CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-642

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

1/13/05

Labeling Comments:

The office of Clinical Pharmacology and Biopharmaceutics has reviewed the package insert labeling for Nascobal® and finds it acceptable pending the following revision:

(~~Strikethrough text~~ is recommended to be deleted and **underlined text** is recommended to be added.)

Proposed Insert for Nascobal® Nasal Spray
Rev. 11/04

7 Page(s) Withheld

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

___ § 552(b)(5) Deliberative Process

✓ § 552(b)(4) Draft Labeling

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Jayabharathi Vaidyanathan
1/12/05 03:47:53 PM
PHARMACOLOGIST

Hae-Young Ahn
1/13/05 09:11:59 AM
BIOPHARMACEUTICS

10/06/04

OFFICE OF CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS

ADDENDUM

NDA: 21-642	Submission Date(s): 12/29/03
Brand Name	Nascobal® nasal spray
Generic Name	Cyanocobalamin nasal spray
Reviewer	Jaya bharathi Vaidyanathan, Ph.D.
Team Leader	Hae-Young Ahn, Ph.D.
OCPB Division	DPEII
ORM Division	Division of Metabolic and Endocrine Drug Products
Sponsor	Nastech Pharmaceutical Company Inc.
Submission Type	Original NDA 505 (b) (1)
Relevant NDA	19-722 (Nascobal® nasal gel)
Relevant IND	25,696
Formulation; Strength(s)	Nasal spray; 500 μg/0.1ml
Indication	Maintenance of normal hematologic status in pernicious anemia patients and supplementation for other vitamin B_{12} deficiencies

1 Table of Contents

2 Appendix

2.1 Study Synopsis

1. SYNOPSIS

NAME OF COMPANY **Nastech Pharmaceutical Co., Inc.** NAME OF FINISHED PRODUCT **Vitamin B_{12} IN Spray/Gel** NAME OF ACTIVE INGREDIENT **Vitamin B_{12}**	SUMMARY TABLE Referring to Part ..... of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:

TITLE OF STUDY:

A Phase I, 3-Way Crossover, Pharmacokinetic Study of vitamin B_{12} Administered via Intranasal Spray at 500-µg, Intranasal Gel (Nascobal®) at 500-µg, and Intramuscular Injection at 100-µg in Fasted Normal Healthy Male and Female Subjects

INVESTIGATOR:

—

STUDY CENTER:

—

PUBLICATION (REFERENCE):

None

STUDIED PERIOD:

First Subject Screened: 5 August 2002

Last Subject Visit: 13 September 2002

PHASE OF DEVELOPMENT:

Phase I

OBJECTIVES:

1) To compare the pharmacokinetic profile of a single intranasally-administered spray, single intranasally-administered gel (Nascobal®), and single intramuscular-administered vitamin B_{12} in a fasted state in normal healthy male and female subjects.

2) To evaluate the bioequivalence of vitamin B_{12} nasal gel versus the nasal spray in a fasted state in normal healthy male and female subjects.

NAME OF COMPANY **Nastech Pharmaceutical Co., Inc.** NAME OF FINISHED PRODUCT **Vitamin B_{12} IN Spray/Gel** NAME OF ACTIVE INGREDIENT **Vitamin B_{12}**	SUMMARY TABLE Referring to Part ..... of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:

3) To evaluate the relative bioavailability of the 2 nasal preparations of vitamin B_{12} in a fasted state in normal healthy male and female subjects.

4) To examine the safety and tolerability of the 3 preparations of vitamin B_{12} in a fasted state in normal healthy male and female subjects.

METHODOLOGY:

This study was a single-site, open-label, 3-way (3-treatment, 6-sequence) crossover, pharmacokinetic study of vitamin B_{12} administered via intranasal (IN) spray at 500-µg, IN gel (Nascobal®) at 500-µg, and intramuscular (IM) injection at 100-µg in fasted normal healthy male and female subjects. Subjects were also on a vitamin B_{12} free diet throughout the confinement period.

NUMBER OF SUBJECTS (PLANNED AND ANALYZED):

Twenty-five (25) normal healthy male and female subjects between 18 and 65 years of age, between 50 and 90 kg, and within ±15% of their ideal body weight for height and frame were dosed. The concentration data from all 25 subjects were analyzed according to the intent-to-treat principle. An attempt was made to enroll approximately equal numbers of male and female subjects into each sequence.

