blue Capsules containing AVELOX® (moxifloxacin hydrochloride) 400 mg Tablets (Batch: 54010K4) and cellulose microcrystalline. NF)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | 400 mg on Day 1 (to correspond to hour 6. last dose of colchicine)                                                                                                           | 54010K4     |
| <b>Colchicine Placebo:</b> DB "B" Gray Opaque Capsules containing cellulose microcrystalline. USP                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Two capsules followed by one capsule hourly for 6 hours on Day -1<br><br>Two capsules followed by one capsule hourly for 5 hours on Day 1                                    | 38061A0     |
| <b>DURATION OF TREATMENT</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | One (1) day (over a 6-hour period for subjects randomized to colchicine and as a single dose for subjects randomized to moxifloxacin). Total study participation is 5½ days. |             |
| <b>ROUTE OF ADMINISTRATION:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | Oral                                                                                                                                                                         |             |
| <b>PRIMARY EFFICACY VARIABLE:</b> Not applicable.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                                                                                                                                              |             |
| <b>SECONDARY EFFICACY VARIABLE:</b> Not applicable.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                              |             |
| <b>CRITERIA FOR EVALUATION:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                                                                                                                                              |             |
| <p><b>Pharmacokinetics:</b> Blood for pharmacokinetic sampling was obtained from all subjects on Day 1 at the following time points (relative to first dose of study drug): pre-dose and 1 (prior to second dose), 3 (prior to fourth dose), 6 (prior to seventh and final dose), 6.17, 6.33, 6.5, 6.75, 7, 7.25, 7.5, 7.75, 8, 10, 12, 23, 36, 48, 72, and 96 hours post-dose. Samples were to be analyzed for colchicine, 2DMC, 3DMC, and 10DMC. Analytical data were used to calculate the following pharmacokinetic parameters: C<sub>max</sub> (highest observed), T<sub>max</sub> (corresponding to time of C<sub>max</sub>, relative to initiation of dosing), AUC<sub>0-1</sub>, AUC<sub>0-∞</sub>, K<sub>el</sub>, V<sub>area</sub>/F, CL/F, and t<sub>½</sub>.</p> <p><b>Electrocardiogram:</b> A 12-lead ECG was obtained at screening and subjects underwent continuous Holter monitoring for 24-hour periods on Day -1 and Day 1, with triplicate ECG recordings obtained from a 5-minute observation period 30 minutes prior to the first dose and at post dose (relative to first dose) study hours 1 (prior to the second dose), 3 (prior to the fourth dose), 6 (prior to the seventh and final dose), 7, 8, 10, 12, and 23.</p> <p><b>Safety:</b> All subjects were monitored throughout the confinement portion of the study. Vital signs were obtained at screening (seated blood pressure, heart rate, respirations, temperature) and on Day -</p> |                                                                                                                                                                              |             |

2 (seated blood pressure and heart rate). On Day -1 and Day 1, blood pressure and pulse were measured with subjects first in a seated position (for at least 5 minutes) and were to be repeated 1 minute after standing at the following time points: prior to the first dose and at post dose (relative to the first dose) study hours 0.5, 1 (prior to second dose), 2 (prior to third dose), 4 (prior to fifth dose), 12 and 24.

Subjects were queried for adverse events at screening, check-in, during the confinement portion of the study, and at study exit (or early termination).

All subjects underwent clinical laboratory testing including hematology, biochemistry, urinalysis and, for women of childbearing potential, pregnancy tests at screening, check-in, and Day 2.

Additionally, physical examinations were performed at screening.

#### **STATISTICAL METHODS:**

##### **Pharmacokinetic Analysis:**

For subjects randomized to colchicine, arithmetic means, standard deviations (SD), coefficients of variation (CV%), median, minimum and maximum values, and number of observations were calculated for each pharmacokinetic parameter. Geometric mean and geometric coefficient of variation were provided for  $AUC_{0-t}$ ,  $AUC_{\infty}$ , and  $C_{max}$ . Results for men and women were also summarized separately.

Plasma levels of 2DMC were all below the limit of quantitation (BLQ 0.2 ng/mL). There were too few quantifiable 3DMC concentrations to allow for meaningful pharmacokinetic parameter summary other than  $C_{max}$ . The analytical method could not be validated for 10DMC.

##### **ECG Analysis:**

Descriptive statistics including mean, SD, median, maximum, minimum and numbers of observations at each time point as well as the change from time-matched baseline were calculated. The parameters analyzed included heart rate (HR) and PR, QRS, QTcF, and QTcB intervals.

##### **Safety Analysis:**

Safety data including treatment-emergent adverse events (coded using the current version of MedDRA), vital sign measurements, laboratory evaluations, and physical examinations were summarized. Descriptive statistics (arithmetic mean, SD, median, minimum, and maximum) were calculated for quantitative safety data as well as for the difference from baseline.

#### **SUMMARY OF RESULTS:**

**Demographic Summary:** The mean age of the subjects was approximately 29 years (Mean = 28.7 years  $\pm$  10.0). Ages ranged from 18 to 50 years. Ten (10) males and 8 females participated. A majority of the subjects were white (approximately 88.89%).

**Pharmacokinetic Summary:** The reported mean colchicine  $C_{max}$  when administered in a standard regimen used to treatment an acute gout flare was 6.84 ng/mL  $\pm$  1.30, occurring on average 4.47 hours following the initiation of dosing (range 3.12 to 7.50 hours). The mean volume of

distribution value was  $1876.09 \text{ L} \pm 456.19$ . The mean half-life was  $31.38 \text{ hours} \pm 8.36$ . The mean clearance was  $43.2 \text{ L/hr} \pm 12.86$  and the mean weight-adjusted clearance was  $0.62 \text{ L/hr/kg} \pm 0.215$ .

Summary parameters for colchicine are presented across all subjects administered the colchicine treatment below.

| Parameter (unit)                         | Arithmetic Mean (%CV)<br>Median (Range) for $T_{max}$<br>(N = 15) |
|------------------------------------------|-------------------------------------------------------------------|
| $C_{max}$ (ng/mL)                        | 6.84 (18.94%)                                                     |
| $AUC_{0-t}$ (ng-hr/mL)                   | 104.95 (23.45%)                                                   |
| $AUC_{0-\infty}$ (ng-hr/mL)              | 118.20 (22.01%)                                                   |
| $T_{max}$ (hr)                           | 4.47 (44.65%)                                                     |
| $K_{el}$ (1/hr)                          | 0.02 (36.59%)                                                     |
| $t_{1/2}$ (hr)                           | 31.38 (26.65%)                                                    |
| CL/F (L/hr)                              | 43.17 (29.79%)                                                    |
| Weight Adjusted Clearance CL/F (L/hr/kg) | 0.62 (34.67%)                                                     |
| $V_{dres}/F$ (L)                         | 1876.09 (24.32%)                                                  |

Source: Table 14.2.1

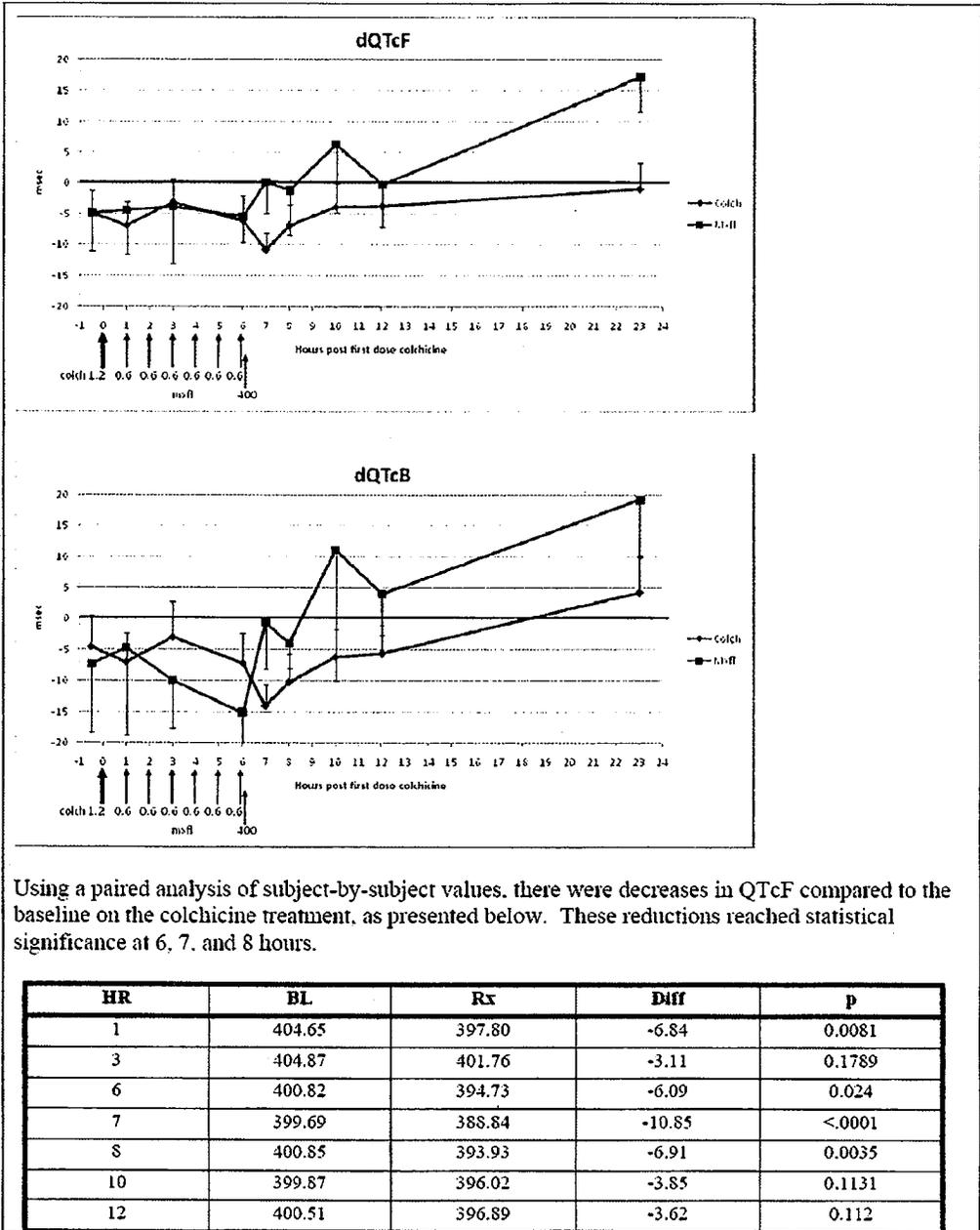
There was no apparent difference in the rate and extent of absorption and the clearance between men and women

**ECG Analysis:** For the colchicine treatment, the upper bounds of the CIs did not reach the 10 msec level for QTcF. In comparison, at hour 23 only, the bounds for QTcB just barely exceeded the referenced level, with a mean of 4.29 msec and an upper bound of \_\_\_\_\_

b(4)

Moxifloxacin response was lower than expected and the time course was not consistent with the usual findings. At all time points, after the moxifloxacin dosage, both dQTcB and dQTcF values for the colchicine treatment group were lower than the dQTcB and dQTcF values for the moxifloxacin group.

