

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**022581Orig1s000**

**STATISTICAL REVIEW(S)**

## STATISTICAL REVIEW

NDA: 22-581	Submission Date(s): 07/20/2009
Brand Name	Phoslyra (Calcium Acetate)
Generic Name	Calcium Acetate
Primary Statistical Reviewer	Donald J. Schuirmann, M.S.
Statistics Division Director	Stella G. Machado, Ph.D.
OCP Division	Clinical Pharmacology -2
OB Division	Division of Biometrics VI
OND division	OND/ODEI/Division of Cardiovascular and Renal Products
Sponsor	Fresenius Medical Care North America
Formulation; Strength(s)	Oral Solution, 667 mg/5 mL
Proposed Indication	Control of Hyperphosphatemia in patients with End Stage Renal Disease

Input for this review was provided by the CDER review team, in particular Rajanikanth Madabushi, Ph.D. of the CDER Office of Clinical Pharmacology and Meiyu Shen, Ph.D. of the CDER Office of Biostatistics.

### 1 Executive Summary

The sponsor carried out an open label crossover bioequivalence (BE) study in healthy volunteers, comparing their proposed Oral Solution formulation of calcium acetate (Liquid) with an approved Gelcap calcium acetate formulation (Gelcaps) and a Calcium Citrate caplet formulation as a positive control (Citrate.)

Serum calcium and urinary calcium levels (as measured by AUC 0-6 hrs in serum and amount excreted 0-6 hrs in urine) were statistically significantly higher under treatment with Citrate than at baseline, establishing validity of the study. The Liquid formulation appeared to have lower serum and urine calcium levels than the Gelcap formulation, and based on the confidence interval the Liquid formulation is at worst comparable to the Gelcap formulation with respect to serum and urine calcium.

The study was essentially inconclusive as regards serum and urine phosphorus levels, though both Liquid and Gelcaps had statistically significantly lower serum phosphorus levels after treatment compared to Citrate.

#### 1.1 Recommendation

The Office of Biostatistics has reviewed the bioequivalence study (protocol number LP-RTG-01-01) submitted in support of NDA 22-581 for PHOSLYRA Oral Solution and finds it acceptable. Based on the review we recommend **approval**.

## 1.2 Summary of Important Findings

Fresenius Medical Care North America is seeking approval of a new formulation (oral solution) of Calcium Acetate in the current New Drug Application (NDA) for the control of hyperphosphatemia in patients with End Stage Renal Disease. The proposed initial dose is 10 mL with each meal.

The key findings from the sponsor's BE study are:

- In the sponsor's crossover BE study, the Calcium Citrate treatment (positive control) demonstrated statistically significantly higher levels of serum calcium (based on AUC 0-6hr) after treatment compared to baseline (p 0.0343), establishing the validity of the study.
- Although designed as a BE study, the study actually appears to show that the levels of serum calcium (based on AUC 0-6hr corrected by subtraction for baseline levels) under the proposed Liquid formulation may be lower than for the approved Gelcap formulation, with an estimated mean ratio of 0.460. The means of serum calcium change from baseline for the Liquid and Gelcap formulations were not statistically significantly different, and based on the confidence interval, it appears that elevation of serum calcium levels is at worst comparable between the Liquid and Gelcap formulation (upper limit of the 90% confidence interval for the mean ratio 1.009.)

## **Description of the Study**

NDA 22-581 - Phoslyra (Calcium Acetate) Oral Solution, 667 mg/5 mL

NAME OF COMPANY: Fresenius USA Manufacturing, Inc., d/b/a  
Fresenius Medical Care North America  
920 Winter Street  
Waltham, MA 02451, USA

NAME OF FINISHED PRODUCT: Calcium Acetate Oral Solution

NAME OF ACTIVE INGREDIENT: calcium acetate

INDICATION: Control of hyperphosphatemia in patients with End Stage Renal Disease.

Protocol Number: LP-RTG-01-01

Title: "Randomized, Controlled, 3-arm, Open Label, Cross-over Bioequivalence Study Comparing Liquid PhosLo Oral Solution vs. PhosLo Gelcaps using Calcium Citrate as a Positive Control in Healthy Volunteers"

Investigator: Antoinette Pragalos, MD

Study Centers: Community Research, Cincinnati, Ohio

Primary Objective as stated by the sponsor: "To demonstrate the bioequivalence of liquid PhosLo to PhosLo Gelcaps with respect to urinary calcium excretion and serum phosphorus levels in healthy subjects."

Secondary Objectives as stated by the sponsor: "To compare change in urinary phosphorus and serum calcium before and after dosing with liquid PhosLo vs. PhosLo Gelcaps in healthy subjects. To compare liquid PhosLo to PhosLo Gelcaps with respect to serum glucose and insulin levels in healthy subjects."

Methodology: This study was designed as a randomized, controlled, 3-arm, open label crossover Phase I study evaluating the bioequivalence of liquid PhosLo (investigational drug) to PhosLo Gelcaps.

## **Inclusion Criteria**

Subjects of both sexes and of any ethnic origin were enrolled in this study if they met all of the following inclusion criteria during Screening:

- Signed and dated ICF
- Aged 18 to 75 years
- Serum calcium level 8.6 to 10.2 mg/dL

- 25-vitamin D level 20 to 100 ng/mL
- 1, 25-dihydroxy vitamin D level 6 to 62 pg/mL
- Fasting glucose level of 65 to 99 mg/dL (min 8 hr fast)
- Intact parathyroid hormone (iPTH) level of 10 to 65 pg/mL
- Serum phosphorus level of 2.5 to 4.5 mg/dL
- Albumin level of 3.6 to 5.1 g/dL
- Sodium level of 135 to 146 mEq/L
- Potassium level of 3.5 to 5.3 mEq/L
- Negative pregnancy test (at Screening and prior to dosing) for women of childbearing potential and subjects agreed to use adequate contraception (hormonal or double barrier method) during the study
- No clinically significant abnormalities on electrocardiogram (ECG) reading as determined by the Investigator
- No clinically significant abnormalities on liver function tests
- No clinically significant abnormalities on complete blood count (CBC) and coagulation studies
- No clinically significant abnormalities on kidney function (estimated glomerular filtration rate (eGFR) using serum creatinine)
- Body Mass Index (BMI) between 18.5 to 30.0 kg/m<sup>2</sup>
- Subjects agreed not to consume alcohol while in the treatment phase of the study

#### **Exclusion Criteria**

Subjects were NOT to be enrolled in this study if they met any of the following criteria during screening:

