

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 20849

STATISTICAL REVIEW(S)

Statistical Report on 20% ProSol™ - sulfite-free (Amino Acid) Injection in PL 146® Plastic Container, Baxter Healthcare Corporation, NDA 20-849

Requestor: Eric Colman

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Request

The request was for a statistical consult for a small (n=16) bioequivalency study comparing the plasma amino acid levels following infusion with ProSol 20% to those after Novamine 15% infusion. In particular, interest was in whether the statistical techniques used were appropriate to the primary objective of the study.

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Study Objectives

The study compares plasma amino acid and protein concentrations resulting from the infusion of the proposed product, 20% ProSol™ - sulfite-free (Amino Acid) Injection in PL 146® Plastic Container (Test), relative to a marketed, lower concentration drug product with the same active ingredients, Novamine® 15% - sulfite-free (Amino Acid) Injection in PL 146® Plastic Container (Reference). The primary objective was to compare plasma amino acid concentrations in normal human volunteers at baseline and at a steady state after receiving the two peripheral amino acid/dextrose infusions. The secondary objective was to evaluate overall nutritional comparability of the two amino acid formulations. Twenty four hour urinary nitrogen excretion were determined for each infusion period. In addition, the concentrations of plasma proteins (albumin, prealbumin, and transferrin) were determined at baseline and at 24 hours after initiation of each infusion period.

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Note: This report will focus only on the primary objective of the study, as requested.

Study Design

The study was a blinded, 2 period, 2 treatment, 2 sequence crossover study in normal human volunteers conducted at a single study site. The study contained 16 subjects, 8 males and 8 females. All subjects completed both arms of the crossover study. Subjects were randomized to a sequence according to a computer generated randomization schedule. On day one, subjects were infused with either ProSol or Novamine and on day two, subjects were infused with the other treatment. Infusion on each day began at 8 or 9 am and continued for 4.5 hours. There were no washout days between treatment days. Subjects on ProSol or Novamine received a peripherally infused dose of amino acids of 0.054 grams of amino acids/kg of body weight/hour. The mean total dose of amino acids over the course of the 4.5 hour infusion period was 0.253 grams/kg of body weight. Plasma amino acid concentrations were measured at baseline prior to infusion and at two hours and at four hours after initiation on infusion day 1 and day 2. The amino acids in the infusion included glycine, alanine, arginine, valine, lysine, proline, histidine, leucine, isoleucine, glutamic acid, aspartic acid, serine, phenylalanine, threonine, methionine, tryptophan, and tyrosine. Glutamine, which is derived metabolically, was determined in plasma, though it was not included in the infusion.

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Two assumptions regarding this study design were made by the sponsor:

1) that baseline levels of amino acids are equal for day 1 and day 2, that there are no carry-over effects from the prior day's infusion of amino acids,

2) that a stable plateau value of plasma amino acids would be reached and be maintained between approximately two hours of infusion and the end of infusion.

The sponsor tested these assumptions in the analysis of the plasma amino acid concentration data. The presence of a carryover effect was tested by comparing baseline values for day one to baseline values for day two. A significant difference was found. The baseline concentration for total amino acid was higher prior to the second infusion ($p < 0.001$). This indicates that there was a carryover from the prior day's infusion. To check the second assumption the sponsor tested for a significant difference between the individual amino acid levels at 2 hours and 4 hours after initiation of the infusion. Six of the amino acids showed a significant difference between 2 and 4 hours. These amino acids were isoleucine, methionine, phenylalanine, serine, tyrosine and valine. All of these amino acids except tyrosine showed an increase at 4 hours over 2 hours.

Note: We checked these two assumptions and obtained qualitatively similar results to those of the sponsor. We tested that the baseline levels on day one were equal to the baseline levels on day two using a paired t-test. Of the 21 tests, 14 had significantly higher values for day 2 over day 1 (increasing levels). All but two (histidine and glutamic acid) of the 21 tests had mean values for day 2 larger than day 1. This is similar to the results obtained from the sponsor.

We tested the second assumption that a stable plateau value was reached and maintained from 2 to 4 hours after the start of infusion by testing the hypotheses that the concentrations at 2 hours and at 4 hours were equal in period 1 and that the concentrations at 2 hours and at 4 hours were equal in period 2 using paired t-tests. The two periods were tested separately because of the carryover detected in the previous analysis. The sample size for these tests was 16. There were 3 significant p-values out of the 21 tests conducted for period 1. These were for methionine ($p=0.0131$), phenylalanine ($p=0.0068$) and valine ($p=0.0180$) where the values were increasing (values at 4 hours were higher than values at 2 hours). Nine of the 21 amino acids showed a decreasing trend. For period 2 there were 9 significant p-values. These were for alanine ($p=0.0001$), asparagine ($p=0.0001$), glutamine ($p=0.0117$), isoleucine ($p=0.0211$), methionine ($p=0.0001$), phenylalanine ($p=0.0002$), serine ($p=0.0420$), tyrosine ($p=0.0001$) and valine ($p=0.0001$). All were increasing except alanine, asparagine and tyrosine. With a conservative adjustment for multiple tests, (i.e., a test is significant with a p-value less than $0.05/21 = 0.002$), six remain significant, 3 with increasing concentrations and 3 with decreasing concentrations. Based on these results it is difficult to determine if a steady state has been reached. With the agreement of the medical officer, we chose to use the average of 2 hours and 4 hours as our primary variable, as an estimate of the steady state value, for all of the amino acids.