MAIN CRITERIA FOR INCLUSION/EXCLUSION:

Healthy, non-smoker, male and non-pregnant female subjects between the ages of 18 and 65 years of age, inclusive, with body weight at Screening within 15% of his/her ideal weight as defined by the 1983 Metropolitan Life Insurance Tables. Subjects with known allergy or sensitivity to vitamin B_{12} or any ingredients in the IN formulations (i.e., methylcellulose, citric acid, sodium citrate, glycerin, benzalkonium chloride) or in the IM formulation (i.e., sodium chloride, benzyl alcohol, sodium hydroxide, and hydrochloric acid) were excluded.

NAME OF COMPANY **Nastech Pharmaceutical Co., Inc.** NAME OF FINISHED PRODUCT **Vitamin B_{12} IN Spray/Gel** NAME OF ACTIVE INGREDIENT **Vitamin B_{12}**	SUMMARY TABLE Referring to Part ..... of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:

TEST PRODUCT, DOSE, AND MODE OF ADMINISTRATION; BATCH NUMBER:

Test Product	Nominal Dose	Actual Dose[a]	Mode	Lot Number
Vitamin B_{12}	500-µg	—	IN spray	01022A
Vitamin B_{12} (Nascobal®)	500-µg	—	IN gel	00753A
Vitamin B_{12}	100-µg	—	IM injection	7518N-00891-1 (2126)

[a] The release assays showed that the IN spray and gel products contained — strength relative to their label claim of 500 µg/0.1 mL, respectively. Therefore, the actual doses for the IN spray and the IN gel formulations were — respectively.

DURATION OF TREATMENT:

Planned Enrollment/Screening Duration: Approximately 4 weeks.

Planned Study Duration: Approximately 7 weeks.

Length of Confinement: During each period, from check-in (approximately 72 hours prior to dose) until approximately 96 hours post dose for a total confinement of approximately 168 hours. At least a 14-day washout period occurred between treatments.

CRITERIA FOR EVALUATION:

Pharmacokinetic Parameters:

For each subject, the following pharmacokinetic parameters were calculated whenever possible, based on the serum concentrations of vitamin B_{12} from Treatments A, B, and C according to the model independent approach: C_{max}, T_{max}, and AUC_{0-t}.

Safety:

No formal statistical analyses were planned.

STATISTICAL METHODS:

Pharmacokinetic:

Pharmacokinetic calculations were performed, if appropriate, using SAS® (SAS Inst., Version 8.02).

For T_{max}, the analyses were run using Wilcoxon's matched pairs method to determine if

differences exist between the test group and each reference group.

A statistical outlier testing procedure utilizing maximum normalized residuals (MNR) was used to evaluate C_{max} and AUC_{0-t} results, to determine if any of the data could be considered extreme observations, if necessary.

Where data was available, relative bioavailability and bioequivalence were examined. Bioequivalence was evaluated for the test (Treatment A - Nasal Spray) versus the reference (Treatment B - Gel) with regard to dose-normalized values of C_{max} and AUC_{0-t} (DN_ C_{max} and DN_AUC_{0-t}). An analysis of variance (ANOVA) was performed and the 90% confidence intervals were generated for the ratio of test/reference. C_{max}, DN_ C_{max}, AUC_{0-t} and DN_ AUC_{0-t} were natural log (ln) transformed prior to ANOVA. The corresponding 90% confidence intervals for the geometric mean ratio were obtained by taking the antilog of the 90% confidence intervals for the difference between the means on the log scale. Relative bioavailability was evaluated based on AUC_{0-t} for the test (Treatments A and B - Nasal Spray and Gel, respectively) and the reference (Treatment C – IM) groups. The following model was used:

Parameter = Sequence + Subject (Sequence) + Period + Treatment + Random error

Where,

Response	=	Given PK parameter
Sequence	=	Sequence term
Subject (Sequence)	=	Subject within sequence term
Period	=	Dosing period term
Treatment	=	A (test): 500-µg IN-Spray B (reference): 500-µg IN-Gel C (reference): 100-µg IM-Injection
Random error	=	Residual Error

Subject (Sequence) was a random effect with all others fixed.