- Women who were pregnant or breast feeding
- Malignancy except squamous cell carcinoma of the skin
- Documented current acute or chronic disease
- Positive for human immunodeficiency virus (HIV), hepatitis B or hepatitis C
- Myocardial infarction within 6 months of study Day 0
- Parathyroidectomy within 6 months of study Day 0
- GI disorder associated with impaired absorption of oral medications
- Inability to swallow tablets or tolerate liquid PhosLo
- Hormonal therapy (except for contraceptives), immunotherapy or corticoid therapy
- Concurrent antibiotic treatment
- Any concurrent investigational treatment within 30 days of Screening
- Unable or unwilling to comply fully with the protocol
- Diuretic therapy such as thiazides, furosemide (International nonproprietary name (INN)) or frusemide (former British approved name (BAN)) within 1 month before screening
- Subjects taking over-the-counter (OTC) or prescribed phosphorus or calcium containing supplements
- Subjects that tested positive for drugs of abuse

**Treatments**

- Liquid PhosLo (Test product) 30 mL with breakfast, lunch, and dinner (5 mL 667 mg calcium acetate or 169 mg elemental calcium)
- PhosLo Gelcaps (Reference product) 6 gelcaps with breakfast, lunch, and dinner (1 caplet 667 mg calcium acetate or 169 mg elemental calcium)
- Calcium Citrate (positive control) 5 caplets with breakfast, lunch, and dinner (1 caplet 950 mg calcium acetate or 200 mg elemental calcium)

Lot Numbers and Use by/Expiration Dates are given in the study report:

**Investigational Product**

Product	Lot Number	Use by/Expiration Date
Liquid PhosLo Oral Solution	XA188B	Use by: 12/2008
PhosLo Gelcaps	1308060	Expiration Date: 4/30/2011
Calcium Citrate 950 mg Caplets	8741450904	Expiration Date: 10/2010

**experimental design**

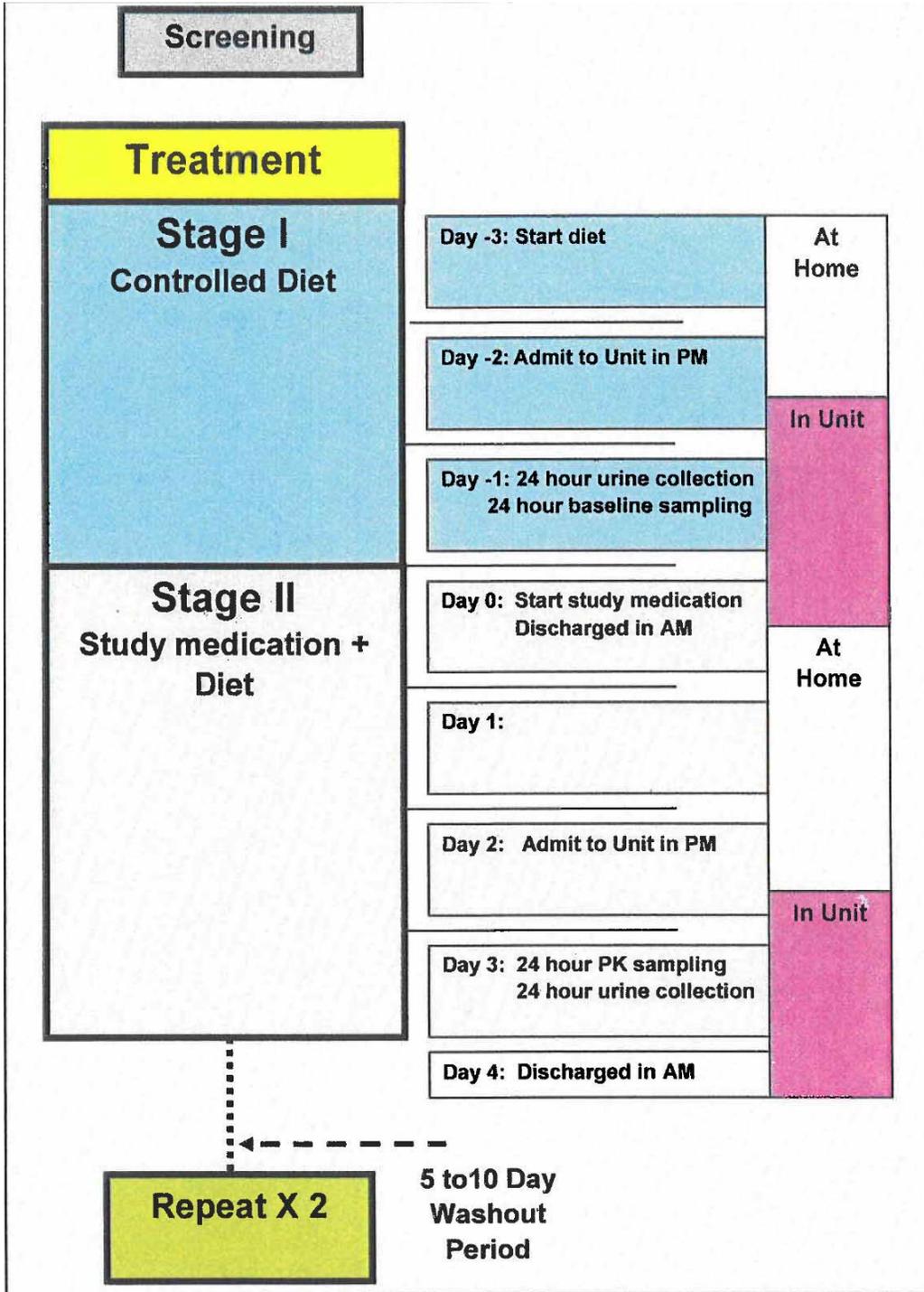
	periods		
	1	2	3
sequence I	A	B	C
sequence II	B	C	A
sequence III	C	A	B

- A Liquid PhosLo
- B PhosLo Gelcaps
- C Calcium Citrate

sequence I 15 subjects randomized, 12 completed  
 sequence II 15 subjects randomized, 12 completed  
 sequence III 16 subjects randomized, 12 completed

- 40 subjects planned, 46 analyzed
- subject participation 60-70 days
- study initiation date 29 July 2008
- study completion date 09 November 2008

**Diagram of a study period (from the sponsor's study report):**



**Analysis data sets**

- Full Analysis data set (ITT) all subjects who met eligibility requirements and were randomized into the study
- PK Analysis data set subset of full analysis data set who completed all PK assessments and the 3 dosing periods (Subjects who experienced emesis during the course of their bioequivalence assessment were excluded from the PK data set.)

Subjects excluded from the PK analysis dataset (10 in all):

subject ID	sequence
(b) (6)-026	3
-053	1
023	1
095	3
006	2
135	2
(b) (6)-056	2
-088	1
-002	3
030	3

**blood sampling times:**

PK serum calcium and phosphorus samples collected at 0.00 (prior to meal and at fasting state), 0.50, 1.00, 1.50, 2.00, 3.00, 4.00, 5.00, 6.00, 7.00, 8.00, 10.00, 12.00, 18.00, & 24.00 hrs relative to morning meal and final dose.