The figures below represent the change in baseline for QTcF and QTcB, respectively.



|    |        |        |       |        |
|----|--------|--------|-------|--------|
| 23 | 400.20 | 399.36 | -0.85 | 0.7345 |
|----|--------|--------|-------|--------|

*THE FIFTEEN (15) SUBJECTS TREATED WITH COLCHICINE DID NOT EXHIBIT EVIDENCE OF PROLONGED REPOLARIZATION. IMMEDIATELY AFTER THE END OF DOSING, THERE WAS A MODERATE, BUT STATISTICALLY SIGNIFICANT, DECREASE OF CHANGE IN QTCF FROM THE TIME-MATCHED BASELINE. THE DECREASES OCCUR AT TIMES 6, 7 AND 8 HOURS AND ARE IN THE RANGE OF -6 TO -12 MSEC. THERE WAS A TREND FOR DQTCF TO DECREASE WITH INCREASING COLCHICINE CONCENTRATION, REACHING STATISTICAL SIGNIFICANCE OF ABOUT -1 MSEC PER NG/ML. AFTER THE MOXIFLOXACIN DOSING BOTH DQTCB AND DQTCF VALUES FOR THE COLCHICINE TREATMENT GROUP WERE LOWER THAN THOSE FOR THE MOXIFLOXACIN GROUP. TREATMENT OF THE THREE (3) SUBJECTS WITH MOXIFLOXACIN FAILED TO DEMONSTRATE CONVINCING POSITIVE CONTROL.*

**Safety Summary:** In general, the clinical portion of the study was completed without any significant sequelae attributable to the investigational drug. The safety monitoring was completed to the satisfaction of the clinical investigators and no SAEs were reported.

No subject was discontinued due to an adverse event (AE). All fifteen (15) subjects randomized to colchicine experienced one or more AEs; a total of 40 adverse events were reported over the course of the study. There were no adverse events experienced by the 3 subjects who were administered the moxifloxacin treatment.

The gastrointestinal system was most affected by adverse events in the study, as all 15 subjects reported diarrhea after administration of the colchicine treatment. Seven (7) of these subjects (46.7%) also had mild nausea, 6 subjects (40.0%) had moderate vomiting, and 1 subject had flatulence (6.7%). All of these AEs were judged by the Investigator to be at least possibly related to treatment. Singular occurrences of dizziness, headache, nasal congestion, nasopharyngitis, pharyngolaryngeal pain, and vessel puncture site pain (arm pain due to phlebotomy) were also reported. Adverse events were mild to moderate in intensity. No subject was discontinued due to an adverse event.

The clinical laboratory values were considered unremarkable and none of the values outside of the reference range at study exit were considered directly attributable to the product.

Seated and standing blood pressure and pulse were measured repeatedly following dosing at time points that encompassed the peak plasma concentrations. There were no apparent effects on either parameter, nor was there any evidence of orthostatic hypotension.

**CONCLUSIONS:** Following the administration of 4.8 mg of colchicine dose over 6 hours in healthy adults, colchicine reached a mean maximum plasma concentration of 6.84 ng/mL at 4.47 hours. For the majority of subjects randomized to colchicine (10/15), the highest observed concentration occurred 3 hours after the first dose (at which time, subjects had received a total of 2.4 mg given as 1.2 mg followed by 0.6 mg hourly), prior to the fourth dose). The mean concentration at this time point for all subjects was 6.46 ng/mL. Values declined thereafter such that there is a

"shoulder" in concentrations through 7.75 hours following the first dose (1.75 hours after the final dose); these mean concentrations range from 4.9 to 5.2 ng/mL. In examining the individual plasma concentrations, 10 subjects (66.6%) had a secondary peak occurring between 6.75 to 7.75 hours after the first dose (0.75 to 1.75 hours after the final dose); in 4 of these subjects, this peak was higher than the initial one. The apparent volume of distribution of colchicine greatly exceeds total body volume (876.09 L). The terminal elimination half-life of a high dose colchicine regimen was 31.38 hours. Colchicine concentrations were similar in male and female subjects.

With respect to metabolites, all levels of 2DMC were below the level of quantification (0.2 ng/mL). 3DMC concentrations were above the LOQ (0.2 ng/mL) on at least one time point for all subjects. The mean  $C_{max}$  was 0.32 ng/mL occurring on average 5.06 hours post dose. These observations suggest that exposure to either metabolite is less than 5% of exposure to parent drug, consistent with *in vitro* findings that colchicine is minimally metabolized hepatically (5 to 20%).

Colchicine given in a standard regimen used in the treatment of acute gout resulted in mild to moderate diarrhea in all subjects. Seven (7) of these subjects (46.7%) also had mild nausea, 6 subjects (40.0%) had moderate vomiting, and 1 subject had flatulence (6.7%). There were no discontinuations due to adverse events.

While statistical power to detect changes in ECG parameters is limited, there was no evidence of ECG repolarization effects following colchicine treatment. There were decreases from baseline in QTcF at all times, reaching statistical significance at the times closest to completion of dosing. The colchicine effect upper bound of a 95% one-sided CI did not reach 10 msec at any of the time points. There were no treatment-emergent values >450 msec or change from baseline > 30 msec. Colchicine was without effect on any other ECG parameter measured (heart rate and PR and QRS intervals).

Colchicine had no apparent effect on clinical laboratory parameters or on vital signs (including an evaluation for possible orthostatic changes).

#### 4.2.4 Synopsis of Study MPC-004-07-1003

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                    |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------|
| <b>SPONSOR:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Mutual Pharmaceutical Company, Inc.<br>1100 Orthodox Street<br>Philadelphia, PA 19124              |
| <b>NAME OF TEST PRODUCT:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Colchicine Tablets USP, 0.6 mg                                                                     |
| <b>ACTIVE INGREDIENTS:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Colchicine                                                                                         |
| <b>STUDY TITLE:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Open-Label Pharmacokinetic Study of a Low-Dose Acute Gout Regimen (Total Dose 1.8 mg Over 2 Hours) |
| <b>PRINCIPAL INVESTIGATOR AND STUDY SITE:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Anthony R. Godfrey, Pharm.D.<br>4801 Amber Valley Parkway<br>Fargo, ND 58104, USA                  |
| <b>STUDY DURATION:</b> 11 September 2007 – 15 September 2007                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                                                                                    |
| <b>STUDY TYPE:</b> Phase 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                                                                                                    |
| <p><b>OBJECTIVE:</b> The objectives of this study were to determine the following:</p> <ul style="list-style-type: none"> <li>• To determine the pharmacokinetic profile of colchicine and its metabolites (2-demethylcolchicine [2DMC] and 3-demethylcolchicine [3DMC] and 10-demethylcolchicine [10DMC]) following a low-dose acute gout regimen (total dose 1.8 mg over 2 hours) of the to-be-marketed product, colchicine tablets USP, 0.6 mg manufactured by Mutual Pharmaceutical Company, Inc. (Mutual).</li> <li>• To evaluate the safety and tolerability profile of this dose regimen in healthy volunteers.</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                                                                    |
| <p><b>METHODOLOGY:</b> This study was a single-center, non-randomized, single-sequence, single-period, open-label pharmacokinetic study of a low dose regimen used in the treatment of patients with gout.</p> <p>On study Days 1, 1.2 mg dose (2 × 0.6 mg tablet) of colchicine was administered to all study subjects following an overnight fast of at least 10 hours. A single 0.6 mg dose of colchicine was then administered to all study subjects 1 hour later. Subjects received a cumulative dose of 1.8 mg (3 × 0.6 mg tablets) of colchicine.</p> <p>Beginning on Day 1, blood samples were collected for analysis of plasma concentrations prior to dosing and at intervals over 96 hours post-dose (for 48 hours while confined to the study unit and then on an outpatient basis). Plasma samples were sent to the bioanalytical laboratory of PRACS Institute, Ltd. – Cetero Research to be analyzed for concentrations of colchicine and its metabolites, 2DMC, 3DMC, and 10DMC. Due to difficulties with method validation, analysis</p> |                                                                                                    |