Relative bioavailability was assessed by examining the 90% confidence intervals for the ratio of the test (Treatments A and B) group means relative to the reference (Treatment C) group mean. It was assumed that the test (Treatment A) was noninferior with respect to the reference (Treatment B) if the lower bound of the 90% confidence intervals from $\log_e$-transformed

NAME OF COMPANY **Nastech Pharmaceutical Co., Inc.** NAME OF FINISHED PRODUCT **Vitamin B_{12} IN Spray/Gel** NAME OF ACTIVE INGREDIENT **Vitamin B_{12}**	SUMMARY TABLE Referring to Part ..... of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:

DN_C_{max} and DN_AUC_{0-t} was greater than or equal to 80%. If the lower bound of the 90% confidence intervals from $\log_e$-transformed DN_C_{max} and DN_AUC_{0-t} was less than 80%, it was assumed that noninferiority could not be established.

Safety:

Adverse events were listed by subject number. No formal statistical analyses were planned.

SUMMARY – CONCLUSIONS

PHARMACOKINETIC RESULTS:

The relative bioavailability for the two IN formulations was 1.04. Relative bioavailability when comparing Treatment A (spray) versus Treatment C (IM) was 0.61, and 0.63 when comparing Treatment B (gel) versus Treatment C (IM).

The pharmacokinetic profiles of the spray formulation and the gel formulation were similar for DN_C_{max} (3.05 pg/mL/μg and 3.24 pg/mL/μg, respectively) and DN_AUC_{0-t} (191.93 pg*hr/mL/μg and 185.99 pg*hr/mL/μg, respectively). Additionally, the median difference for T_{max} between the spray and gel IN formulation was about one half hour (-0.48 hr). The C_{max} value for the IM formulation was significantly higher than the C_{max} values for the two IN formulations ($p<0.0001$).

Bioequivalence was established for the Vitamin B_{12} IN spray with regard to the IN gel formulation based on dose-normalized C_{max} and AUC_{0-t}. The 90% confidence intervals for the $\log_e$-transformed dose-normalized C_{max} and AUC_{0-t} for the spray and gel formulations fell within the range of 80% to 125%. Thus, noninferiority can be assumed when comparing the two IN formulations because the lower bounds of the confidence intervals are greater than 80% for both AUC_{0-t} and C_{max}.

SAFETY RESULTS:

The safety results indicate that all three Vitamin B_{12} formulations were well tolerated. Overall, the incidence of adverse events was low for all three treatments. There were no deaths, serious or severe adverse events. No adverse event led to subject discontinuation and all events were

resolved by the end of the study The total number of adverse events following treatment was similar for the Treatment A (13 adverse events), Treatment B (17 adverse events), and Treatment C (19 adverse events). Sneezing in one subject was the only adverse event determined by the Principal Investigator to be drug-related.

CONCLUSIONS:

- Compared to the IN gel formulation, the relative bioavailability for the IN spray formulation was 1.04. Relative bioavailability for Treatment A (spray) versus Treatment C (IM) was 0.61, and 0.63 when comparing Treatment B (gel) versus Treatment C (IM).
- The pharmacokinetic profiles of the spray formulation and the gel formulation were similar for DN_C_{max} (3.05 pg/mL/µg and 3.24 pg/mL/µg, respectively) and DN_AUC_{0-t} (191.93 pg*hr/mL/µg and 185.99 pg*hr/mL/µg, respectively). Additionally, the median difference for T_{max} between the spray and gel IN formulation was about one half hour (-0.48 hr). The C_{max} value for the IM formulation was significantly higher than the C_{max} values for the two IN formulations ($p<0.0001$)
- Bioequivalence between the Vitamin B_{12} spray formulation and the Vitamin B_{12} gel formulation was established using 90% confidence intervals for $\log_e$-transformed dose-normalized values of AUC_{0-t} and C_{max}. The 90% confidence intervals for the $\log_e$-transformed DN_C_{max} and DN_AUC_{0-t} for the spray and gel formulations fell within the range of 0.80 to 1.25. Noninferiority can be assumed for the two IN formulations (Treatment A versus Treatment B).
- A second pharmacokinetic analysis was performed in the per-protocol PK population, that is, data from Subjects 003, 015 and 008 were excluded from the analysis since these three subjects experienced possible dosing irregularities or rhinorrhea after intranasal administration which might affect the rate and extent of drug absorption. The results can be found in Appendix 4.
- In addition, a third pharmacokinetic analysis was performed in the intent-to-treat PK population using protocol-specified nominal doses for bioequivalence evaluation of the two intranasal formulations. The detailed results can be found in Appendix 5.
- All three pharmacokinetic analyses showed that bioequivalence could be established for

NAME OF COMPANY **Nastech Pharmaceutical Co., Inc.** NAME OF FINISHED PRODUCT **Vitamin B_{12} IN Spray/Gel** NAME OF ACTIVE INGREDIENT **Vitamin B_{12}**	SUMMARY TABLE Referring to Part ..... of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:

the intranasal spray formulation as compared to the intranasal gel formulation.