**urinary collection intervals:**

24 hr urine samples were collected in fractions every 2 hr, up to 8 hr, then from 8-12 hr, and then from 12-24 hr. If subjects could not void every 2 hr, urine was collected as close to the defined time interval as possible.

**Datasets submitted by the sponsor**

This review utilized the following SAS datasets submitted by the sponsor (Location: [\\FDSWA150\NONECTD\N22581\N\\_000\2009-07-20](#))

- s\_ca\_p individual serum calcium and phosphorus concentrations
- s\_6\_pk pharmacokinetic parameters, notably area under the curve, for serum calcium and serum phosphorus over 0 to 6 hours
- u\_ca\_p volumes and amounts of calcium and phosphorus for each urinary collection interval
- u\_6\_pk pharmacokinetic parameters, notably total amount excreted, for urinary calcium and urinary phosphorus over 0 to 6 hours.

**Endpoints Considered in this Review**

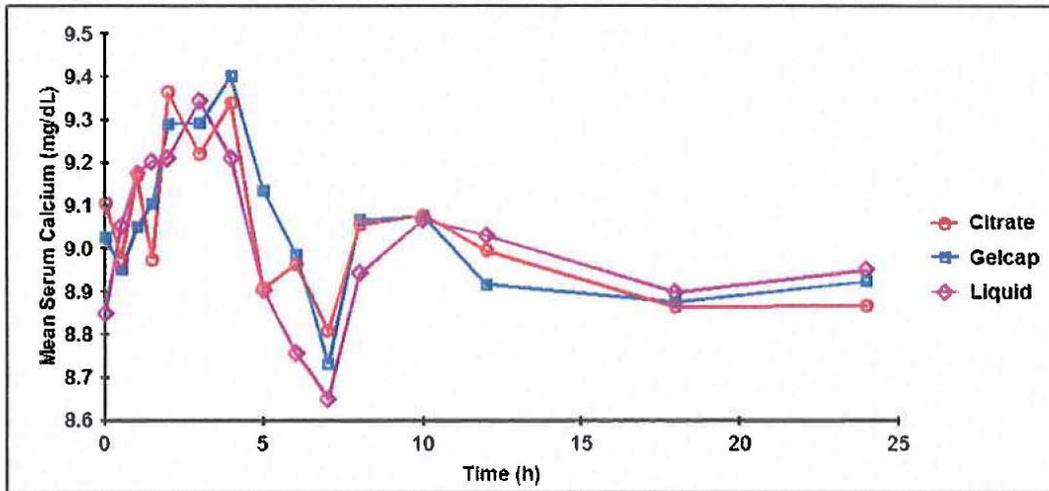
Based on discussions between the CDER review team and the sponsor during the IND phase, the endpoints considered in this review are

- Serum Calcium AUC 0-6hr
- Urinary Calcium Ae 0-6hr
- Serum Phosphorus AUC 0-6hr
- Urinary Phosphorus Ae 0-6hr

Hereafter in this review, AUC 0-6hr (area under the serum concentration (serum calcium or phosphorus)-time curve from 0 to 6 hours) will be called “AUC06” and Ae 0-6hr (amount of urinary calcium or phosphorus excreted from 0 to 6 hours) will be called “Ae06”.

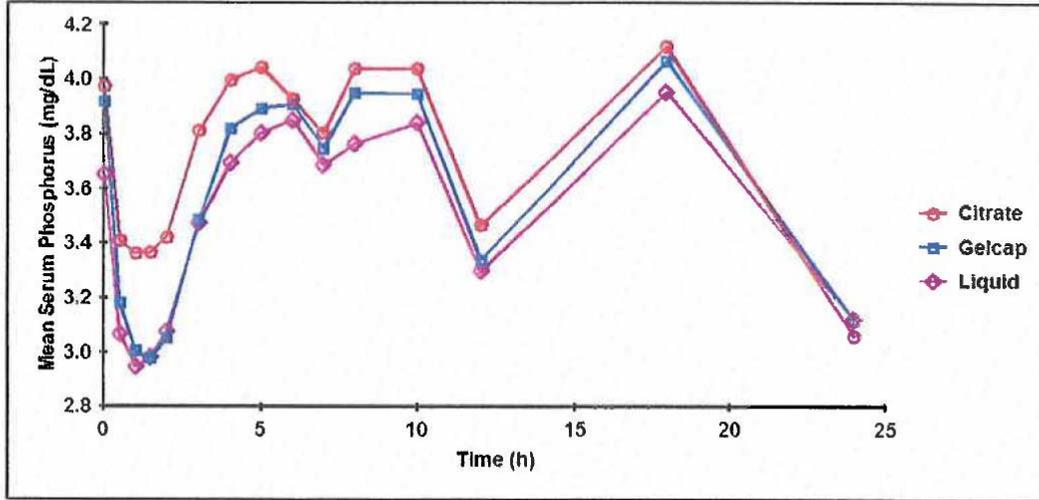
The review team noted that serum levels of calcium and phosphorus appeared to have returned to starting (i.e. time 0 hr) levels by about 6 hours, as illustrated by these figures from the sponsor’s study report:

**Figure 4 - Linear Plot of Mean Serum Calcium on Treatment Day (Day 3)**



Source: Listing 16.4, Appendix 9

**Figure 2 - Linear Plot of Mean Serum Phosphorus on Treatment Day (Day 3)**



Source: Listing 16.4, Appendix 9

For assessing the relative performance of the treatments in the study, particularly Liquid vs. Gelcaps, the CDER review team wished to look at AUC06 values adjusted for baseline by subtraction. That is