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                                                                  |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------|
| for 10DMC was not performed.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                                                                                  |
| <b>NUMBER OF SUBJECTS:</b> A total of 15 healthy adult subjects were to participate in this study. Due to enrollment difficulties, 13 healthy adult subjects participated in this study. Since this was not a comparative study, no sample size calculation was performed. The sample size was considered adequate to characterize the pharmacokinetic profile of colchicine when given in this treatment regimen.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                                  |
| <b>MAIN DIAGNOSIS FOR ENTRY:</b> All subjects were to be asymptomatic, healthy, non-smoking adults who were non-obese (within 15% of ideal weight). Subjects were not allowed to use concomitant medication (either prescription, over-the-counter), or vitamins or supplements in supra-pharmacologic doses.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                                                                                  |
| <b>TEST PRODUCT:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Colchicine Tablets USP, 0.6 mg;<br>Lot #: BB 374 0215; Exp. N/A;<br>Manufacturer: Mutual Pharmaceutical Co., Inc |
| <b>ROUTE OF ADMINISTRATION:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Oral                                                                                                             |
| <b>DURATION OF TREATMENT:</b> The subjects received 1.2 mg of colchicine (2 × 0.6 mg tablets) on Day 1. One (1) hour after receiving this initial dose, subjects received an additional of 0.6 mg colchicine (1 × 0.6 mg tablet).                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                                                                                                                  |
| <b>PRIMARY EFFICACY VARIABLE:</b> Not applicable.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                                                                                                                  |
| <b>SECONDARY EFFICACY VARIABLE:</b> Not applicable.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                                                                                                  |
| <b>CRITERIA FOR EVALUATION:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                                                                  |
| <p><b>Pharmacokinetics:</b> Blood for pharmacokinetic sampling was obtained from all subjects within one hour prior to dosing (0 hour) and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 18, 24, 36 and 48 while confined, 72 and 96 on an outpatient basis. Analytical data from the blood samples collected during the study conduct were used to calculate the following pharmacokinetic parameters: <math>C_{max}</math> (highest observed), <math>T_{max}</math> (relative to first dose), <math>AUC_{0-t}</math>, <math>AUC_{\infty}</math>, <math>K_{el}</math>, <math>V_{area}/F</math>, <math>CL/F</math>, <math>t_{1/2}</math>. The statistical analyses were performed using WinNonlin<sup>®</sup> Version 5.0.1. .</p> <p><b>Safety:</b> All subjects were monitored throughout the confinement portion of the study for safety assessments. Subjects were queried for adverse events and they were observed. On Day -1 and Day 2, blood samples were collected for pregnancy screens (females only) and for a CBC with differential and clinical chemistry, a urine sample was collected for urinalysis. On Day 1, sitting (5 minutes) and standing (taken 1 minute after collection of sitting vitals) blood pressure and heart rate were measured prior to dosing and at 0.5, 1, 2, 4, 12 and 24 hours post-dose. Triplicate 12-lead ECGs were also collected at these time points approximately 2-4 minutes apart.</p> |                                                                                                                  |
| <b>SUMMARY OF RESULTS:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                                                                                  |

**Demographic Summary:** The mean age of the subjects was approximately 29 years (Mean = 28.9 yrs  $\pm$  8.4 yrs). Ages ranged from 20 to 49 years. Sex was evenly distributed (7 females; 6 males) and a majority of the subjects (69%) were white (not Hispanic or Latino = approximately 92.3%; Hispanic or Latino = 7.7%).

**Pharmacokinetic Summary:** The following table presents the mean colchicine pharmacokinetic parameter values: The lower limit of quantification was 0.20 ng/mL for colchicine, 2DMC, and 3DMC. For statistical analysis, subject sample values below the lower limit of quantification (BLQ) were reported as zero. All of the plasma concentration values for 2DMC were BLQ (0.200 ng/mL). There were 29 plasma concentration values for 8 subjects reported for 3DMC that were not BLQ. Within a subject, the highest concentrations ranged from \_\_\_\_\_ Statistical analyses were not done for 2DMC and 3DMC due to a lack of plasma concentration values.

b(4)

Pharmacokinetic parameter values calculated based on colchicine plasma levels are summarized below.

| Parameter (unit)               | Arithmetic Mean (%CV) Median (Range for T <sub>max</sub> ) |                       |                     |
|--------------------------------|------------------------------------------------------------|-----------------------|---------------------|
|                                | All Subjects (N=13)                                        | Females Fasting (N=7) | Males Fasting (N=6) |
| AUC <sub>0-1</sub> (ng-hr/mL)  | 43.79 (26.12)                                              | 46.43 (25.23)         | 40.70 (27.8)        |
| AUC <sub>0-∞</sub> (ng-hr/mL)  | 52.07 (26.29)                                              | 54.55 (25.0)          | 49.18 (29.4)        |
| C <sub>max</sub> (ng/mL)       | 6.19 (39.30)                                               | 7.15 (36.8)           | 5.07 (34.8)         |
| T <sub>max</sub> (hr)          | 1.81 (1.00 - 2.50)                                         | 1.79 (1.00-2.50)      | 1.83 (1.50-2.00)    |
| k <sub>el</sub> (1/hr)         | 0.03 (30.80)                                               | 0.03 (26.5)           | 0.03 (37.81)        |
| t <sub>1/2</sub> (hr)          | 23.63 (39.10)                                              | 22.58 (26.1)          | 24.86 (50.9)        |
| CL/F (L/hr)                    | 36.95 (27.04)                                              | 35.2 (29.1)           | 39.1 (26.1)         |
| Weight-Adjusted CL/F (L-hr/kg) | 0.52 (25.31)                                               | 0.53 (31.1)           | 0.511 (18.6)        |
| V <sub>nes</sub> /F (L)        | 1188.72 (26.88)                                            | 1101.93 (24.3)        | 1289.97 (28.6)      |

**Safety Summary:** In general, the clinical portion of the study was completed without any significant sequelae attributable to the investigational drug. The safety monitoring was completed to the satisfaction of the clinical investigators and no SAEs were reported.

Five (5) of 13 subjects (38.5%) experienced a total of 8 AEs following 1.8 mg of colchicine administered over 1 hour. These AEs included 2 subjects (15.4%) with diarrhea or headache and single subjects (7.7%) with upper abdominal pain, dyspepsia, nausea or hot flush. There were 3 subjects who experienced a gastrointestinal AE. All AEs were mild in intensity and considered by the Investigator to be "probably" related to the administration of colchicine. No subject was discontinued due to an AE.

The clinical laboratory values were considered unremarkable and none of the values outside of the reference range at study exit were considered directly attributable to the product. No consistent or clinically relevant abnormalities or changes *versus* baseline in ECG intervals or morphology were observed in any subject. QTcF values remained within normal limits in all

subjects and no relevant changes in QTcF *versus* baseline were observed.

**CONCLUSIONS:**

**Pharmacokinetic:** Mean peak plasma concentration of 6.19 ng/mL was observed 1.81 hours after the initial dose. The apparent volume of distribution exceeded total body water, a mean of 1188.72 L. Apparent oral clearance was 36.95 L/hour. The mean plasma elimination half-life was estimated as 23.63 hours.

Exposure to colchicine was higher in women as compared to men.  $C_{max}$  concentrations were approximately 40% higher and AUC concentrations were approximately 14% higher. CL/F, when normalized for weight, was similar between females and males, 0.53 and 0.51 L/hr/kg, suggesting that the difference is due to differences in body weight rather than inherent pharmacokinetics.

Plasma samples were also analyzed for the two putative primary metabolites, 2DMC and 3DMC. Pharmacokinetic parameter values could not be calculated as 2DMC levels were all BLQ and 8 subjects had only 29 single time points total at which 3DMC was just above BLQ. These results suggest that less than 5% of total exposure is to metabolites, an observation that is consistent with the overall conclusion that colchicine is minimally metabolized hepatically (approximately 5 to 20% biotransformation *via* CYP3A4 pathway).

**Safety:** Overall, colchicine was well tolerated as a cumulative oral 1.8 mg dose (3 × 0.6 mg tablet). Eight (8) adverse events occurred in 5 out of 13 subjects and all AEs were considered by the Investigator to be "probably" related to the study drug. All AEs were mild in intensity and did not result in any subject discontinuations.

#### 4.2.5 Synopsis of Study MPC-004-07-1004

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                             |
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| <b>SPONSOR:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Mutual Pharmaceutical Company, Inc.<br>1100 Orthodox Street<br>Philadelphia, PA 19124-3131                                                  |
| <b>NAME OF TEST PRODUCT:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Colchicine Tablets USP, 0.6 mg                                                                                                              |
| <b>ACTIVE INGREDIENTS:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Colchicine                                                                                                                                  |
| <b>STUDY TITLE:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | An Open-Label, Two-Period, Sequential, Single- and Multiple-Dose Pharmacokinetic Study with 0.6 mg Colchicine Tablets in Healthy Volunteers |
| <b>PRINCIPAL INVESTIGATOR AND STUDY SITE:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Anthony R. Godfrey, Pharm. D.<br>PRACS Institute, Ltd. – Cetero Research<br>4801 Amber Valley Parkway<br>Fargo, ND 58104, USA               |
| <b>PHASE OF DEVELOPMENT: I</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                                                                                             |
| <b>STUDY DURATION:</b> The time from when the first subject dosed to when the last subject completed the study (i.e. the date the last pharmacokinetic sample was collected) was 29 days (Period I: 06 September 2008 – 10 September 2008; Period II: 20 September 2008 – 04 October 2008).                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                             |
| <p><b>OBJECTIVE:</b> The objectives of this study were to:</p> <ul style="list-style-type: none"> <li>determine the single- and multiple-dose pharmacokinetics of colchicine (administered as colchicine tablets USP, 0.6 mg, manufactured by Mutual Pharmaceutical Company, Inc.), following the gout prophylaxis multiple dose regimen in healthy adult volunteers; and,</li> <li>evaluate the safety and tolerability of colchicine tablets USP, 0.6 mg administered to healthy volunteers as a single-dose and multiple-dose (every 12 hours in a 10-day regimen).</li> </ul>                                                                                                                                                                                                                                                               |                                                                                                                                             |
| <p><b>METHODOLOGY:</b> This open-label, single-sequence, two-period, single-center study was conducted to determine the pharmacokinetics of colchicine (Colchicine Tablets USP, 0.6 mg, Mutual Pharmaceutical Company, Inc.) following single and multiple oral doses.</p> <p>On study Days 1 and 25, a single 0.6 mg (1 × 0.6 mg tablet) dose of colchicine was administered to all study subjects following an overnight fast of at least 13 hours. A single dose of colchicine was re-administered to all study subjects 12 hours later for Days 15 through 24, such that each subject received 0.6 mg of colchicine every 12 hours on Days 15-24.</p> <p>On Days 1 and 25, blood samples were collected for analysis of colchicine plasma concentrations prior to dosing and at intervals over 96 hours post-dose. On Days 15-24, blood</p> |                                                                                                                                             |