- All Vitamin B_{12} formulations were safe and well tolerated by healthy male and female volunteers.

14 Page(s) Withheld

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

___ § 552(b)(5) Deliberative Process

___ § 552(b)(4) Draft Labeling

B/P Review
10/01/04

OFFICE OF CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

NDA: 21-642	Submission Date(s): 12/29/03
Brand Name	Nascobal® nasal spray
Generic Name	Cyanocobalamin nasal spray
Reviewer	Jaya bharathi Vaidyanathan, Ph.D.
Team Leader	Hae-Young Ahn, Ph.D.
OCPB Division	DPEII
ORM Division	Division of Metabolic and Endocrine Drug Products
Sponsor	Nastech Pharmaceutical Company Inc.
Submission Type	NDA 505 (b) (2)
Relevant NDA	19-722 (Nascobal® nasal gel)
Relevant IND	25,696
Formulation; Strength(s)	Nasal spray; 500 μg/0.1ml
Indication	Maintenance of normal hematologic status in pernicious anemia patients and supplementation for other vitamin B_{12} deficiencies

1 Table of Contents

2 Executive Summary

Nastech Pharmaceutical Company, Inc. submitted an NDA for Nascobal® (cyanocobalamin) spray for intranasal administration. Nascobal® (cyanocobalamin, USP) is a synthetic form of vitamin B_{12} with equivalent vitamin B_{12} activity. This NDA is for a new dosage form (a nasal spray) for the marketed Nascobal® nasal gel (cyanocobalamin, USP NDA 19-722) product. The suggested dose of Nascobal® (cyanocobalamin, USP) is 500-µg administered intranasally once weekly. This NDA contains one bioequivalence study comparing the nasal spray to the approved nasal gel (NDA 19-722) and intramuscular cyanocobalamin. (Note: Many intramuscular cyanocobalamin products are available in the U.S.) There were no clinical trials conducted with the Nascobal® spray and the clinical studies performed in support of NDA 19-722 (approved nasal gel) is being referenced in support of this NDA.

The reference drug, nascobal nasal gel® was approved based on a bioavailability study conducted in patients with pernicious anemia who were stable on intramuscular B_{12} for at least 6 months. A sequential trial was conducted wherein the patients were given a 100 µg IM injection followed by blood sampling. This was followed by treatment with IN gel (500 µg) for a month (4 doses) with blood sampling. Baseline uncorrected method was used to determine relative bioavailability to IM.

Intranasal cyanocobalamin gel is approved for a dose of 500-µg. The BE study submitted also utilized a cyanocobalamin nasal spray at the same dose (500-µg) and an intramuscular dose of 100-µg.

Required office level CPB briefing was held on 10/1/04. The attendees were Drs. Chandra Sahajwalla, John Hunt, Mary Parks, Arzu Selen, Atiqur Rahman, Hae-Young Ahn and Jayabharathi Vaidyanathan.

2.1 Recommendation

The Office of Clinical Pharmacology and Biopharmaceutics, Division of Pharmaceutical Evaluation II (OCPB/DPE-2) reviewed NDA 21-642 and finds the results unacceptable due to lack of demonstration of bioequivalence between the nasal spray and nasal gel formulations using baseline corrected analysis. The nasal spray had 10% less AUC than the nasal gel using baseline corrected analysis. Since there are no clinical trials conducted, the clinical significance of the reduced exposure obtained with the nasal spray is not known. Before it is approved, it is recommended to change the formulation of the nasal spray and conduct a bioequivalence trial with baseline corrected analysis.

2.2 Phase IV Commitments

None

2.3 Summary of CPB Findings

Relative bioavailability of Nascobal® Nasal Spray to that of Nascobal® Nasal gel.

Strict bioequivalence between the two intranasal formulations was not established using log-transformed 90% confidence intervals for baseline uncorrected AUC_{0-t} and C_{max}. The confidence intervals were 91.22-103.84 and 79.8-108.96 respectively for AUC_{0-t} and C_{max}.