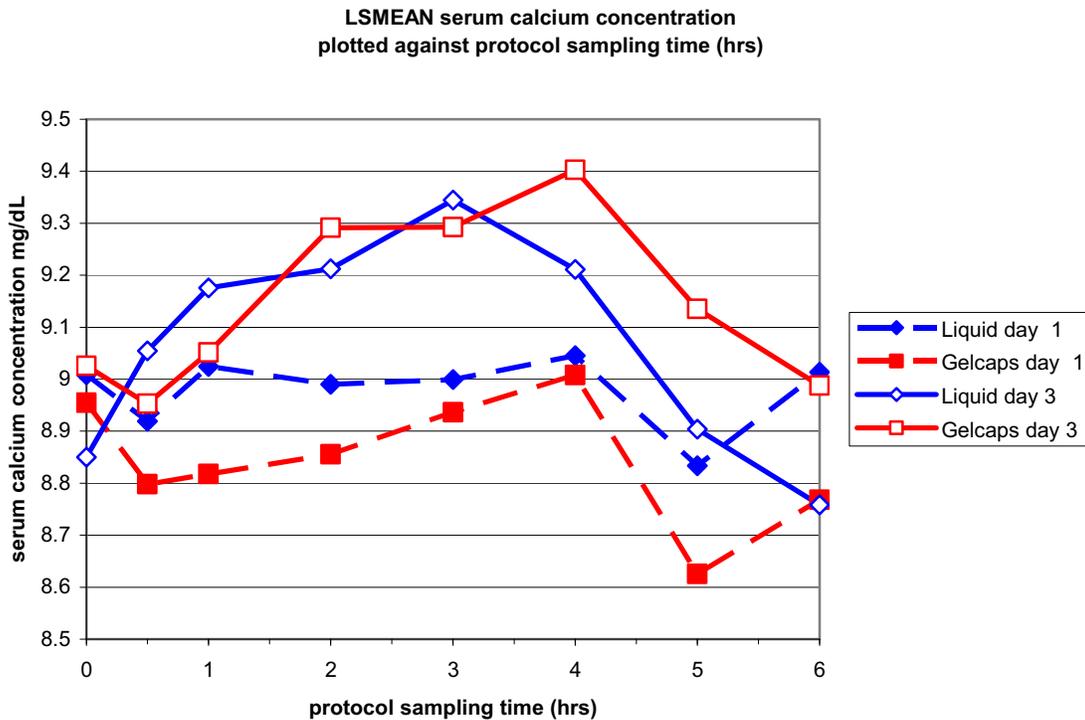
$$\text{adjusted AUC06} = \text{AUC06}(\text{study day 3}) - \text{AUC06}(\text{study day -1})$$

This endpoint represents the extent, over the first 6 hours, of change in serum levels under treatment (study day 3) relative to serum levels at baseline (study day -1).

As previously noted, the protocol called for serum samples (for Ca and P) to be taken at 0.00 (prior to meal and at fasting state), 0.50, 1.00, 1.50, 2.00, 3.00, 4.00, 5.00, 6.00, 7.00, 8.00, 10.00, 12.00, 18.00, & 24.00 hrs relative to morning meal and final dose. These are the *protocol time postdose* (hereafter called “protocol time”) values. In the datasets, the sponsor also makes note of the *actual time postdose* (hereafter called “actual time”.)

The sponsor presents their calculated AUC06 values in the s\_6\_pk SAS dataset. When the adjusted AUC06 values (AUC06(under treatment) - AUC06(baseline)) for serum calcium are calculated using the sponsor’s AUC06s, they are almost all negative, with a negative mean.

Looking at the least square mean (lsmean) serum calcium concentrations, as obtained from the sponsor’s s\_ca\_p SAS dataset, for the baseline samples (study day -1) and under treatment samples (study day 3) for the Liquid treatment and the Gelcap treatment, plotted against the protocol time, we have



There is some crossing of the day -1 curve and the day 3 curve for the Liquid treatment (between 0 and 0.5 hrs and between 5 and 6 hrs, blue lines.) However, for both the Liquid and the Gelcap treatments the overall trend is for the under treatment lsmeans to be higher than the baseline lsmeans. It therefore seems unlikely that the great majority of the individual adjusted AUC06s would be negative, with a negative mean. This led us to question the sponsor about how exactly they calculated their AUC06 values. Based on the sponsor's response, it appears that for the initial (0-0.5 hr) trapezoidal area, the sponsor *replaced* the actual time corresponding to protocol time 0 hr with 0. For the final (5-6 hr) trapezoidal area, the sponsor calculated the area from actual time corresponding to protocol time 5 hr to actual time corresponding to protocol time 6 hr. In-between trapezoidal areas used actual sampling times.

Using this method, we have been able to reproduce the sponsor's AUC06s, as presented in the sponsor's s\_6\_pk SAS dataset and also in Appendix 9, Listings 16.4.6.1 (pp. 425-430, module 5, volume 3) and 16.4.6.5 (pp. 458-463, module 5, volume 3) of the study report.

In most cases, the actual sampling time corresponding to protocol time 6 hr was later in the baseline period (study day -1) than in the treatment period (study day 3), as illustrated with these results using SAS PROC MEANS:

SAS PROC MEANS - Analysis Variable : t6 (actual sampling time postdose corresponding to protocol sampling time 6 hr.)

The MEANS Procedure all data

treat- ment	study day	N	Mean	Std Dev	Minimum	Maximum
A	-1	37	6.43514	0.08765	6.2500	6.65
A	3	37	6.01261	0.06672	5.9000	6.3667
B	-1	38	6.41184	0.09119	6.2167	6.5833
B	3	37	6.01396	0.04366	5.9167	6.0833
C	-1	37	6.43198	0.09674	6.2000	6.6667
C	3	38	6.00176	0.03236	5.9167	6.0833

The MEANS Procedure PK analysis dataset (pk 'Yes')

treat- ment	study day	N	Mean	Std Dev	Minimum	Maximum
A	-1	35	6.43477	0.08906	6.2500	6.6500
A	3	36	6.01574	0.06485	5.9667	6.3667
B	-1	36	6.40371	0.08638	6.2167	6.5667
B	3	36	6.01620	0.04206	5.9167	6.0833
C	-1	36	6.43703	0.09302	6.2000	6.6667
C	3	36	6.00417	0.02991	5.9333	6.0833

- A Liquid
- B Gelcaps
- C Citrate

Thus, the sponsor's method tended to calculate areas over a longer time interval for baseline (study day -1) than for under treatment (study day 3). This, we are convinced, accounts for the preponderance of negative serum calcium adjusted AUC06 values on the part of the sponsor.

We also carried out our own calculation of AUC06, and found that with our calculations, while there were some individual negative adjusted serum calcium AUC06s, the mean adjusted serum calcium AUC06 was positive.

Our method of calculating AUC06 (programmed by Meiyu Shen, Ph.D. of CDER's Office of Biostatistics, Division of Biometrics VI, and independently verified by Donald J. Schuirmann of CDER's Office of Biostatistics, Division of Biometrics VI) was as follows: For the initial (0-0.5 hr) trapezoidal area, we *interpolated* the area from protocol time 0 (note that the actual times corresponding to protocol time 0 were negative in all cases) to actual time corresponding to 0.5 hr. For the final (5-6 hr) trapezoidal area,

we *interpolated* or *extrapolated* the area from actual time corresponding to 5 hr to protocol time 6 hr.

In-between trapezoidal areas used actual sampling times.

Note on specific AUC06s:

For subject (b) (6), receiving the Liquid treatment in period 1 of the study, the actual times were not available. We followed the sponsor in using the protocol times to calculate this AUC06.