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                                                                                            |
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| <p>samples were collected for analysis of colchicine within 3 minutes prior to dosing. Plasma samples were sent to the bioanalytical laboratory of PRACS Institute, Ltd. – Cetero Research for concentration determination of colchicine and its metabolites 2-, 3-, and 10-demethylcolchicine (2DMC, 3DMC, and 10DMC, respectively).</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                                                                                            |
| <p><b>NUMBER OF SUBJECTS:</b> A total of 13 healthy adult subjects participated in this study. No intra-subject coefficients of variation were available. Based on the published literature, the inter-subject %CV for colchicine pharmacokinetic parameter values appears to be 25-50%. Based on this information, 14 subjects were judged to be a sufficient number to characterize the multiple-dose pharmacokinetic profile.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                                                                                                            |
| <p><b>MAIN DIAGNOSIS FOR ENTRY:</b> Diagnosis was not required for this study. All subjects were asymptomatic, healthy, adult subjects who met the inclusion/exclusion criteria for this study.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                                            |
| <p><b>TEST PRODUCT:</b></p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | <p>Colchicine Tablets USP, 0.6 mg;<br/>Lot #: BB 374 0215; Exp. N/A;<br/>Manufacturer: Mutual Pharmaceutical Co., Inc.</p> |
| <p><b>ROUTE OF ADMINISTRATION:</b></p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | <p>Oral</p>                                                                                                                |
| <p><b>DURATION OF TREATMENT:</b> The subjects received a single 0.6 mg colchicine tablet on Day 1 and then completed a 14-day washout period. On Day 15, subjects received 0.6 mg colchicine tablet twice daily for 10 days; therefore, total study duration, including dosing, washout period, and pharmacokinetic sampling, was completed in a 29-day period.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                                            |
| <p><b>PRIMARY EFFICACY VARIABLE:</b> Not applicable.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                            |
| <p><b>SECONDARY EFFICACY VARIABLE:</b> Not applicable.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                            |
| <p><b>CRITERIA FOR EVALUATION:</b></p> <p><b>Pharmacokinetics:</b> Blood for pharmacokinetic sampling was obtained from all subjects within one hour prior to dosing (0 hour) and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 hours post-dose, while confined; and, at 36, 48, 72, and 96 hours post-dose on an outpatient basis. Analytical data from the blood samples collected during the study conduct were used to calculate the following pharmacokinetic parameters: <math>AUC_{0-t}</math>, <math>AUC_{0-\infty}</math>, <math>AUC_{0-inf}</math>, <math>C_{max}</math>, <math>C_{min}</math>, <math>C_{ave}</math>, <math>CL/F</math>, <math>T_{max}</math>, <math>K_{el}</math>, <math>R</math>, <math>t_{1/2}</math>, and <math>V_d</math>.</p> <p>Analyses of Variance (ANOVA) were performed on the ln-transformed <math>AUC_{0-t}</math>, <math>AUC_{0-\infty}</math>, and <math>C_{max}</math> as appropriate. The ANOVA model included factors accounting for the following sources of variation: sequence, subjects within sequence, period, and treatment. The above statistical analyses were performed using the SAS® procedure.</p> <p><b>Safety:</b> All subjects were monitored throughout the confinement portion of the study for safety assessments. Subjects were queried for adverse events and they were observed. On Day -1 and Day 14, blood samples were collected for pregnancy screens (females only) and for a CBC with</p> |                                                                                                                            |

differential and clinical chemistry; these assessments were repeated prior to study exit at 24 hours post-dose (Day 26). Pregnancy screens were repeated at study exit (or early termination) on Day 29. An additional pregnancy screen was administered to all female subjects on Day 17. On Day 1 and Day 25, sitting (5 minutes) blood pressure and heart rate were measured prior to dosing and at 1, 2, and 4 hours post-dose; 12-lead ECGs were also collected at these time points. On Days 15-24, sitting (5 minutes) blood pressure and heart rate were measured prior to the morning dose administration. Blood pressure and heart rate measurements were also repeated prior to study exit (i.e. 24 hours post-dose).

**SUMMARY OF RESULTS:**

**Demographic Summary:** The mean age of the subjects was approximately 25 years (Mean = 25.5 yrs ± 5.4 yrs). Ages ranged from 19 to 38 years. However, sex was not evenly distributed (1 female: 12 males) and a majority of the subjects were white (not Hispanic or Latino = approximately 69.2%; Hispanic or Latino = 15.38%).

**Pharmacokinetic Summary:** The table below presents the mean colchicine pharmacokinetic parameters for Day 1 (i.e., after a single 0.6 mg dose) and Day 25 (i.e., after 10 days of dosing with colchicine 0.6 mg every 12 hours). Pharmacokinetic parameter values could not be calculated for 2DMC or 3DMC due to the number of samples below the level of quantitation (BLQ); blood samples could not be analyzed to 10DMC due to variability during validation.

| Parameter (units)               | Arithmetic Mean (%CV)<br>Mean (Range) for T <sub>max</sub> |                    |
|---------------------------------|------------------------------------------------------------|--------------------|
|                                 | Day 25*<br>(N = 13)                                        | Day 1<br>(N = 13)  |
| AUC <sub>0-t</sub> (ng·hr/mL)   | 43.58 (21.42)                                              | 10.49 (33.77)      |
| AUC <sub>0-inf</sub> (ng·hr/mL) | 54.20 (17.00)                                              | 12.27 (36.05)      |
| AUC <sub>0-t</sub> (ng·hr/mL)   | 20.37 (16.31)                                              | NA*                |
| C <sub>max</sub> (ng/mL)        | 3.55 (23.74)                                               | 2.45 (28.66)       |
| C <sub>min</sub> (ng/mL)        | 0.907 (16.79)                                              | NA*                |
| C <sub>ave</sub> (ng/mL)        | 1.70 (16.31)                                               | NA*                |
| T <sub>max</sub> (hr)           | 1.31 (0.50 – 3.00)                                         | 1.50 (1.00 – 3.00) |
| K <sub>d</sub> (l/hr)           | 0.0267 (16.34)                                             | 0.1829 (32.38)     |
| T <sub>1/2</sub> (hr)           | 26.60 (16.26)                                              | 4.95 (89.54)       |
| CL/F (L/hr)                     | 30.31 (18.95)                                              | 54.05 (30.98)      |
| Weight-Adjusted CL/F (L/hr/kg)  | 0.3916 (18.31)                                             | 0.6902 (29.11)     |
| V <sub>d</sub> (L)              | 1150.95 (18.73)                                            | 341.54 (54.35)     |

\*Not Applicable

\*After 10 days of dosing with colchicine 0.6 mg twice daily

**Safety Summary:** No serious adverse events (SAEs) were reported over the course of this study. No subject was discontinued due to an adverse event (AE). Four (4) of 13 subjects experienced a total of six AEs over the course of the study. No subject experienced an AE following the administration of colchicine single doses. Following the administration of multiple doses of colchicine (twice daily dosing), four subjects reported these AEs: stomach discomfort, dizziness, decreased appetite, muscle spasms, dry mouth, and diarrhea. Adverse events were mild in intensity.

No clinically significant findings from an assessment of clinical laboratory test results, vital signs data, or physical examination results were observed. No consistent or clinically relevant abnormalities or changes versus baseline in ECG intervals or morphology were observed in any subject. QTcF values remained within normal limits in all subjects and no relevant changes in QTcF versus baseline were observed.

#### **SUMMARY CONCLUSIONS:**

**Pharmacokinetic:** In healthy adults, colchicine appears to be readily absorbed when given orally as Mutual's colchicine tablets, USP 0.6 mg, reaching a mean maximum plasma concentration of 2.5 ng/mL in 1.5 hours after a single dose, and distributing widely with an apparent volume of distribution of 342 L, greatly exceeding total body water. The elimination half-life following a single oral dose is approximately 5 hours. Levels were not detectable by 24 hours post-dose after a single 0.6 mg dose and therefore the half-life and AUC are underestimated.

With 10 days of repeated twice daily dosing, steady state conditions are approached but do not appear to be entirely achieved as trough concentrations appear to be increasing to a small extent. In review of trough concentrations, there is a suggestion of diurnal variation with evening concentrations approximately 12% higher than morning concentrations. Alternatively, this could be attributed to evening dosing within 90 minutes following a meal as opposed to morning dosing in a fasting condition.

On the final day of dosing, near steady state, the mean peak plasma concentration is 3.5 ng/mL occurring, on average, approximately 1.3 hours post-dose. With the higher plasma levels, a longer terminal elimination half-life of 26.6 hours is apparent. After the last dose of a steady state regimen, concentrations remained quantifiable for five of 13 subjects (38.46%) out to 72 hours post-dose.

Based on *in vitro* studies, colchicine is minimally metabolized hepatically. This was confirmed in this study by the measurement of plasma concentrations of the two primary metabolites, 2DMC and 3DMC. All but two values were below the level of assay quantification (0.2 ng/mL). This suggests that exposure to these metabolites, even at steady state, is less than 5% of the parent drug. 10DMC, an even less prevalent metabolite, could not be assayed due to excessive variability in the analytical method.

**Safety:** Overall, colchicine was well tolerated as a single oral dose (1 × 0.6 mg tablet) or as a twice daily dose (1 × 0.6 mg tablet b.i.d.). Other than gastrointestinal adverse events occurring in 3 subjects (23.1%), there were no treatment-related adverse events. Colchicine administered for 10 days does not appear to result in changes in clinical laboratory results, vital sign measurements, or ECG parameters.