Sponsor did not conduct analysis with baseline correction. Baseline corrected analysis was conducted during review process. The relative bioavailability of the nasal spray as compared to nasal gel was measured after single nasal administration of 500-µg of both the formulations. The two intranasal formulations had a relative bioavailability of 0.061 (spray) and 0.071 (gel) to the intramuscular injection formulation used as a reference. When the plasma concentrations were corrected for baseline levels, the nasal spray had 10% less AUC_{0-t} values than the nasal gel. The log-transformed 90% confidence intervals for AUC_{0-t} and C_{max} were 71.71-114.19 and 71.6-118.66 respectively. Therefore the two formulations are not bioequivalent.

3 QBR

3.1 General Attributes

Q. What is the therapeutic indication of the drug? What is the proposed dosage and route of administration? What is the to-be-marketed formulation of Nascobal® Nasal Spray?

Cyanocobalamin is a synthetic form of vitamin B_{12} with equivalent B_{12} activity. Vitamin B_{12} deficiency results in magaloblastic anemia and is followed by gradual degradation of the axon and nerve head. Cyanocobalamin is the most stable and widely used form of vitamin B_{12}.

The proposed indication for Nascobal® nasal spray is for the maintenance of normal hematologic status in pernicious anemia patients who are in remission following intramuscular vitamin B_{12} therapy and who have no nervous system involvement. Nascobal® nasal spray is also indicated as a supplement for other vitamin B_{12} deficiencies including dietary deficiency of vitamin B_{12}, malabsorption of Vitamin B_{12}, and inadequate secretion of intrinsic factor.

Nascobal® nasal spray is an aqueous solution of cyanocobalamin, USP for administration as a metered nasal spray. The suggested dose of Nascobal® (cyanocobalamin, USP) is 500-µg administered intranasally once weekly. Each bottle of Nascobal® nasal spray contains 2.3 ml of 500 µg/0.1 ml solution of cyanocobablamin with sodium citrate, USP, citric acid, USP, glycerin, USP and benzylkonium chloride solution, NF in purified water, USP. Table 1 shows the composition of Nascobal® nasal spray.

Table 1: Composition of Nascobal® Nasal Spray

Component	Reference to Quality Standard	Function	% W/W	mg per 0.1 mL spray	mg per 2.3 mL bottle
Cyanocobalamin*	USP	active ingredient	—	0.50	11.5
Citric acid	USP				
Sodium citrate	USP				
Glycerin	USP				
Benzalkonium chloride solution	NF				
Purified water	USP	diluent			
Total	—	—	100.00	100.00	2300.00

3.2 General Clinical Pharmacology

Not applicable.

3.3 Intrinsic Factors

Not applicable.

3.4 Extrinsic Factors

Not applicable.

3.5 General Biopharmaceutics

Q. Is the Nascobal® nasal spray bioequivalent to the currently marketed Nascobal® nasal gel in healthy volunteers?

The objectives of the study were to compare the bioavailability of vitamin B_{12} nasal gel versus the nasal spray and to evaluate the relative bioavalilability of the 2 nasal preparations as compared to intramuscularly-administered vitamin B_{12}. The only difference between the two nasal products is that Nascobal® nasal spray is reformulated without methylcellulose , and . The clinical trial formulation is identical to the proposed commercial formulation.

Table 2: Composition of Nascobal® Nasal Gel versus Nasal Spray

Ingredient	Nasal Gel Quantity (g/100 g)	Nasal Spray Quantity (g/100 g)
Cyanocobalamin, USP		0.50
Citric Acid, USP		
Sodium Citrate, USP		
Methylcellulose, USP		none
Glycerin, USP		
Benzalkonium Chloride Solution, NF		
Purified Water, USP q.s.	100	100

In order to test bioequivalence, an open-label, 3-way crossover study was conducted in fasting healthy adult subjects (25 subjects dosed). Each subject received a single intranasal spray (500-µg), or a single intranasal gel (Nascobal®) (500-µg), or a single intra-muscular injection (100-µg). A washout period of 14 days separated the three dosing periods. Blood samples for PK analysis of vitamin B12 were collected on day (-1) at -24, -18, -12 hr and on Day 1 at pre-dose, 30 min, 1, 1.5, 2, 4, 6, 8, 10, 12, 18, 24, 36, 48, 60, 72, 84 and 96h post-dose in each period.

