

Determination of Water- and Fat-Soluble Vitamins in Functional Waters by HPLC with UV-PDA Detection

INTRODUCTION

Functional beverages are vitamin-enhanced waters that have gained consumer popularity for convenience, perceived health benefits, and improved flavor over tap water. These beverages are typically enriched with Vitamin C, B-complex vitamins, and Vitamins A and E, with the advertised benefits of increased energy from the B vitamins and antioxidant benefits from Vitamins A, C, and E. Industry forecasts predict the sales of these beverages to increase to 4,388.9 million liters per year by 2011.¹

Labeling the nutritional content of these beverages is regulated by the U.S. Food and Drug Administration (US FDA). Therefore, methods are needed to assay the vitamins to support product labeling. Determination of vitamins in foods is inherently difficult and deviation of the determined amounts of a vitamin from labeled amounts has been observed.² Analysis of these beverages presents a challenge due to the presence of both water- and fat-soluble vitamins. Proprietary formulations of vitamins that remain soluble and shelf-stable are used to enrich these beverages. Additionally, gums, preservatives, and other additives are used to emulsify and stabilize the drink.

Traditional analysis of vitamin products requires several different methods to quantify the additives. Water-soluble vitamins are often determined with RP-HPLC using an aqueous mobile phase, while the fat-soluble vitamins use organic solvent mobile phases in both reversed and normal-phase HPLC methods.³ Combined methods evaluating both types of vitamins pose a challenge due to the difference in solubility limits of the two classes of vitamins and the many different biologically equivalent compounds that can be added, but are listed as a single vitamin. For example, niacin is available as nicotinic acid and nicotinamide, which are both biologically active and referred to as niacin in product labeling.

The simultaneous determination of a wide range of vitamins increases the complexity of an analytical method. Vitamin structures range from small unconjugated organic acids, such as pantothenic acid (Vitamin B5) that are minimally UV active, to large complexes that absorb at different wavelengths, such as cyanocobalamine (Vitamin B12). Multiple detection wavelengths are needed to optimize sensitivity due to the chemical diversity of vitamins.

In this application note, an Acclaim® PolarAdvantage II column is used to determine water- and fat-soluble vitamins in a single method. This column contains a high-efficiency, silica-based, polar-embedded stationary phase manufactured by bonding a proprietary amide-embedded ligand to high-purity spherical silica. It is compatible with 100% aqueous mobile phases over a wide pH range (1.5–10), and provides excellent peak shapes and efficiencies for both basic and acidic compounds. The gradient method in this application uses the aqueous compatibility of this column by beginning with a 100% aqueous mobile phase and ending with a 100% organic solvent mobile phase. Linearity, detection limits, precision, and recovery are demonstrated. This method provides a rapid means of analyzing a sample for both water- and fat-soluble vitamins in a single injection using a low flow rate that minimizes mobile phase preparation time and reduces waste.

EQUIPMENT

Dionex UltiMate® 3000 Intelligent LC system:

SRD-3200 Solvent Rack (Dionex P/N 5035.9250)

HPG-3200M pump (Dionex P/N 5035.0018)

WPS-3000TSL Micro Autosampler
(Dionex P/N 5822.0025)

Sample Loop, 25 µL (Dionex P/N 6820.2415)

TCC-3200 column compartment
(Dionex P/N 5722.0025)

PDA-3000 detector (Dionex P/N 5080.0020)

Semi-Micro PEEK™ flow cell, 3 µL
(Dionex P/N 064169)

Chromeleon 6.8 Chromatography Data System

Glass injection vials with caps and septa, 1.5 mL
(Dionex P/N 055427)

Nalgene® Filter Unit, 0.2 µm nylon membrane,
1L capacity (Nalgene P/N 164-0020)

REAGENTS AND STANDARDS

Deionized water, Type I reagent grade, 18 MΩ-cm resistivity or better

Formic Acid, purris. p.a. grade or better
(Fluka P/N 06440)

Acetonitrile, HPLC grade or better (B&J P/N 015-4)

Methanol, HPLC grade or better (B&J P/N 230-4)

Folic Acid (AccuStandard P/N VIT-0007N)