For subject (b) (6), the serum phosphorus concentration at protocol time 6 hr. was missing for the Liquid treatment in period 2 of study day 3, and so the AUC06 was missing for this case.

### Urinary Endpoints

The sponsor's u\_ca\_p SAS dataset contained two variables, VOLUME\_M (labeled "Volume(mL)") and URINE\_CO (labeled "Urine Measurement".) Taking these values for the 0-2 hour, 2-4 hour, and 4-6 hour collections, the sponsor's Ae06 values, as presented in their u\_6\_pk SAS dataset, may be obtained for urinary calcium by taking

$$[(\text{VOLUME\_M} \times \text{URINE\_CO})_{0-2} + (\text{VOLUME\_M} \times \text{URINE\_CO})_{2-4} + (\text{VOLUME\_M} \times \text{URINE\_CO})_{4-6}]/1000$$

and for urinary phosphorus by taking

$$[(\text{VOLUME\_M} \times \text{URINE\_CO})_{0-2} + (\text{VOLUME\_M} \times \text{URINE\_CO})_{2-4} + (\text{VOLUME\_M} \times \text{URINE\_CO})_{4-6}]/100$$

The different divisors (1000 for calcium, 100 for phosphorus) were apparently due to different units for URINE\_CO (presumably mg/L for calcium and mg/dL for phosphorus.)

We used the sponsor's Ae06 values for our analyses.

### Subtraction adjustment vs. Ratio adjustment

As already noted, the CDER review team wishes to look at AUC06s with adjustment by *subtraction*

$$\text{adjusted AUC06} = \text{AUC06}(\text{under treatment}) - \text{AUC06}(\text{baseline}).$$

The sponsor, on the other hand, argues for adjustment by *ratio*

$$\text{adjusted AUC06} = \text{AUC06}(\text{under treatment})/\text{AUC06}(\text{baseline}).$$

With some (or in the case of the sponsor, almost all) negative values, standard analysis of subtraction-adjusted AUC06 after log transformation cannot be used. The sponsor argues that analysis after log transformation is the standard analysis for bioequivalence studies. It is true that use of the log transformation is standard for the analysis of pharmacokinetic bioequivalence studies where the active ingredient being studied does not occur naturally in the body. Log transformation analysis is also sometimes used for the case where the active ingredient does occur naturally in the body, but the blood levels under treatment are substantially higher than at baseline. But for a case like this, where the baseline levels of serum calcium or serum phosphorus are almost as high as the levels seen under treatment, use of the log transformation is by no means standard.

While the log transformation cannot be used for these data that are both positive and negative, inference about the ratio of means can still be made using *Fieller's Method*.

When we use subtraction adjustment, we are making inference about

$$\frac{\mu_T - \mu_{TB}}{\mu_R - \mu_{RB}}$$

With ratio adjustment, we are making inference, at least approximately, about

$$\frac{\mu_T / \mu_{TB}}{\mu_R / \mu_{RB}} = \frac{\mu_T}{\mu_R} \times \frac{\mu_{RB}}{\mu_{TB}}$$

where

$\mu_T$  mean AUC06 under treatment (study day 3) for Liquid

$\mu_{TB}$  mean AUC06 at baseline (study day -1) for Liquid

$\mu_R$  mean AUC06 under treatment for Gelcaps

$\mu_{RB}$  mean AUC06 at baseline for Gelcaps

If  $\mu_{TB} \approx \mu_{RB}$  (as we would expect), ratio adjustment essentially makes inference about  $\mu_T/\mu_R$ , the ratio of means for *unadjusted* AUC06s. To illustrate the difficulty with making inference about the ratio of means for unadjusted AUC06s, The least square mean (lsmean) serum calcium AUC06 for the Gelcap product on study day 3 (under treatment) was 55.087 hr\*mg/dL (analyzing just Gelcap data, PK analysis dataset.) The

least square mean (lsmean) serum calcium AUC06 for the Liquid product on study day -1 (at baseline) was 53.787 hr\*mg/dL (analyzing just Liquid data, PK analysis dataset.) Suppose a hypothetical product were given (instead of the Liquid treatment) that would not change the serum calcium levels *at all*, compared to baseline, so that the mean after treatment would be about the same as the baseline mean for Liquid. The ratio of mean unadjusted AUC06s for this hypothetical product over the Gelcap product would be around 53.787/55.087, or around 97.6%. This would be regarded as being indicative of equivalence! This argues for the use of subtraction adjustment for baseline, both for AUC06 and for Ae06.

## Statistical Methods

Although the sponsor does not provide SAS code for their statistical analyses, they do say that for the comparison of the Liquid and Gelcap treatments they used a linear mixed effects model with subjects(sequence) as a random effect. Such an analysis might be accomplished, for example, with these SAS statements:

```
proc mixed;
class seq subj per trt;
model y = seq per trt;
random subj(seq);
run;
```

where y is the endpoint being analyzed (e.g. serum calcium AUC06), and the variables seq, subj, per, and trt identify the sequence, subject, period, and treatment, respectively. There might also be additional program statements to provide estimates, standard errors, and confidence intervals, and possible other details.

For our own analyses comparing the Liquid and Gelcap treatments, we used

```
proc mixed data=nocpk; by analyte;
class subj seq per trt;
model adj auc =
seq per trt/ddfm=satterth;
repeated trt/type=un sub=subj r;
estimate '0.1353 ' intercept 0.8647 trt 1 -0.1353;
estimate '0.1354 ' intercept 0.8646 trt 1 -0.1354;
estimate '0.15 ' intercept 0.85 trt 1 -0.15;
estimate '0.45990 ' intercept 0.54010 trt 1 -0.45990;
estimate '0.59421 ' intercept 0.40579 trt 1 -0.59421;
estimate '1.0093 ' intercept -0.0093 trt 1 -1.0093;
estimate '1.0094 ' intercept -0.0094 trt 1 -1.0094;
estimate '1.0095 ' intercept -0.0095 trt 1 -1.0095;
estimate '1.681 ' intercept -0.681 trt 1 -1.681;
lsmeans trt;
title1 'without treatment C (Citrate)';
title2 'adjusted auc06 values';
title3 'PK analysis dataset';
title4 'using the repeated statement';
run;
```

The use of the REPEATED statement in SAS PROC MIXED allowed for the possibility that the variances associated with the two treatments might not be equal.

The several ESTIMATE statements were used to carry out *Fieller's Method* of obtaining a confidence interval for the ratio of means. The method looks for all values of  $\lambda$  for which we do not reject

$H_0: \mu_T - \lambda \mu_R = 0$   
in favor of  
 $H_1: \mu_T - \lambda \mu_R \neq 0$

testing at a level of significance equal to 1 minus the confidence level (i.e. for a 90% confidence interval we test at  $\alpha = 0.10$ .)