The mean plasma concentration profiles over time (uncorrected for baseline activity) for all the three formulations are shown in linear scale in Figure 1 and those for the intranasal formulations in Figure 2.

Figure 1: Mean serum concentration versus time of vitamin B_{12} over time

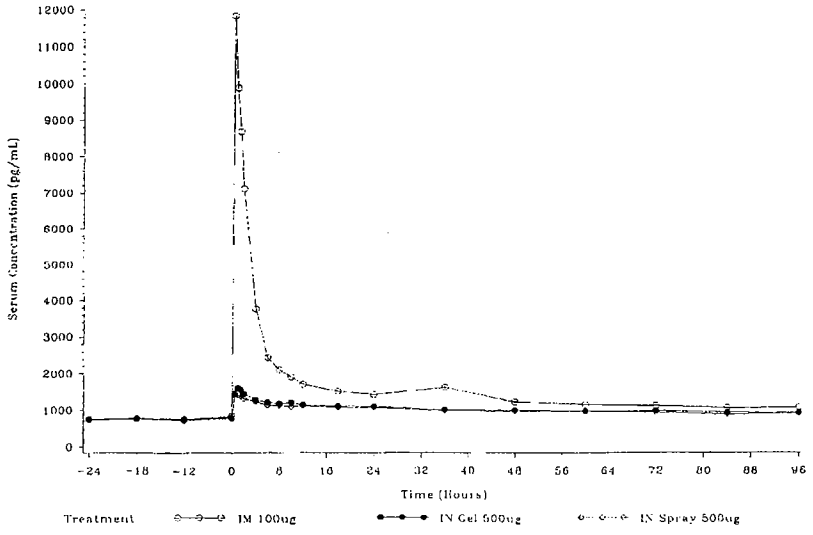
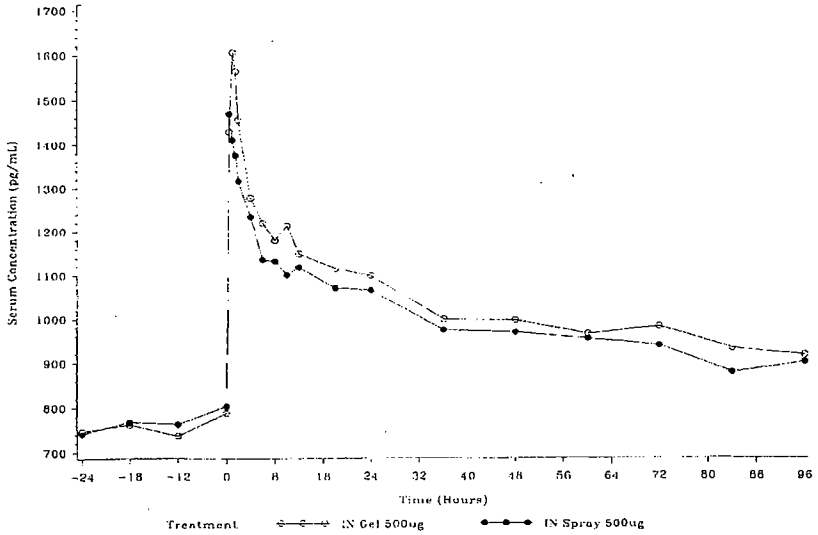


Figure 2: Vitamin B_{12} mean serum concentration profiles (uncorrected for baseline levels)



The pharmacokinetic parameters obtained for the three Vitamin B_{12} formulations (uncorrected for baseline activity) are summarized in Table 3. The relative bioavailability of the intranasal spray and gel was 0.12 and 0.126 as compared to that with the IM injection. The relative bioavailability of the nasal spray to the nasal gel was 0.97. The 90% confidence intervals for the log-transformed AUC and C_{max} were 91.22-103.84% and 79.8-108.96% respectively.