Study variables

The primary outcome was the change in plasma amino acids concentrations from a baseline fasted state. Concentration changes of individual amino acids, the total concentration of amino acids, and the total concentration of nutritionally essential amino acids were evaluated. The concentrations were determined at baseline and at two and four hours after initiation of the infusion on day 1 and day 2. The amino acids in the infusion included:

glycine	alanine	arginine	
valine	lysine	proline	
histidine	leucine	isoleucine	APPEARS THIS WAY
glutamic acid	aspartic acid	serine	ON ORIGINAL
phenylalanine	threonine	methionine	
tryptophan	tyrosine		

glutamine (derived metabolically from some of the infused amino acids).

Asparagine and cystine concentrations were included for completeness. However, the analytical method for these two amino acids may be unreliable. The analytical method used for plasma amino acid concentration does not accurately measure tryptophan concentrations. They were not reported by the analytical laboratory.

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Note: The sponsor chose to use a change from baseline to steady state value to correct for the carryover effect. It is not clear, however, that this variable completely corrects the problem of carryover.

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Statistical analysis plan

As stated in the final study report, comparability between treatments was evaluated by determining if the 90 or 95% confidence intervals of the difference between test and reference of each amino acid were contained within a comparability interval. This interval, defined as 25% of the reference range, was calculated by determining the width of the normal range for each amino acid during a fasted state and dividing it by 4, delta. The comparability interval ranged from -delta to +delta. If the 90% confidence interval on the difference between test and reference was contained within the interval -delta to +delta, then the amino acid was considered to be comparable for the two solutions. The sponsor's results are give in Table 1 (from table 9 of the final study report). Column 1 states the amino acid. Columns 2 and 3 give the means of the change from baseline variable for ProSol and Novamine. Column 4 gives the difference in the means and column 5 gives the sponsor's 90% confidence interval for the difference. Columns 6 gives the reference range for that particular amino acid and column 7 states one quarter of the width of the reference range, delta. Column 8 states whether or not the 90% confidence interval was contained within -delta and +delta. All but glycine, histidine and methionine were considered comparable by this test. The last column contains our calculations of the 90%

confidence interval. Note that the sponsor did not run the analysis on asparagine or cystine because the analytical methods was unreliable.

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Note: Due to the presence of a carryover from period 1 to period 2, it is difficult to come up with an adequate test to compare the test and reference products. The sponsor's test focuses on the difference between the test product response and the reference product response and is based on the assumption that correcting for baseline overcomes the carryover effects. Under this assumption, we also calculated these intervals, given in the last column of table 1. The intervals we obtained are slightly larger. Since there are no typical regulatory boundaries for declaring equivalence for the difference in responses, we will not consider the sponsor's test of comparability.

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Typically, bioequivalence tests are based on the ratio of responses of test to reference. A 90% confidence interval is calculated for the ratio and the products are considered bioequivalent if the interval is contained within the regulatory bounds of *If we can assume that subtracting baseline corrects for the obvious carryover problem and that there is no unequal carryover (i.e., carryover for test is different than carryover for reference) we can use a variation of this method for performing a bioequivalence test. Note that we cannot use the typical method based on using log transformed data because the change from baseline variable contains negative values. The method is described below.*

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The test is a two one-sided test procedure. It tests both that test response was not significantly higher than 125% or lower than 80% of the response. The null and alternative hypotheses are

$$H_0: \text{Test} \leq .80 \text{ Ref or } \text{Test} \geq 1.25 \text{ Ref}$$

$$H_A: .80 \text{ Ref} < \text{Test} < 1.25 \text{ Ref}$$

The lower and upper confidence limits are

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$$\text{Test} - 1.645 \sqrt{\text{MSE}(1/n_T + .64/n_R)} \quad \text{and} \quad \text{Test} + 1.645 \sqrt{\text{MSE}(1/n_T + 1.56/n_R)}$$

where Test is the mean response for test product and Ref is the mean response for the reference product, n_T and n_R are the number of subjects in test treatment group and in the reference treatment group. The lower limit would need to be larger than 80% of Ref to pass. The upper limit would need to be smaller than 125% of Ref to pass. Since the standard errors are different for the high and low bounds a traditional confidence interval could not be calculated.