For comparing each treatment's mean AUC06 under treatment to the mean AUC06 at baseline, we used, for example

```
proc glm data=compck; by analyte;
class subj seq study da;
model auc06 =
seq subj(seq) study da;
lsmeans study da;
title1 'only treatment C (Citrate)';
title2 'PK analysis dataset';
run;
```

where the variable study\_da identifies the study day (-1 or 3.)

For comparing the mean AUC06 at baseline across the three treatments, we used

```
proc mixed data=bothpk; by analyte;
class subj seq per trt;
model bauc06 =
seq per trt/ddfm=satterth;
repeated trt/type=un sub=subj r;
lsmeans trt;
title1 'our baseline AUCs';
title2 'PK analysis set';
title3 'with repeated statement';
run;
```

For all of these analyses, we used only the data from treatments involved in the analysis. That is, for example, to compare Liquid to Gelcaps, we only used data from Liquid and Gelcaps, not from Citrate. To compare AUC06 under treatment to AUC06 at baseline for Citrate, we only used data from Citrate. To compare baseline AUC06 across all three treatments, and to compare Citrate adjusted AUC06s to Liquid and Gelcap adjusted AUC06s, data from all three treatments were used. This is a standard analysis strategy for analysis of bioequivalence studies.

**Sponsor’s Analyses**

The sponsor carried out a number of analyses using either ratio adjusted AUC06s and Ae06s or unadjusted AUC06s and Ae06s. Conclusions were similar for these two sets of analyses:

- For serum Calcium and Serum Phosphorus, Liquid was found to be equivalent to Gelcaps (the 90% confidence interval for the ratio of means fell within [80%, 125%.])
- For urinary Calcium and urinary Phosphorus, Liquid was not found to be equivalent to Gelcaps, but the upper limit of the 90% confidence interval fell below 125% and the point estimate fell below 100%.

As we have argued, we are actually interested in subtraction-adjusted AUC06s and Ae06s. The sponsor reports results for these endpoints, based on Fieller’s Method. Their results tables are

Sponsor’s Table 11

**Table 11 - Bioequivalence Assessment (Fieller’s Theorem) of Adjusted Serum Calcium (0 to 6 hr)**

Parameter	C <sub>max</sub> (mg/dL)		AUC <sub>0-6</sub> (hr*mg/dL)	
	Liquid PhosLo (A) (n=36)	PhosLo Gelcap (B) (n=36)	Liquid PhosLo (A) (n=36)	PhosLo Gelcap (B) (n=36)
Mean	0.27	0.34	0.88	1.99
Ratio (A)/(B) (in %)	80.78		44.46	
90% CI	(13.40, 238.76)		(13.65, 96.81)	

Source: Appendix 10, Table 14.4.4

Ratio (%) = (Mean test/ Mean Reference) x 100

Some subjects had no serum concentration at 6 hour

C<sub>max</sub>=peak concentration; AUC<sub>0-6</sub>=area under the curve from time 0 to 6 hr;

LS=least squares

CI=confidence interval

PK parameters were calculated from 0 to exactly 6 hours post meal/dose.

Interpolation was employed if the actual time was not exactly at 6 hours.

Sponsor’s Table 12

**Table 12 - Bioequivalence Assessment (Fieller’s Theorem) of Adjusted Serum Phosphorus (0 to 6 hr)**

Parameter	C <sub>max</sub> (mg/dL)		AUC <sub>0-6</sub> (hr*mg/dL)	
	Liquid PhosLo (A) (n=36)	PhosLo Gelcap (B) (n=36)	Liquid PhosLo (A) (n=36)	PhosLo Gelcap (B) (n=36)
Mean	0.23	0.42	0.66	1.18
Ratio (A)/(B) (in %)	54.44		56.29	
90% CI	(28.35, 88.67)		(13.38, 143.48)	

Source: Appendix 10, Table 14.4.4

Ratio (%) = (Mean test/ Mean Reference) x 100

Some subjects had no serum concentration at 6 hour

C<sub>max</sub>=peak concentration; AUC<sub>0-6</sub>=area under the curve from time 0 to 6 hr;

CI=confidence interval

PK parameters were calculated from 0 to exactly 6 hours post meal/dose.

Interpolation was employed if the actual time was not exactly at 6 hours.

Sponsor’s Table 13

**Table 13 - Bioequivalence Assessment (Fieller’s Theorem) of Adjusted Urinary Calcium (0 to 6 hr)**

Parameter	R <sub>max</sub> (mg/hr)		Ae <sub>0-6</sub> (mg)	
	Liquid PhosLo (A) n=34	PhosLo Gelcap (B) n=33	Liquid PhosLo (A) n=34	PhosLo Gelcap (B) n=33
Mean	6.90	10.07	35.28	46.07
Ratio (A)/(B) (in %)	68.53		76.58	
90% CI	(21.33, 174.65)		(45.10, 125.66)	

Source: Appendix 10, Table 14.4.4

Ratio (%) = (Mean test/ Mean Reference) x 100

Some subjects had no urine collection at 4-6 hour interval, or concentration levels <LOQ in urine for all 3 collection intervals

R<sub>max</sub>=maximal rate of urinary excretion; Ae<sub>0-6</sub>=cumulative urinary excretion from 0 – 6;

CI=confidence interval

Sponsor’s Table 14

**Table 14 - Bioequivalence Assessment (Fieller’s Theorem) of Adjusted Urinary Phosphorus (0 to 6 hr)**

Parameter	$R_{max}$ (mg/hr)		$Ae_{0-6}$ (mg)	
	Liquid PhosLo (A) n=31	PhosLo Gelcap (B) n=29	Liquid PhosLo (A) n=31	PhosLo Gelcap (B) n=29
Mean	-12.81	-7.04	-47.56	-53.90
Ratio (A)/(B) (in %)	181.91		88.24	
90% CI	(17.67, 825.68)		(34.17, 175.04)	

Source: Appendix 10, Table 14.4.4

Ratio (%) = (Mean test/ Mean Reference) x 100

Some subjects had no urine collection at 4-6 hour interval, or concentration levels <LOQ in urine for all 3 collection intervals

$R_{max}$ =maximal rate of urinary excretion;  $Ae_{0-6}$ =cumulative urinary excretion from 0 – 6;

CI=confidence interval

The sponsor’s results for serum Calcium and serum Phosphorus subtraction-adjusted AUC06s, as reported in these tables, are puzzling. We cannot reproduce these results using the sponsor’s AUC06 values as given in the s\_6\_pk SAS dataset. We also note the footnote at the end of the two serum tables (sponsor’s Tables 11 and 12): “PK parameters were calculated from 0 to exactly 6 hours post meal/dose. Interpolation was employed if the actual time was not exactly at 6 hours.” This is in contradiction to what the sponsor informed us they had done in calculating their AUC06 values.