Table 3: Summary of pharmacokinetic parameters and treatment comparisons for the different formulations (N=27)

Parameter	Arithmetic mean (SD)	Geometric mean	Ratio (%B)	Ratio (%C)	90% CI
Nasal Spray (A) $AUC_{(0-t)}$ (pg*hr/ml)	95582 (32784)	90933	97.33	60.99	91.22-103.84
C_{max} (pg/ml)	1518 (645)	1555	93.01		79.8-108.96
Nasal Gel (B) $AUC_{(0-t)}$ (pg*hr/ml)	99224 (40545)	93430		62.66	
C_{max} (pg/ml)	1731 (1036)	1671			
IM injection (C) $AUC_{(0-t)}$ (pg*hr/ml)	153905 (45803)	149105			
C_{max} (pg/ml)	12074 (3623)	12441			

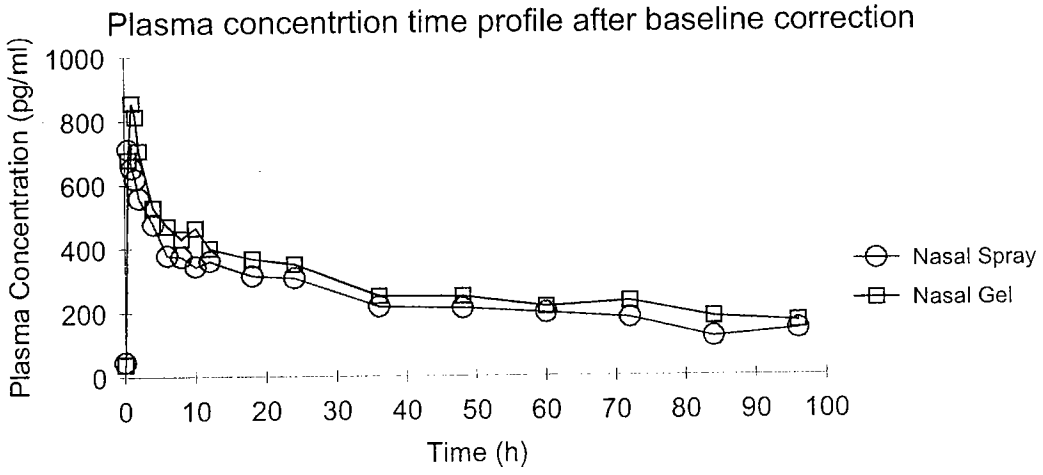
(Note: 24 subjects were randomized, but 25 subjects were dosed. One subject (#115) was a replacement for subject 015 and subjects 003 and 015 added one additional period due to dosing irregularities. Therefore it changed the total number of samples to N =27.)

As seen in the above figures, pre-dose plasma cyanocobalamin levels contribute significantly to the AUC. Baseline corrected analysis was conducted during the review process. The sponsor did not conduct this analysis. Baseline correction was done by taking the average of the individual baseline levels (3 points in this study) and subtracting it from the observed values at each time point for each treatment. The following Figure 3 shows the plasma concentration time profiles and Table 4 shows the statistical comparisons for the nasal spray and gel after correcting for baseline levels. The nasal spray exhibited a 10% lower AUC than the intranasal gel. The 90% confidence intervals for both $AUC_{(0-t)}$ and C_{max} were not within the 80-125% range, therefore bioequivalence was not established. The two intranasal formulations had a relative bioavailability of 0.061 (spray) and 0.071 (gel) to the intramuscular injection formulation used as a reference.

Table 4: Summary of treatment comparisons for the intranasal formulations after baseline correction (N=27)

Parameter	Geometric mean	Ratio (%B)	90% CI	Ratio (%C)
Nasal Spray (A) $AUC_{(0-t)}$ (pg*hr/ml)	20121.13	90.49	71.71-114.19	25.61
C_{max} (pg/ml)	743.95	92.18	71.60-118.66	28.06
Nasal Gel (B) $AUC_{(0-t)}$ (pg*hr/ml)	22236.45			
C_{max} (pg/ml)	807.10			
IM injection (C) $AUC_{(0-t)}$ (pg*hr/ml)	79392.87			
C_{max} (pg/ml)	12067.3			

Figure 3:



In the memorandum dated Sept 17, 2004, DSI recommended that the BE study be accepted for agency review with the exception of subject samples stored for 7-8 months prior to analysis (subjects 003/periods 2 and 4, and 015/period 4). The following analysis was done after exclusion of subjects 3 and 15.

Table 5 shows the statistical comparisons of pharmacokinetic parameters (uncorrected for baseline) obtained after removing the above mentioned subjects. The ratio of AUC and C_{max} for the nasal spray to gel was 95.68% and 89.97% respectively. As shown the 90% CI for AUC is within the 80-125% range, while the 90% CI for C_{max} does not fall within the range.