#### 4.2.6 Synopsis of Study MPC-004-07-1005

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                                                                                                                                                      |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>SPONSOR:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Mutual Pharmaceutical Company, Inc.<br>1100 Orthodox Street<br>Philadelphia, PA 19124-3131                                                                                           |
| <b>NAME OF TEST PRODUCT:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | Colchicine Tablets USP, 0.6 mg                                                                                                                                                       |
| <b>ACTIVE INGREDIENTS:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Colchicine                                                                                                                                                                           |
| <b>STUDY TITLE:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | A Pharmacokinetic Study to Evaluate the Effect of Colchicine on the Pharmacokinetic Profile of an Oral Contraceptive Containing Ethinyl Estradiol and Norethindrone in Healthy Women |
| <b>PRINCIPAL INVESTIGATOR AND STUDY SITE:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Barrie March, M.D.<br>PRACS Institute, Ltd. – Cetero Research<br>4801 Amber Valley Parkway<br>Fargo, ND 58104, USA                                                                   |
| <b>PHASE OF DEVELOPMENT: 1</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                                                                                                                                                      |
| <b>STUDY DURATION:</b> 26 August 2007 – 27 January 2008.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                      |
| <p><b>OBJECTIVE:</b> The objectives of this study were to determine the following:</p> <ul style="list-style-type: none"> <li>• Whether steady-state dosing of colchicine tablets USP, 0.6 mg (manufactured by Mutual Pharmaceutical Company, Inc.) influences the steady-state pharmacokinetic profile of ethinyl estradiol or norethindrone (McNeil Pharmaceutical, Inc.'s Ortho-Novum® 1/35)</li> <li>• Steady-state colchicine plasma concentrations in women on oral contraceptives</li> <li>• Safety and tolerability of concurrent administration of colchicine tablets USP, 0.6 mg and an estrogen/progesterone-containing oral contraceptive</li> </ul>                                                                                                                                                                                                                                                     |                                                                                                                                                                                      |
| <p><b>METHODOLOGY:</b> This was a double-blind, randomized, two-sequence, single-center, two-cycle crossover study to characterize the steady-state pharmacokinetic profile of ethinyl estradiol and norethindrone administered without colchicine, and following concomitant administration with colchicine (Colchicine Tablets USP, 0.6 mg, Mutual Pharmaceutical Company, Inc.), also administered to steady-state conditions.</p> <p>On study Days 1 through 7 of each cycle subjects received one active tablet of Ortho-Novum® 1/35. On Days 8 through 21, subjects received one active tablet Ortho-Novum® 1/35 in the morning with the test product administered twice daily (b.i.d., one capsule in the morning followed by one capsule in the evening); the test product was either over-encapsulated colchicine tablets USP, 0.6 mg or a matching placebo capsule administered in a randomly assigned</p> |                                                                                                                                                                                      |

sequence). On Day 22 through 28 (Cycle 1 only), subjects received one inert tablet of Ortho-Novum<sup>®</sup> 1/35.

On Day 21 of each cycle, serial pharmacokinetic blood samples to measure ethinyl estradiol, norethindrone, and colchicine plasma concentrations were collected immediately prior to dosing (0 hour) and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 (prior to colchicine/placebo dose) and 24 hours post-dose. Plasma samples to be analyzed for ethinyl estradiol and norethindrone were shipped to \_\_\_\_\_ for analysis. Plasma samples to be analyzed for colchicine were transferred to the bioanalytical laboratory at PRACS Institute, Ltd. – Cetero Research for analysis

b(4)

**NUMBER OF SUBJECTS:** A total of 30 healthy adult women participated in this study. The sample size of 30 was considered adequate based on the sample sizes used in other published interaction studies with oral contraceptives (range 18 to 37 subjects).

**MAIN DIAGNOSIS FOR ENTRY:** All subjects were asymptomatic, healthy, adult, non-smoking, non-obese female subjects who met the inclusion/exclusion criteria for this study. A main criterion for inclusion was that they already be using oral hormonal contraception. They were not using concomitant medications that could interfere with the pharmacokinetic interpretations of the study and general health was ascertained by medical history, physical examination, clinical laboratory testing, and ECG measurement.

**TEST PRODUCT:**

Colchicine Tablets USP, 0.6 mg;  
Lot #: BB 374 0215; Exp. N/A;  
Manufacturer: Mutual Pharmaceutical  
Company, Inc.

**ROUTE OF ADMINISTRATION:**

Oral

**DURATION OF TREATMENT:** A one-cycle lead-in period preceded the pharmacokinetic period in subjects who did not enter the study already receiving Ortho-Novum<sup>®</sup> 1/35 or a generic equivalent. During the Pharmacokinetic Period, one cycle of Ortho-Novum<sup>®</sup> 1/35 active tablets was administered on Days 1 through 21 of each of two study periods, followed by 7 days of inert tablets (Period 1 only). Colchicine (over-encapsulated) or matching placebo was co-administered on Days 8-21 in a randomly assigned sequence (14 days total). Total study duration including dosing for both cycles and pharmacokinetic sampling was 49 days; if a run-in period was required, total study duration was 77 days.

**PRIMARY EFFICACY VARIABLE:** Not applicable.

**SECONDARY EFFICACY VARIABLE:** Not applicable.

**CRITERIA FOR EVALUATION:**

**Pharmacokinetics:** Analytical data from the blood samples collected during the study conduct were used to calculate the following pharmacokinetic parameters for norethindrone, ethinyl

estradiol, and colchicine:  $AUC_{\tau}$ ,  $C_{max, ss}$ ,  $C_{min, ss}$ ,  $C_{av, ss}$ ,  $T_{max, ss}$ ,  $T_{1/2}$ , Flux, CL/F, Weight-Adjusted CL/F, and  $V_d$ .

Analyses of variance (ANOVA) were performed on the ln-transformed  $AUC_{\tau}$  and  $C_{max, ss}$  of ethinyl estradiol and norethindrone when given with and without colchicine.  $T_{max}$  was analyzed without transformation. The ANOVA model included treatment, sequence, treatment-within-sequence as fixed effects, and subject as a random effect. Each ANOVA included calculation of least-squares means (LSM), the difference between regimen LSM, and the standard error associated with this difference. The above statistical analyses were done using the appropriate SAS<sup>®</sup> procedure.

**Safety:** All subjects were monitored throughout the study. Subjects were queried for adverse events at screening, check-in, during the confinement portion of the study, and at study exit (or early termination). Subjects were contacted once daily via telephone on study Days 9 through Day 20 regarding the tolerability of the study drug. All subjects underwent clinical laboratory testing including hematology, serum chemistry, urinalysis, and serum pregnancy tests at screening check-in for each period, and at study discharge (24 hours after the last dose of study drug). Vital signs (blood pressure and pulse) were measured on Day 21 pre-dose and 1, 2, and 4 hours post-dose. Additionally, physical examinations were performed at screening and at study exit (or early termination).

#### SUMMARY OF RESULTS:

**Demographic Summary:** The mean age of the subjects was approximately 24 years (Mean = 23.9 yrs  $\pm$  6.3 yrs). Ages ranged from 18 to 45 years. The majority of the subjects were white (approximately 96.7%), with one individual reported being Hispanic or Latino (3.3%).

**Pharmacokinetic Summary:** When colchicine (0.6 mg tablet) and Ortho-Novum<sup>®</sup> 1/35 were co-administered at steady-state, the mean norethindrone  $C_{max, ss}$  was 24.17 ng/mL  $\pm$  6.63, occurring on average 1 hour post-dose (range 1 to 4 hours). In comparison, when Ortho-Novum<sup>®</sup> 1/35 was administered alone, the mean norethindrone  $C_{max, ss}$  was 24.42 ng/mL  $\pm$  6.11, occurring on average 1 hour post-dose (range 0.5 to 4 hours). Norethindrone  $AUC_{\tau}$  was 175.57 ng·hr/mL  $\pm$  60.74 when co-administered with colchicine as compared to  $AUC_{\tau}$  of 178.08 ng·hr/mL  $\pm$  51.69 when Ortho-Novum<sup>®</sup> 1/35 was administered alone. Total apparent oral clearance was 6.3 L/hr when co-administered with colchicine as compared to total apparent oral clearance of 6.1 L/hr when administered alone.

Summary mean norethindrone steady-state pharmacokinetic parameter values following multiple doses of Ortho-Novum<sup>®</sup> 1/35 tablets with and without co-administration of multiple doses of colchicine tablets are presented in the table below.

| Parameter (units)            | Arithmetic Mean (%CV)<br>Median (Range) for T <sub>max</sub> |                                        |
|------------------------------|--------------------------------------------------------------|----------------------------------------|
|                              | Co-Administered with Colchicine<br>(N=27)                    | Co-Administered with Placebo<br>(N=27) |
| AUC <sub>t</sub> (ng·hr/mL)  | 175.57 (34.60)                                               | 178.08 (29.03)                         |
| C <sub>max, ss</sub> (ng/mL) | 24.17 (27.44)                                                | 24.42 (25.03)                          |
| C <sub>min, ss</sub> (ng/mL) | 2.45 (47.75)                                                 | 2.62 (45.15)                           |
| C <sub>av, ss</sub> (ng/mL)  | 7.32 (34.60)                                                 | 7.42 (29.03)                           |
| T <sub>max, ss</sub> (hr)    | 1.00 (1.00 – 4.00)                                           | 1.00 (0.50 – 4.00)                     |
| T <sub>1/2</sub> (hr)        | 10.93 (26.23)                                                | 10.85 (22.49)                          |
| FLUX1 (%) <sup>1</sup>       | 308.14 (20.20)                                               | 302.86 (22.19)                         |
| FLUX2 (%) <sup>2</sup>       | 1060.05 (46.55)                                              | 982.38 (47.34)                         |
| CL/F (L/hr)                  | 6.30 (31.21)                                                 | 6.08 (29.48)                           |
| Weight-Adjusted CL/F (L/hr)  | 0.09 (30.19)                                                 | 0.09 (27.46)                           |
| V <sub>d</sub> (L)           | 96.21 (31.91)                                                | 93.93 (32.48)                          |

<sup>1</sup> Relative to C<sub>min, ss</sub>

<sup>2</sup> Relative to C<sub>av, ss</sub>

When colchicine (0.6 mg tablet) and Ortho-Novum<sup>®</sup> 1/35 were co-administered at steady-state, the mean ethinyl estradiol C<sub>max, ss</sub> was 0.14 ng/mL ± 0.05, occurring on average 1.5 hour post-dose (range 1 to 4 hours). In comparison, when Ortho-Novum<sup>®</sup> 1/35 was administered alone, the mean ethinyl estradiol C<sub>max, ss</sub> was 0.15 ng/mL ± 0.05, occurring on average 1.5 hour post-dose (range 1 to 4 hours). Ethinyl estradiol AUC<sub>t</sub> was 1.24 ng·hr/mL ± 0.41 when co-administered with colchicine as compared to AUC<sub>t</sub> of 1.29 ng·hr/mL ± 0.40 when Ortho-Novum<sup>®</sup> 1/35 was administered alone. Total apparent oral clearance was 30.7 L/hr when co-administered with colchicine as compared to total apparent oral clearance of 29.6 L/hr when administered alone.