In the case of urinary Calcium and Phosphorus Ae06s, we used the sponsor’s values to do our analyses, so the results should have been comparable. A possible source of difference is the statistical model and specific SAS program statements used by the sponsor and us. Although the sponsor states that they used SAS, they do not provide any actual SAS code to document their methods.

**Summary of our analyses using PK analysis dataset – serum endpoints, analyzing our AUC06**

**under treatment (study day 3) vs. baseline (study day -1)**

Table 1. Serum Calcium and Serum Phosphorus AUC06, study day 3 vs. study day -1

Serum Calcium AUC06 <sup>1</sup>			
study day	Citrate LSMEAN	Liquid LSMEAN	Gelcaps LSMEAN
-1	53.545	53.787	53.076
3	54.785	54.712	55.087
	p <sup>2</sup> 0.0343	p 0.0123	p 0.0003
Serum Phosphorus AUC06			
study day	Citrate LSMEAN	Liquid LSMEAN	Gelcaps LSMEAN
-1	19.852	19.869	19.758
3	22.351	20.556	20.878
	p<0.0001	p 0.0289	p 0.0036

<sup>1</sup>units for AUC06 are hr\*mg/dL

<sup>2</sup>all p-values are *two-sided* p-values

**Summary:**

For *our* AUC06s, mean AUC06 for serum calcium and for serum phosphorus on day 3 (under treatment) was statistically significantly higher (two-sided  $p \leq 0.05$ ) than on day -1 (baseline) for all three treatments.

**Liquid vs. Gelcaps – equivalence assessment, using Fieller’s Method**

We analyzed AUC06s adjusted by subtraction, i.e.

$$\text{adjusted AUC06} = \text{AUC06}(\text{under treatment day 3}) - \text{AUC06}(\text{baseline day -1})$$

Table 2. Serum Calcium and Serum Phosphorus subtraction-adjusted AUC06, point estimate and 90% confidence interval for ratio of means, using Fieller’s method

mean adjusted AUC06(Liquid) / mean adjusted AUC06(Gelcaps)			
serum calcium		serum phosphorus	
point estimate	90% conf. interval	point estimate	90% conf. interval
0.460	(0.150, 1.009)	0.540	(0.081, 1.582)
LSMEAN adjusted AUC06 <sup>1</sup>			
Liquid 0.9250	Gelcaps 2.0112	Liquid 0.6653	Gelcaps 1.1197

<sup>1</sup>units for adjusted AUC06 are hr\*mg/dL

**Summary:**

For serum calcium

Using our AUC06s, the mean subtraction-adjusted AUC06 for the Liquid formulation appears to be lower than for the Gelcap formulation (estimated ratio of means 0.460), and based on the confidence interval it appears at worst to be comparable (upper 90% confidence bound on ratio of means 1.009.)

For serum phosphorus

Using our AUC06s, the mean subtraction-adjusted AUC06 for the Liquid formulation appears to be lower than for the Gelcap formulation (estimated ratio of means 0.540), however based on the 90% confidence interval it could possibly be higher (upper confidence bound on ratio of means 1.582.), so the study must be regarded as inconclusive for this endpoint.

**Citrate vs. Liquid and Gelcaps**

Table 3. Serum Calcium and Serum Phosphorus subtraction-adjusted AUC06<sup>1</sup>s, comparing Citrate to Liquid and Gelcaps

	two-sided p-values	
	Serum Calcium	Serum Phosphorus
Liquid vs. Gelcaps	0.1115	0.4253
Citrate vs. calcium acetate treatments	0.7141	0.0001
Citrate vs. Liquid	0.6313	0.0001
Citrate vs. Gelcaps	0.3113	0.0070
	LSMEANS <sup>1,2</sup>	
Liquid	0.925	0.718
Gelcaps	2.011	1.120
Citrate	1.240	2.500

<sup>1</sup>units for adjusted AUC06 are hr\*mg/dL

<sup>2</sup>all LSMEANS were statistically significantly (p < 0.05) greater than zero.

For serum calcium

Using our AUC06s, there were no statistically significant differences (p > 0.05) between the lsmean subtraction-adjusted AUC06s for the three treatments.

For serum phosphorus

Using our AUC06s, both the Liquid and the Gelcap lsmean subtraction-adjusted AUC06s were statistically significantly (p < 0.05) lower than the Citrate lsmean. The Liquid and Gelcap lsmeans were not statistically significantly different.

**Analyses of Baseline AUC06s**

Using our AUC06s:

Table 4. Analysis of Serum Calcium and Serum Phosphorus baseline AUC06s

factor	type-III two-sided p-value	
	serum calcium AUC06	serum phosphorus AUC06
sequence	0.5241 (2 d.f.)	0.9213 (2 d.f.)
period	0.0007 (2 d.f.)	0.3863 (2 d.f.)
treatment	0.5272 (2 d.f.)	0.9425 (2 d.f.)

## Summary:

In the case of serum calcium, there was a statistically significant *period effect* for baseline AUC06s. However, there was no evidence of a *treatment effect* i.e. no evidence of imbalance at baseline with respect to treatment. There was no evidence of period effect or treatment effect for serum phosphorus AUC06s.

**Urinary Endpoints**

Summary of analyses using PK analysis dataset analyzing Ae06, amount excreted in urine 0-6 hours.

**under treatment (study day 3) vs. baseline (study day -1)**

Table 5. Urinary Calcium and Urinary Phosphorus Ae06, study day 3 vs. study day -1

Urinary Calcium Ae06			
study day	Citrate LSMEAN	Liquid LSMEAN	Gelcaps LSMEAN
-1	50.364	49.182	50.203
3	72.791	83.750	100.305
	p 0.0026	p 0.0003	p<0.0001
Urinary Phosphorus Ae06			
study day	Citrate LSMEAN	Liquid LSMEAN	Gelcaps LSMEAN
-1	155.485	139.709	161.999
3	143.995	92.147	108.097
	p <sup>1</sup> 0.5825	p 0.0072	p 0.0005

<sup>1</sup>all p-values are *two-sided* p-values

In Summary:

For all three treatments, mean urinary calcium Ae06 after treatment was higher than at baseline these differences were statistically significant (p<0.05) for all three treatments. For all three treatments, mean urinary phosphorus Ae06 after treatment was *lower* than at baseline these differences were statistically significant (p<0.05) for Gelcaps and Liquid, but not for Calcium Citrate (p 0.5825.)