Table 5: Summary of pharmacokinetic parameters and treatment comparisons for the different formulations (N=23)

Parameter	**Geometric mean**	**Ratio (%B)**	**Ratio (%C)**	**90% CI**
Nasal Spray (A) $AUC_{(0-t)}$ (pg*hr/ml)	85590	95.68	60.77	88.94-102.93
C_{max} (pg/ml)	1369	89.97		75.00-107.93
Nasal Gel (B) $AUC_{(0-t)}$ (pg*hr/ml)	89453		63.51	
C_{max} (pg/ml)	1521			
IM injection (C) $AUC_{(0-t)}$ (pg*hr/ml)	140849			
C_{max} (pg/ml)	11003			

Table 6 shows the statistical comparisons of pharmacokinetic parameters (corrected for baseline) obtained after removing the above mentioned subjects. The ratio of AUC and C_{max} for the nasal spray to gel was 89.97% and 88.62% respectively. As shown the 90% CI for AUC and C_{max} do not fall within the range of 80-125%.

Table 6: Summary of treatment comparisons for the intranasal formulations after baseline correction (N=23)

Parameter	Arithmetic mean (SD)	Geometric mean	Ratio (%B)	Ratio (%C)	90% CI
Nasal Spray (A) $AUC_{(0-t)}$ (pg*hr/ml)	2243.39 (11617)	18635	89.97	25.26	70.38-115.0
C_{max} (pg/ml)	748.04 (549)	640	88.62		66.53-118.05
Nasal Gel (B) $AUC_{(0-t)}$ (pg*hr/ml)	20713.5 (24085)	20713.5		28.08	
C_{max} (pg/ml)	1063.04 (1029)	722			
IM injection (C) $AUC_{(0-t)}$ (pg*hr/ml)	73771 (19615)	73771			
C_{max} (pg/ml)	10742 (3439)	10205			

Conclusions:

- The 90% confidence interval for baseline corrected AUC and C_{max} was outside the 80-125% range, the intranasal spray had 10% less AUC as compared to the intranasal gel.
- Since there are no clinical trials conducted, the clinical significance of the reduced exposure (10%) obtained with the nasal spray is not known.
- The relative bioavailability (baseline corrected) of the two intranasal formulations as compared to the intramuscular injection was found to be 0.061 (Spray) and 0.071 (Gel).
- The 90% confidence interval for AUC (baseline uncorrected) was within the 80-125% range. However the lower 90% interval for C_{max} (baseline uncorrected) was slightly below 80%, 79.8-108.96.
- The 90% confidence interval for AUC (baseline uncorrected) after exclusion of subjects 003 and 015 (recommended by DSI) was within the 80-125% range. However the lower 90% interval for C_{max} (baseline uncorrected) was slightly below 80%, 75.0-107.93.

- The 90% confidence interval for baseline corrected AUC and C_{max} was outside the 80-125% range after exclusion of subjects 003 and 015 (recommended by DSI).

3.5 Analytical

Q. Was the analytical assay for the cyanocobalamin plasma concentration validated?

The vitamin B_{12} in human serum was measured using the vitamin B_{12} — — : (commercially available competitive enzyme immunoassay). All assays were performed by — The lower limit of quantitation was 78.8 pg/ml and the upper limit of quantitation was 2140 pg/ml. Numerous subject samples required dilution because the vitamin B_{12} concentration exceeded the upper limit if the calibration curve (2140 pg/ml). Most of the dilutions were performed by the — DSI made the observations that the firm established dilution linearity for manual dilutions and similar experiments were not conducted for automated dilutions. In response to this the firm conducted these experiments and found analytical recoveries in the range of 87.3 – 108%.

The within and between batch precision and accuracy for vitamin B_{12} in stripped and unstripped serum was as follows:
Precision (%CV) for LOQ and low QC $\leq$ 7%.
Precision (%CV) for medium and high QC $\leq$ 6%.
Accuracy (%nominal) for LOQ and low QC within 96-120%.
Accuracy (%nominal) for medium and high QC within 101-130%.

4 Labeling Comments

Not applicable

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Jayabharathi Vaidyanathan
10/1/04 05:29:33 PM
PHARMACOLOGIST

Hae-Young Ahn
10/1/04 05:31:59 PM
BIOPHARMACEUTICS