Summary mean ethinyl estradiol steady-state pharmacokinetic parameter values following a multiple doses of Ortho-Novum<sup>®</sup> 1/35 tablets with and without co-administration of multiple doses of colchicine tablets are presented in the table below.

| Parameter (units)            | Arithmetic Mean (%CV)<br>Median (Range) for T <sub>max</sub> |                                        |
|------------------------------|--------------------------------------------------------------|----------------------------------------|
|                              | Co-Administered with Colchicine<br>(N=27)                    | Co-Administered with Placebo<br>(N=27) |
| AUC <sub>t</sub> (ng·hr/mL)  | 1.24 (33.08)                                                 | 1.29 (31.10)                           |
| C <sub>max, ss</sub> (ng/mL) | 0.14 (35.47)                                                 | 0.15 (32.70)                           |
| C <sub>min, ss</sub> (ng/mL) | 0.02 (44.76)                                                 | 0.02 (39.37)                           |
| C <sub>av, ss</sub> (ng/mL)  | 0.05 (33.07)                                                 | 0.05 (31.10)                           |
| T <sub>max, ss</sub> (hr)    | 1.50 (1.00 – 4.00)                                           | 1.50 (1.00 – 4.00)                     |
| T <sub>1/2</sub> (hr)        | 12.37 (16.38)                                                | 14.87 (16.72)                          |
| FLUX1 (%) <sup>1</sup>       | 222.57 (19.12)                                               | 232.59 (16.22)                         |
| FLUX2 (%) <sup>2</sup>       | 590.48 (31.57)                                               | 579.02 (25.70)                         |
| CL/F (L/hr)                  | 30.73 (26.78)                                                | 29.58 (29.54)                          |
| Weight-Adjusted CL/F (L/hr)  | 0.45 (23.22)                                                 | 0.43 (26.96)                           |
| V <sub>d</sub> (L)           | 541.41 (28.95)                                               | 620.48 (27.78)                         |

<sup>1</sup> Relative to  $C_{max, ss}$   
<sup>2</sup> Relative to  $C_{av, ss}$

The following table summarizes the results of the analyses performed on the ln-transformed pharmacokinetic parameters for the comparison of norethindrone co-administered with colchicine and co-administered with placebo.

| Geometric Means, Ratio of Means, and 90% Confidence Interval of Ln-Transformed Data for Norethindrone |                                 |                              |         |                 |
|-------------------------------------------------------------------------------------------------------|---------------------------------|------------------------------|---------|-----------------|
| Parameter                                                                                             | Co-Administered with Colchicine | Co-Administered with Placebo | % Ratio | 90% CI          |
| $AUC_T$<br>(ng-hr/mL)                                                                                 | 167.52                          | 171.95                       | 97.43   | (92.82, 102.26) |
| $C_{max, ss}$<br>(ng/mL)                                                                              | 23.40                           | 23.71                        | 98.67   | (91.90, 105.95) |

The following table summarizes the results of the analyses performed on the ln-transformed pharmacokinetic parameters for the comparison of ethinyl estradiol co-administered with colchicine and co-administered with placebo.

| Geometric Means, Ratio of Means, and 90% Confidence Interval of Ln-Transformed Data for Ethinyl Estradiol |                                 |                              |         |                 |
|-----------------------------------------------------------------------------------------------------------|---------------------------------|------------------------------|---------|-----------------|
| Parameter                                                                                                 | Co-Administered with Colchicine | Co-Administered with Placebo | % Ratio | 90% CI          |
| $AUC_T$<br>(ng-hr/mL)                                                                                     | 1.18                            | 1.24                         | 95.71   | (91.23, 100.41) |
| $C_{max, ss}$<br>(ng/mL)                                                                                  | 0.13                            | 0.14                         | 91.20   | (85.93, 96.79)  |

When colchicine (0.6 mg tablet) and Ortho-Novum<sup>®</sup> 1/35 were co-administered at steady-state, the mean colchicine  $C_{max, ss}$  was 3.11 ng/mL  $\pm$  0.86, occurring on average 1.37 hour post-dose (range 1 to 3 hours). Colchicine  $AUC_T$  was 18.40 ng-hr/mL  $\pm$  4.15 when co-administered with Ortho-Novum<sup>®</sup> 1/35 and total apparent oral clearance was 34.19 L/hr.

Summary mean colchicine steady-state pharmacokinetic parameter values following multiple doses of Ortho-Novum<sup>®</sup> 1/35 tablets are presented in the table below

| Parameter (units)           | Arithmetic Mean (%CV)<br>Median (Range) for T <sub>max</sub> |
|-----------------------------|--------------------------------------------------------------|
|                             | Co-Administered with Ortho-Novum 1/35<br>(N=27)              |
| AUC <sub>t</sub> (ng-hr/mL) | 18.40 (22.55)                                                |
| C <sub>max,ss</sub> (ng/mL) | 3.10 (27.75)                                                 |
| C <sub>min,ss</sub> (ng/mL) | 0.76 (23.45)                                                 |
| C <sub>av,ss</sub> (ng/mL)  | 1.53 (22.55)                                                 |
| T <sub>max,ss</sub> (hr)    | 1.50 (1.00 -3.00)                                            |
| T <sub>1/2</sub> (hr)       | 14.74 (27.18)                                                |
| FLUX1 (%) <sup>1</sup>      | 152.19 (20.67)                                               |
| FLUX2 (%) <sup>2</sup>      | 321.08 (33.48)                                               |
| CL/F (L/hr)                 | 34.19 (22.41)                                                |
| Weight-Adjusted CL/F (L/hr) | 0.51 (28.46)                                                 |
| V <sub>d</sub> (L)          | 737.60 (40.15)                                               |

<sup>1</sup> Relative to C<sub>min,ss</sub>

<sup>2</sup> Relative to C<sub>av,ss</sub>

Steady-state colchicine concentrations were approximated as evidenced by the means of the three last pre-dose values (prior to the morning and evening doses on Day 21 and 12 hours after the evening dose on Day 21): these were 0.861, 0.812, and 0.986 ng/mL, respectively. Review of individual subject data is consistent with this. The mean C<sub>max</sub> at steady state was 3.10 ng/mL, occurring on average 1.0 hour post-dose.

**Safety Summary:** In general, the clinical portion of the study was completed without any significant sequelae attributable to the co-administration of study drug and Ortho-Novum<sup>®</sup> 1/35. The safety monitoring was completed to the satisfaction of the clinical investigators and no SAEs were reported.

No subject was discontinued due to an AE. The most common adverse events were gastrointestinal, occurring in 6 of 27 subjects (22.2%) who received colchicine (0.6 mg twice daily dosing for 14 days) co-administered with Ortho-Novum<sup>®</sup> 1/35 (1 "active" tablet daily). Diarrhea occurred in 5 subjects or 18.5%, nausea and upper abdominal pain in 3 subjects each or 11.1%, and cold sweat in 2 subjects or 7.4%. The remaining adverse events, enumerated on Table 12.3, occurred in single subjects. Of these adverse events, 18 were judged to be at least possibly related to treatment by the medical investigator. All events were mild or moderate in severity.

The clinical laboratory values were considered unremarkable and none of the values outside of the reference range at study exit were considered directly attributable to the product. In the opinion of the clinical investigators, the clinical portion of the study was successfully completed.

**SUMMARY CONCLUSIONS:**

**Pharmacokinetic:** When ethinyl estradiol and norethindrone are co-administered with steady-state colchicine (0.6 mg b.i.d. × 14 days) hormone concentrations are not affected as compared to hormone concentrations observed when ethinyl estradiol and norethindrone are administered with placebo, concluding no drug interaction is present when colchicine and Ortho-Novum<sup>®</sup> are administered concomitantly.

The mean  $C_{max, ss}$  of colchicine at steady-state is 3.1 ng/mL, occurring on average 1.0 hour post-dose.

**Safety:** Overall, colchicine, when administered to steady-state, was well tolerated when co-administered with Ortho-Novum<sup>®</sup> 1/35, also administered to steady-state. The most common adverse events were gastrointestinal (22.2%). All adverse events were mild or moderate in intensity and none resulted in discontinuation.

#### 4.2.7 Synopsis of Study MPC-004-07-1006

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| <b>SPONSOR:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Mutual Pharmaceutical Company, Inc.<br>1100 Orthodox Street<br>Philadelphia, PA 19124-3131                                      |
| <b>NAME OF TEST PRODUCT:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Colchicine Tablets USP, 0.6 mg                                                                                                  |
| <b>ACTIVE INGREDIENT:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Colchicine                                                                                                                      |
| <b>STUDY TITLE:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | A Pharmacokinetic Study to Evaluate the Effect of Clarithromycin on the Pharmacokinetic Profile of Colchicine in Healthy Adults |
| <b>PRINCIPAL INVESTIGATOR AND STUDY SITE:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Anthony R. Godfrey, Pharm.D.<br>PRACS Institute, Ltd. – Cetero Research<br>4801 Amber Valley Parkway<br>Fargo, ND 58104, USA    |
| <b>STUDY DURATION:</b> 03 November 2007 to 05 December 2007                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                 |
| <p><b>OBJECTIVES:</b> The objectives of this study were the following:</p> <ul style="list-style-type: none"> <li>• To confirm the extent to which multiple oral doses of clarithromycin influence the single-dose pharmacokinetic profile of colchicine and its metabolites in healthy adult subjects.</li> <li>• To assess the safety and tolerability of concurrent administration of clarithromycin and a single dose of colchicine</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                                 |
| <p><b>METHODOLOGY:</b> This was an open-label, one-sequence, two-period pharmacokinetic drug-drug interaction study with colchicine and clarithromycin. Subjects received a single 0.6 mg colchicine tablet on Day 1 and then completed a 21-day washout period. Beginning the evening of Day 22, subjects received 250 mg of clarithromycin twice daily for 14 doses (7 days). On Day 29, the subjects received a single 0.6 mg colchicine tablet co-administered with a single 250 mg clarithromycin tablet.</p> <p>On Days 1–5 (Period I) and Days 29–33 (Period II), blood samples were collected prior to dosing through 96 hours post-dose for determination of pharmacokinetic profiles of colchicine and its metabolites 2-demethylcolchicine (2DMC), 3-demethylcolchicine (3DMC), and 10-demethylcolchicine (10DMC). Clarithromycin plasma concentrations were not measured.</p> <p>Plasma samples were sent to the bioanalytical laboratory of PRACS Institute, Ltd. – Cetero Research, for determination of colchicine and metabolite plasma concentrations.</p> |                                                                                                                                 |
| <b>NUMBER OF SUBJECTS:</b> A total of 24 healthy adult subjects participated in this study. A                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                                                 |