**Liquid vs. Gelcaps – equivalence assessment, using Fieller’s Method**

We analyzed Ae06s adjusted by subtraction, i.e.

$$\text{adjusted Ae06} = \text{Ae06}(\text{under treatment day 3}) - \text{Ae06}(\text{baseline day -1})$$

Table 6. Urinary Calcium and Urinary Phosphorus subtraction-adjusted Ae06, point estimate and 90% confidence interval for ratio of means, using Fieller’s method

mean adjusted Ae06(Liquid) / mean adjusted Ae06(Gelcaps)			
urinary calcium		urinary phosphorus	
point estimate	90% conf. interval	point estimate	90% conf. interval
0.676	(0.410, 1.039)	0.947	(0.415, 1.934)
LSMEAN adjusted Ae06			
Liquid 34.133 mg	Gelcaps 50.502 mg	Liquid -50.499 mg	Gelcaps -53.302 mg

Summary:

For urinary calcium

The mean adjusted Ae06 for the Liquid formulation appears to be lower than for the Gelcap formulation (estimated ratio of means 0.676), and based on the confidence interval the mean for the Liquid formulation appears at worst to be equivalent to that for the Gelcap formulation (upper 90% confidence bound on ratio of means 1.039.)

For both treatments the mean adjusted urinary calcium Ae06 was positive i.e. urinary calcium levels were higher under treatment than at baseline.

For urinary phosphorus

The mean adjusted Ae06 for the Liquid formulation appears to be roughly comparable to or slightly lower than that for the Gelcap formulation (estimated ratio of means 0.947), however based on the 90% confidence interval (0.415, 1.934) the study must be judged inconclusive in this case - the mean for the Liquid formulation might be substantially lower or substantially higher than for the Gelcap formulation, based on these data.

For both treatments the mean adjusted urinary phosphorus Ae06 was negative i.e. urinary phosphorus levels were lower under treatment than at baseline.

**Citrate vs. Liquid and Gelcaps**

Table 7. Urinary Calcium and Urinary Phosphorus subtraction-adjusted Ae06<sup>1</sup>s, comparing Citrate to Liquid and Gelcaps

	two-sided p-values	
	Urinary Calcium	Urinary Phosphorus
Liquid vs. Gelcaps	0.1284	0.8837
Citrate vs. calcium acetate treatments	0.0364	0.0834
Citrate vs. Liquid	0.2472	0.1065
Citrate vs. Gelcaps	0.0161	0.1208
	LSMEANS <sup>1</sup>	
Liquid	34.302	-50.337
Gelcaps	50.753	-53.359
Citrate	22.491	-12.403 <sup>2</sup>

<sup>1</sup>units for adjusted Ae06 are mg

<sup>2</sup>The Citrate Urinary Phosphorus LSMEAN, -12.403, was not statistically significantly (p > 0.05) different from zero. All other LSMEANS were statistically significantly different from zero.

Summary:

For urinary calcium

There was no statistically significant difference ( $p = 0.1284$ ) between the Liquid and Gelcap lsmean subtraction-adjusted Ae06s. Taken individually, the Citrate lsmean was not statistically significantly different from the Liquid lsmean ( $p = 0.2472$ ), but was statistically significantly less than the Gelcap lsmean ( $p = 0.0161$ .) The Citrate lsmean was also statistically significantly less than the mean of the two calcium acetate treatment (Liquid and Gelcaps) lsmeans ( $p = 0.0364$ .)

For urinary phosphorus

There was no statistically significant difference ( $p = 0.8837$ ) between the Liquid and Gelcap lsmean subtraction-adjusted Ae06s. Taken individually, the Citrate lsmean was not statistically significantly different from either the Liquid lsmean ( $p = 0.1065$ ) or the Gelcap lsmean ( $p = 0.1208$ ), nor was it statistically significantly different from the mean of the two calcium acetate treatment (Liquid and Gelcaps) lsmeans ( $p = 0.0834$ .)

**Analyses of Baseline Ae06s**

Table 8. Analysis of Urinary Calcium and Urinary Phosphorus baseline Ae06s

factor	type-III two-sided p-value <sup>1</sup>	
	urinary calcium Ae06	urinary phosphorus Ae06
sequence	0.6245 (2 d.f.)	0.4186 (2 d.f.)
period	0.2561 (2 d.f.)	0.6543 (2 d.f.)
treatment	0.9981 (2 d.f.)	0.5080 (2 d.f.)

<sup>1</sup>all p-values are *two-sided* p-values

Summary:

For both urinary calcium and urinary phosphorus baseline Ae06, there was no evidence of a *treatment effect* i.e. no evidence of imbalance at baseline with respect to treatment. There was also no evidence of sequence or period effects.

## Summary and Discussion

Calcium acetate is administered to patients with End Stage Renal Disease to bind with dietary phosphate, thus reducing absorption of dietary phosphate, to control hyperphosphatemia. However, one of the possible adverse events associated with calcium acetate treatment is *hypercalcemia*. For this reason, the CDER review team wished to determine if the proposed Liquid formulation would increase serum calcium levels compared to the approved Gelcap formulation.

In the study the Calcium Citrate positive control had statistically significantly higher levels under treatment (study day 3) than at baseline (study day -1.) This establishes that in this study calcium present in the treatments was being absorbed, establishing validity of the study. Indeed, mean serum calcium AUC06 was statistically significantly higher under treatment than at baseline for all three treatments in the study.

Based on the results for subtraction-corrected AUC06 for serum calcium from the sponsor's bioequivalence study, it appears that serum calcium increase over baseline levels is actually lower with the Liquid formulation than for the Gelcap formulation, with a point estimate of 0.460 for the ratio of mean increases over baseline, Liquid over Gelcaps. Based on the confidence interval, it appears that increase in calcium levels with the Liquid formulation is at worst comparable to that for Gelcaps (upper limit of the 90% confidence interval for the ratio of means = 1.009.) Results for urinary calcium, as measured by subtraction-corrected amount excreted from 0 to 6 hours (Ae06), are similar, with a point estimate of 0.676 and an upper limit of the 90% confidence interval of 1.039.

This study did not show the Liquid and Gelcap formulations to be bioequivalent with respect to increase over baseline in serum and urinary calcium levels. As already noted, the data suggest that increase in serum and urinary calcium levels from the Liquid may be lower than for the Gelcaps. If the Liquid formulation is approved, the possibility may exist that a patient might use the Liquid formulation successfully for a time, and then if that patient were to switch to the Gelcap formulation (for whatever reason), he/she might exhibit higher serum calcium levels.

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Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22581	ORIG-1	FRESENIUS BIOTECH NORTH AMERICA INC	PHOSLYRA(CALCIUM ACETATE)ORAL SOL 667MG/

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