The results of this analysis are given below in Table 2. The model contained factors for period, sequence and subject and was run in SAS Proc GLM. The first column states the amino acid. The second and third columns states the mean for ProSol and the mean for Novamine. Columns 4 and 5 give the sample size and the residual variability. The lower confidence limit along with the lower bound (.80*Ref) and the upper confidence limit along with the upper bound (1.25*Ref)

are given in columns 6 - 9. Column 10 states whether that amino acid passed the test for bioequivalence (p=pass, f=fail). Of the 21 tests run only 1 passed for equivalence, methionine.

If we do not want to make the assumption that test and reference carryover are equal and that correcting for baseline is adequate for the lack of the washout period, we can use the data from the first period only, not correct for baseline, and treat the data as if it were from a parallel design. The sample size is reduced to 8 for each treatment arm and each subject only contributes one measurement. When we do this we are able to use the typical bioequivalence analysis using log transformed data. The outcome variable was the average of 2 hours and 4 hours rather than a change from baseline to equilibrium value. Table 3 contains bioequivalence analyses for these data. The first column states the amino acid. Column two gives the estimate of the ratio of test to reference. The third column states the 90% confidence interval about the ratio of the test product to the reference product. Often times drugs are considered bioequivalent if the 90% confidence interval is contained within 0.8 and 1.25. Column 4 states whether or not it passed the 0.80 to 1.25 boundaries. With this test, 11 out of the 21 tests passed the test for bioequivalency. Note that a problem with this test is that the sample size is essentially cut in half, greatly reducing the power of determining bioequivalence.

Conclusions

There are three analyses to look for equivalence between these two products. The first is based on the sponsor's analysis and is given in table 1. This analysis calculates a confidence interval about the difference between the test response and the reference response, using the change from baseline variable to correct for the effects of carryover. The second analysis, which also uses the change from baseline variable, tests whether or not the ratio of responses for test and reference is contained within . . . This also assumes that the change from baseline value corrects for the effects of carryover. The third analysis uses only the period one data and uses the average of the 2 hour and 4 hour measurements. This calculates the typical bioequivalence analysis based on log transformed data.

In summary, using the bounds . . . for the ratio, our analysis using period one data only found 9 amino acids, total and total essential bioequivalent while using all the data but with change from baseline as the primary variable found only one amino acid to be bioequivalent. The reason for this difference may be due to increased variability of the change from baseline variable over the average of 2 and 4 hours and to the problems associated with carryover effects due to the lack of a washout period. The estimate of the direct effect of the drug may be confounded with carryover effects.

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July 30, 1998

The primary request was in whether the statistical techniques used were appropriate to the primary objective of the study. Due to the presence of carryover and to the non standard test that was proposed, the sponsor's final statements of comparable versus not comparable do not seem appropriate for this study. The decision of whether or not these two products are comparable should be decided based on a clinical opinion of the confidence intervals of the difference stated in table 1 and on the bioequivalence tests reported in tables 2 and 3. It should be kept in mind that the analysis in tables 1 and 2 may be inaccurate due to the effect of carryovers.

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Table 1: Differences in amino acids concentrations with confidence intervals

<i>Amino Acid</i>	<i>ProSol LSmean</i>	<i>Novamine LSmean</i>	<i>Difference (ProS.-Nov.)</i>	<i>Sponsor's 90% C.I.</i>	<i>Reference Range</i>	<i>Range/4</i>	<i>Comparable ?</i>	<i>Our 90% C.I.</i>
Alanine	144.8	87.1	57.69	(32.8, 82.6)		101.8	Yes	(23.3, 92.1)
Arginine	60.8	58.9	1.88	(-7.2, 11.0)		29.75	Yes	(-10.5, 14.2)
Aspartic Acid	2.6	2.8	-0.16	(-0.9, 0.6)		2.5	Yes	(-1.1, 0.8)
Glutamic Acid	13.2	13.0	0.19	(-3.9, 4.3)		27.75	Yes	(-5.5, 5.9)
Glutamine	40.1	-19.0	59.03	(28.7, 89.4)		127.3	Yes	(16.2, 101.8)
Glycine	166.4	66.2	100.2	(81.8, 118.6)		76.5	No	(75.5, 124.8)
Histidine	42.7	33.1	9.66	(-0.8, 20.1)		19.5	No	(-7.2, 26.5)
Isoleucine	41.1	26.5	14.94	(10.9, 19.0)		28.5	Yes	(10.2, 19.7)
Leucine	14.9	24.6	-9.66	(-15.4, -3.9)		32.5	Yes	(-16.6, -2.7)
Lysine	62.0	59.0	3.00	(-17.1, 23.1)		39.25	Yes	(-27.0, 33.0)
Methionine	32.4	36.5	-4.13	(-6.8, -1.5)		6.25	No	(-7.6, -0.6)
Phenylalanine	27.2	36.8	-9.59	(-13.1, -6.1)		17.50	Yes	(-13.9, -5.3)
Proline	60.0	22.9	37.06	(18.0, 56.1)		71.50	Yes	(8.1, 66.0)
Serine	39.2	24.1	15.09	(3.4, 26.8)		29.00	Yes	(4.7, 25.5)
Threonine	58.0	32.7	25.31	(16.3, 34.3)		35.00	Yes	(13.1, 37.5)
Tyrosine	-10.9	-11.7	0.87	(-1.5, 3.3)		20.50	Yes	(-2.4, 4.2)
Valine	106.3	67.4	38.88	(26.2, 51.6)		62.00	Yes	(23.3, 54.5)
Total	901.1	560.9	340.2	(209.8, 470.7)		727.0	Yes	(168.2, 512.3)
Total Essential	385.0	316.6	68.41	(16.2, 120.6)		240.5	Yes	(-2.0, 138.9)