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                                                                                                                    |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------|
| formal sample size calculation has not been performed.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                                                                                                    |
| <b>MAIN DIAGNOSIS FOR ENTRY:</b> All subjects were healthy, non-smoking, non-obese adult subjects who were not using concomitant medications or other products that might interfere with the pharmacokinetic interpretation. General health was confirmed by medical history, physical examination, clinical laboratory testing, and vital sign and ECG measurements.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                                                                                                                    |
| <b>INVESTIGATIONAL PRODUCTS:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Colchicine Tablets USP, 0.6 mg;<br>Lot #: BB 374 0215; Exp. N/A;<br>Manufacturer: Mutual Pharmaceutical Company, Inc                               |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | Clarithromycin Tablets, USP 250 mg<br>Lot 48597AA21; Exp. 1MAR2009;<br>Mfd. by: Abbott Pharmaceuticals PR Ltd.;<br>For: DAVA Pharmaceuticals, Inc. |
| <b>ROUTE OF ADMINISTRATION:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | Oral                                                                                                                                               |
| <b>DURATION OF TREATMENT:</b> The study subjects received a single 0.6-mg colchicine tablet on two separate occasions (administered alone and co-administered with steady-state clarithromycin). The two doses of colchicine were separated by a 28-day washout period, during the last 7 days of which a 250-mg b.i.d. regimen of clarithromycin was administered. Total study participation, exclusive of screening, was 33 days (Period I: 03 November – 07 November 2007; Period II: 24 November 2007 – 05 December 2007).                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                                                    |
| <b>PRIMARY EFFICACY VARIABLE:</b> Not applicable.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                                                                                                                    |
| <b>SECONDARY EFFICACY VARIABLE:</b> Not applicable.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                    |
| <b>CRITERIA FOR EVALUATION:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                                                                                                                    |
| <p><b>Pharmacokinetics:</b> Plasma concentration data from the blood samples collected during the study conduct were used to calculate values for the following pharmacokinetic parameters: <math>C_{max}</math>, <math>T_{max}</math>, <math>AUC_{0-t}</math>, <math>AUC_{0-\infty}</math>, <math>k_{el}</math>, <math>V_{area}/F</math>, <math>CL/F</math>, and <math>t_{1/2}</math>. Samples were also separately calculated for men and women.</p> <p>Analyses of variance (ANOVA) were performed on the ln-transformed <math>AUC_{0-t}</math>, <math>AUC_{inf}</math>, and <math>C_{max}</math> for colchicine with clarithromycin (Test) versus colchicine alone (Reference). <math>T_{max}</math> was analyzed without transformation. The ANOVA model included treatment as a fixed effect, and subject as a random effect. Each ANOVA included calculation of least-squares means (LSM), the difference between treatment LSM, and the standard error associated with this difference. The above statistical analyses were done using the appropriate SAS<sup>®</sup> procedure.</p> <p><b>Safety:</b> All subjects were monitored throughout the confinement and ambulatory portions of the study. Sitting (5 minutes) blood pressure and pulse were measured prior to dosing (time 0) and 1 hour post-dose; 12-lead ECGs were obtained in triplicate prior to dosing (time 0) and 1 hour</p> |                                                                                                                                                    |

after each colchicine dose. Subjects were also queried for adverse events at screening, check-in, during the confinement portion of the study, during the ambulatory portion of the study, and at study exit (or early termination). All subjects underwent clinical laboratory testing including hematology, biochemistry, and for post-menopausal women at screening only, follicle stimulating hormone; and, for females, pregnancy tests. Clinical laboratory testing (hematology and serum chemistry) was repeated prior to check-in for each period and prior to discharge from the study unit during the second period (48 hours after the last dose of study drug; repeat colchicine administered with a steady state clarithromycin regimen); testing was to be repeated at study exit only if deemed necessary in response to adverse events or changes in medical history. Additionally, vital signs were taken and physical examinations were performed at screening and at study exit (or early termination) if deemed necessary.

#### SUMMARY OF RESULTS:

**Demographic Summary:** The mean age of the subjects was approximately 23 years (Mean = 22.8 yrs  $\pm$  5.3 yrs). Ages ranged from 18 to 38 years. Sex was not evenly distributed (N = 13 females, N = 11 males). All subjects were white (100%).

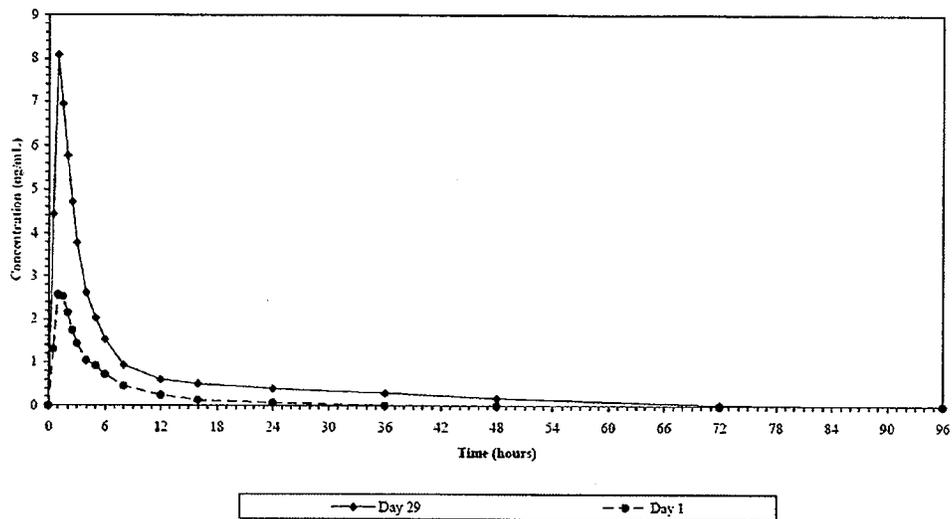
**Pharmacokinetic Summary:** Three of 24 subjects each had one measurable 2DMC concentration. The 2DMC concentrations ranged from \_\_\_\_\_ (near the LOQ) and were observed at the 0.5-, 5-, and 96-hour time-points. This is the only colchicine study in which 2DMC concentrations were observed. Nine of 24 subjects had at least one measurable 3DMC concentration (16 total 3DMC measurable concentrations). The 3DMC concentrations ranged from \_\_\_\_\_ (near the LOQ) to \_\_\_\_\_ ( $2.8 \times$  LOQ) and were observed 1 to 5 hours post-dose.

b(4)

Data for 23 of 24 subjects were used in the statistical and pharmacokinetic analysis for colchicine. When colchicine (0.6 mg single-dose) is co-administered with steady-state clarithromycin, colchicine concentrations increase to a maximum 8.4 ng/mL, which is approximately 200% higher than the maximum colchicine concentration when colchicine is administered alone as a single 0.6 mg dose ( $C_{max}$  = \_\_\_\_\_).  $T_{max}$  was not affected by the co-administration with clarithromycin (1.0 hours with and without clarithromycin co-administration). Colchicine  $AUC_{0-t}$  concentrations are increased approximately 240% (41.9 ng·h/mL) when co-administered with clarithromycin as compared to the reported colchicine  $AUC_{0-t}$  concentrations when administered alone (12.4 ng·h/mL). Total apparent oral clearance was decreased by 75% when colchicine was co-administered with clarithromycin as compared to the reported total apparent oral clearance when colchicine was administered alone (12 L/hr versus 46.8 L/hr). The terminal elimination half-life was 30 hours when colchicine was administered with clarithromycin; half-life was not accurately measured following administration of colchicine alone as plasma concentrations were not quantifiable for sufficiently long to estimate the terminal elimination slope.

b(4)

The following figure illustrates the mean colchicine plasma concentration of single doses of colchicine before and after a 7-day regimen of clarithromycin.



The following table summarizes colchicine descriptive statistics (mean (%CV)) for all subjects.

| Parameter (units)              | Arithmetic Mean (%CV)<br>Median (Range) for $T_{max}$ |                            |
|--------------------------------|-------------------------------------------------------|----------------------------|
|                                | Day 29<br>(N=23)                                      | Day 1<br>(N=23)            |
| $AUC_{0-t}$ (ng·hr/mL)         | 41.95 (23.31)                                         | 12.37 (37.64)              |
| $AUC_{0-inf}$ (ng·hr/mL)       | 52.62 (22.84)                                         | 15.53 (49.6)               |
| $C_{max}$ (ng/mL)              | 8.44 (17.63)                                          | 2.84 (30.97)               |
| $T_{max}$ (hr)                 | 1.00 (0.50-2.00)                                      | 1.50 (0.50-2.00)           |
| $K_d$ (1/hr)                   | 0.0296 (87.99)                                        | 0.1324 (46.87)             |
| $t_{1/2}$ (hr)                 | 30.31 (41.31)                                         | 8.89 (126.38) <sup>1</sup> |
| CL/F (L/hr)                    | 12.0 (23.75)                                          | 46.8 (43.68)               |
| Weight-Adjusted CL/F (L/hr/kg) | 0.17 (24.07)                                          | 0.66 (42.86)               |
| $V_d$ (L)                      | 493.43 (33.59)                                        | 431.89 (56.11)             |

There was no difference in pharmacokinetics between men and women: women had a slightly higher exposure to colchicine, attributed to differences in body weight as there was no difference in weight-adjusted clearance. The differences in volume of distribution are consistent with this interpretation. The magnitude of the drug interaction was similar between men and women. The ratios of Day 29 to Day 1 for  $C_{max}$ ,  $AUC_{0-t}$ , and  $AUC_{0-inf}$  for males were 2.81, 3.51, and 3.85, respectively, and for females were 3.36, 3.63, and 3.49, respectively.