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Table 2: Two one-sided tests of Change from Baseline variable

Amino Acid	mean of Prosol	mean of Novamine	N	sigma ²	.80*Ref		1.25*Ref		pass or fail?
					lower CI	lower limi	upper CI	upper limit	
Alanine	144.8	87.1	16	3045	115.74	69.68	181.13	108.88	f
Arginine	60.8	58.9	16	393	50.36	47.12	73.85	73.63	f
Asparagine	-4.7	-22.3	16	237	-12.81	-17.84	5.43	-27.88	f
Aspartic Acid	2.59	2.75	16	2.46	-1.76	2.20	3.62	3.44	f
Cystine	1.75	2.75	16	36.5	-1.43	2.20	5.73	3.44	f
Glutamic Acid	13.2	13	16	83.8	-8.38	10.40	-19.23	16.25	f
Glutamine	40.1	-19	16	4722.7	3.91	-15.20	85.34	-23.75	f
Glycine	166.4	66.2	16	1526.4	145.82	52.96	192.12	82.75	f
Histidine	42.8	33.1	16	729.3	28.58	26.48	60.58	41.38	f
Isoleucine	41.4	26.5	16	57.9	37.39	21.20	46.41	33.13	f
Leucine	14.9	24.6	16	124.8	-9.02	19.68	22.25	30.75	f
Lysine	62	59	16	2323.5	-36.61	47.20	93.73	73.75	f
Methionine	32.4	36.5	16	31.7	29.43	29.20	36.11	45.63	p
Phenylalanine	27.2	36.8	16	46.9	23.59	29.44	31.71	46.00	f
Proline	60	22.9	16	2165.3	35.49	18.32	90.63	28.63	f
Serine	39.2	24.1	16	281.2	30.37	19.28	50.24	30.13	f
Threonine	58	32.7	16	385.9	47.65	26.16	70.93	40.88	f
Tyrosine	-10.9	-11.8	16	28.2	-13.70	-9.44	-7.40	-14.75	f
Valine	106.3	67.4	16	629	93.09	53.92	122.81	84.25	f
Total	901.1	560.9	16	76321.5	755.60	448.72	1082.97	701.13	f
Total Essential	385	316.6	16	12797	325.42	253.28	459.47	395.75	f

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Table 3: Two one-sided test on Average of 2 and 4 hours with period 1 only

<i>Amino Acid</i>	<i>Test to Reference Ratio</i>	<i>90% C. I.</i>	<i>p/f?</i>
Alanine	1.23	(1.05, 1.45)	f
Arginine	1.11	(0.99, 1.25)	p
Asparagine	1.27	(0.99, 1.63)	f
Aspartic Acid	0.83	(0.61, 1.13)	f
Cystine	1.09	(0.88, 1.35)	f
Glutamic Acid	1.05	(0.73, 1.50)	f
Glutamine	1.08	(0.98, 1.20)	p
Glycine	1.24	(1.02, 1.51)	f
Histidine	0.97	(0.89, 1.07)	p
Isoleucine	1.25	(1.12, 1.40)	f
Leucine	0.97	(0.85, 1.11)	p

<i>Amino Acid</i>	<i>Test to Reference Ratio</i>	<i>90% C. I.</i>	<i>p/f?</i>
Lysine	0.98	(0.86, 1.13)	p
Methionine	0.91	(0.82, 1.02)	p
Phenylalanine	0.92	(0.83, 1.02)	p
Proline	1.08	(0.93, 1.26)	f
Serine	1.05	(0.85, 1.29)	f
Threonine	1.07	(0.91, 1.24)	p
Tyrosine	1.00	(0.84, 1.19)	p
Valine	1.17	(1.04, 1.30)	f
Total	1.10	(1.01, 1.21)	p
Total Essential	1.04	(0.94, 1.16)	p

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