**Safety Summary:** No serious adverse events (SAEs) were reported over the course of the study.

Fourteen of 24 subjects experienced a total of 37 AEs over the course of the study; all were mild or moderate in intensity. Headache and gastrointestinal disorders were the most common treatment-emergent adverse events following administration of colchicine, occurring in 5 subjects (20.8%) and 4 subjects (16.6%), respectively. With respect to GI events, there were 4 subjects (16.7%) with nausea and 2 subjects (8.3%) with diarrhea, the latter occurring only when subjects received colchicine in combination with clarithromycin. Other than diarrhea, there was no clear pattern of increased incidence between the two treatment periods. Of the remaining events that occurred in 1 or 2 subjects each, chest pain, pain in the extremity, and blurred vision were deemed possibly treatment-related adverse events. There were no discontinuations due to adverse events.

The clinical laboratory values were considered unremarkable and none of the values outside of the reference range at study exit were considered directly attributable to the product. No consistent or clinically relevant abnormalities or changes versus baseline in ECG intervals or morphology were observed in any subject. QTcF values remained within normal limits in all subjects and no relevant changes in QTcF versus baseline were observed. Colchicine is without effect on blood pressure or pulse.

Overall, colchicine was tolerated as a single oral dose of 0.6 mg (1 × 0.6 mg) administered under fasting conditions, either alone or with clarithromycin at steady state.

#### **SUMMARY CONCLUSIONS:**

**Pharmacokinetic:** When a single 0.6 mg dose of colchicine is co-administered with steady-state clarithromycin (250 mg b.i.d. × 7 days) to healthy volunteers under fasted conditions, colchicine plasma concentrations are increased significantly as compared to a single 0.6-mg colchicine dose administered alone. Mean  $C_{max}$  and AUC concentrations are increased by approximately 200% and 240%, respectively, and the apparent total oral clearance is decreased by 75% during combined administration. These results indicate a significant drug interaction is present when colchicine and clarithromycin are given concomitantly, and depending on indication, concomitant use should be contraindicated (when there are alternate therapies such as in the management of gout) or colchicine dose reductions are warranted when both drugs are co-administered (when there are no alternate therapies for serious indications such as FMF).

**Safety:** Overall, colchicine was tolerated as a single oral dose of 0.6 mg (1 × 0.6 mg) administered under fasting conditions, either alone or with clarithromycin at steady state. The most common adverse events are headache and gastrointestinal events, with diarrhea occurring only in subjects receiving the combination regimen, consistent with the higher exposure.

#### 4.2.8 OCP NDA Filing Memo

| Office of Clinical Pharmacology<br><i>New Drug Application Filing and Review Form</i> |                            |                             |                                           |                          |
|---------------------------------------------------------------------------------------|----------------------------|-----------------------------|-------------------------------------------|--------------------------|
| General Information About the Submission                                              |                            |                             |                                           |                          |
|                                                                                       | Information                |                             | Information                               |                          |
| NDA Number                                                                            | 22-352                     | Brand Name                  |                                           |                          |
| OCP Division (I, II, III, IV, V)                                                      | II                         | Generic Name                | Colchicine Oral Tablets                   |                          |
| Medical Division                                                                      | DAARP                      | Drug Class                  | Anti-Inflammatory Agent                   |                          |
| OCP Reviewer                                                                          | Srikanth C. Nallani, Ph.D. | Indication(s)               | Treatment of Familial Mediterranean Fever |                          |
| OCP Team Leader                                                                       | Suresh Doddapaneni, Ph.D.  | Dosage Form                 | Tablet                                    |                          |
|                                                                                       |                            | Dosing Regimen              | 0.6 mg – 2.4 mg                           |                          |
| Date of Submission                                                                    | 6/20/2008                  | Route of Administration     | Oral                                      |                          |
| Estimated Due Date of OCP Review                                                      |                            | Sponsor                     | Mutual Pharmaceutical Company, Inc.       |                          |
| PDUFA Due Date                                                                        |                            | Priority Classification     |                                           |                          |
| Division Due Date                                                                     |                            |                             |                                           |                          |
| Clin. Pharm. and Biopharm. Information                                                |                            |                             |                                           |                          |
|                                                                                       | "X" if Included at filing  | Number of studies submitted | Number of studies reviewed                | Critical Comments If any |
| <b>STUDY TYPE</b>                                                                     |                            |                             |                                           |                          |
| Table of Contents present and sufficient to locate reports, tables, data, etc.        | X                          |                             |                                           |                          |
| Tabular Listing of All Human Studies                                                  | X                          |                             |                                           |                          |
| HPK Summary                                                                           |                            |                             |                                           |                          |
| Labeling                                                                              |                            |                             |                                           |                          |
| Reference Bioanalytical and Analytical Methods                                        |                            | 1                           | 1                                         |                          |
| <b>I. Clinical Pharmacology</b>                                                       |                            |                             |                                           |                          |
| Mass balance:                                                                         |                            |                             |                                           |                          |
| Isozyme characterization:                                                             | X                          |                             |                                           |                          |
| Blood/plasma ratio:                                                                   |                            |                             |                                           |                          |
| Plasma protein binding:                                                               |                            |                             |                                           |                          |
| <b>Pharmacokinetics (e.g., Phase I) -</b>                                             |                            |                             |                                           |                          |
| <i>Healthy Volunteers-</i>                                                            |                            |                             |                                           |                          |
| single dose:                                                                          | X                          | 3                           | 3                                         |                          |
| multiple dose:                                                                        | X                          | 2                           | 2                                         |                          |
| <b>Patients-</b>                                                                      |                            |                             |                                           |                          |
| single dose:                                                                          |                            |                             |                                           |                          |
| multiple dose:                                                                        |                            |                             |                                           |                          |
| <b>Dose proportionality -</b>                                                         |                            |                             |                                           |                          |
| fasting / non-fasting single dose:                                                    |                            |                             |                                           |                          |
| fasting / non-fasting multiple dose:                                                  |                            |                             |                                           |                          |
| <b>Drug-drug interaction studies -</b>                                                |                            |                             |                                           |                          |
| In-vivo effects on primary drug:                                                      | X                          | 1                           | 1                                         | Clarithromycin DDI study |
| In-vivo effects of primary drug:                                                      | X                          | 1                           | 1                                         | Oral contraceptive study |
| In-vitro:                                                                             | X                          | 2                           | 2                                         | CYP induction studies    |
| <b>Subpopulation studies -</b>                                                        |                            |                             |                                           |                          |
| ethnicity:                                                                            |                            |                             |                                           |                          |
| gender:                                                                               |                            |                             |                                           |                          |
| pediatrics:                                                                           |                            |                             |                                           |                          |
| geriatrics:                                                                           |                            |                             |                                           |                          |
| renal impairment:                                                                     | X                          | 2                           | 2                                         | Publications             |
| hepatic impairment:                                                                   | X                          | 2                           | 2                                         | Publications             |
| PD:                                                                                   |                            |                             |                                           |                          |
| Phase 2:                                                                              |                            |                             |                                           |                          |
| Phase 3:                                                                              |                            |                             |                                           |                          |
| PK/PD:                                                                                |                            |                             |                                           |                          |
| Phase 1 and/or 2, proof of concept:                                                   |                            |                             |                                           |                          |

|                                                         |                   |                                                                                                                                                               |          |  |
|---------------------------------------------------------|-------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------|----------|--|
| Phase 3 clinical trial:                                 |                   |                                                                                                                                                               |          |  |
| <b>Population Analyses -</b>                            |                   |                                                                                                                                                               |          |  |
| Data rich:                                              |                   |                                                                                                                                                               |          |  |
| Data sparse:                                            |                   |                                                                                                                                                               |          |  |
| <b>2II. Biopharmaceutics</b>                            |                   |                                                                                                                                                               |          |  |
| <b>Absolute bioavailability:</b>                        |                   |                                                                                                                                                               |          |  |
| <b>Relative bioavailability -</b>                       |                   |                                                                                                                                                               |          |  |
| solution as reference:                                  |                   |                                                                                                                                                               |          |  |
| alternate formulation as reference:                     |                   |                                                                                                                                                               |          |  |
| <b>Bioequivalence studies -</b>                         |                   |                                                                                                                                                               |          |  |
| traditional design; single / multi dose:                |                   |                                                                                                                                                               |          |  |
| replicate design; single / multi dose:                  |                   |                                                                                                                                                               |          |  |
| <b>Food-drug interaction studies:</b>                   |                   |                                                                                                                                                               |          |  |
| Dissolution:                                            |                   |                                                                                                                                                               |          |  |
| (IVIVC):                                                |                   |                                                                                                                                                               |          |  |
| Bio-wavier request based on BCS                         |                   |                                                                                                                                                               |          |  |
| BCS class                                               |                   |                                                                                                                                                               |          |  |
| <b>III. Other Clin Pharm Studies</b>                    |                   |                                                                                                                                                               |          |  |
| <b>Genotype/phenotype studies:</b>                      |                   |                                                                                                                                                               |          |  |
| Chronopharmacokinetics                                  |                   |                                                                                                                                                               |          |  |
| Pediatric development plan                              |                   |                                                                                                                                                               |          |  |
| Literature References                                   |                   |                                                                                                                                                               |          |  |
| <b>Total Number of Studies</b>                          |                   | <b>13</b>                                                                                                                                                     | <b>9</b> |  |
| Filability and QBR comments                             |                   |                                                                                                                                                               |          |  |
|                                                         | <b>"X" if yes</b> | <b>Comments</b>                                                                                                                                               |          |  |
| Application filable ?                                   |                   | Reasons if the application <u>is not</u> filable (or an attachment if applicable)<br>For example, is clinical formulation the same as the to-be-marketed one? |          |  |
| Comments sent to firm ?                                 |                   | Comments have been sent to firm (or attachment included). FDA letter date if applicable.                                                                      |          |  |
| <b>QBR questions (key issues to be considered)</b>      |                   |                                                                                                                                                               |          |  |
| <b>Other comments or information not included above</b> |                   |                                                                                                                                                               |          |  |
| <b>Primary reviewer Signature and Date</b>              |                   |                                                                                                                                                               |          |  |
| <b>Secondary reviewer Signature and Date</b>            |                   |                                                                                                                                                               |          |  |

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/s/

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Srikanth Nallani  
11/26/2008 11:48:42 AM  
BIOPHARMACEUTICS

Suresh Doddapaneni  
11/26/2008 12:12:57 PM  
BIOPHARMACEUTICS