Vitamin B6 as Pyridoxine-HCl
(AccuStandard P/N VIT-003N)

Vitamin E as DL-α-Tocopherol Acetate
(AccuStandard P/N VIT-015N)

Vitamin A as Retinol Palmitate
(AccuStandard P/N VIT-014N)

Niacin as Nicotinic Acid (AccuStandard P/N VIT-005N)

Niacin as Nicotinamide (AccuStandard P/N VIT-006N)

Vitamin B5 as D-Pantothenic Acid
(AccuStandard P/N VIT-008N)

Vitamin C as Ascorbic Acid
(AccuStandard P/N VIT-004N)

Vitamin B12 as Cyanocobalamine
(AccuStandard P/N VIT-010N-R1)

Samples

Three fruit-flavored vitamin-enhanced beverages (Labeled Brands A, B, and C) were analyzed for vitamin content. Samples were diluted 1:1 with 0.015% formic acid prior to analysis. The ingredients are listed in Table 1.

Table 1. Ingredients in Vitamin-Enhanced Functional Beverages

Brand A	Brand B	Brand C
water	water	purified water
sucrose syrup	cane sugar	sugar
citric acid	crystalline fructose	citric acid
natural flavors	natural flavors	natural flavors
sodium citrate	citric acid	potassium citrate
potassium citrate	ascorbic acid (vitamin C)	calcium lactate
sucralose	vitamin E acetate	calcium gluconate
vitamin C	fruit and vegetable juice (color)	magnesium lactate
vitamin E acetate	magnesium lactate	modified corn starch
niacinamide	calcium lactate	ginseng extract
calcium disodium EDTA	niacin	caffeine
calcium pantothenate	monopotassium phosphate	guarana seed extract
pyridoxine hydrochloride	pantothenic acid	vegetable juices (color)
acesulfame potassium	pyridoxine hydrochloride	acacia gum
vitamin B12	vitamin B12	calcium disodium EDTA
	folic acid	ribose
		niacinamide
		vitamin E acetate
		calcium pantothenate
		zinc gluconate
		pyridoxine hydrochloride
		manganese gluconate
		EGCG (epigallocatechin gallate)
		vitamin A palmitate
		vitamin B12

CONDITIONS

Column: Acclaim PolarAdvantage II 3 μ m, 2.1 \times 150 mm (Dionex P/N 063187)

Gradient: Mobile Phase A:
0.015% Formic Acid in DI water
Mobile Phase B:
17/83 Methanol/Acetonitrile
100% A for 3 min, 0-45% B in 5 min,
45-100% B in 0.1 min,
100% B for 16.9, 5 min of equilibration
at 100% A prior to injection

Flow Rate: 0.21 mL/min

Temperature: 40 °C (column compartment)

Inj. Volume: 5 μ L

Detection: Photodiode Array; 210, 280, and 350 nm

Noise: ~0.28 mAU at 210 nm

~0.12 mAU at 280 nm and 350 nm

System

Backpressure: ~1120 psi at 100% A, 510 psi at 100% B

PREPARATION OF SOLUTIONS AND REAGENTS

Mobile Phase A (0.015% Formic Acid)

To prepare this solution, measure 2 L (2000 g) of DI water in a 2 L glass eluent bottle. Using a 1 mL graduated pipet, add 0.30 mL of formic acid to the water. Mix well and briefly degas the solution.

Mobile Phase B (17/83 methanol/acetonitrile)

To prepare this solution, transfer 340 mL methanol into a 2 L volumetric flask. Bring to volume with acetonitrile. Mix well. Do not adjust the volume of solution after mixing. Transfer the solution to a 2 L glass eluent bottle and briefly degas the solution.

Water-Soluble Vitamin Standards

Vitamin standards of pyridoxine HCl, nicotinic acid, nicotinamide, D-pantothenic acid, and cyanocobalamin were prepared by accurately weighing 10–20 mg of the vitamin powder and adding DI water to a total of 10–20 g to form a stock solution of 1.0 mg/mL for each individual vitamin. Due to the limited stability of ascorbic acid, a stock solution of 2.0 mg/mL was freshly prepared weekly. Folic acid is not soluble in water as the free acid. To convert the vitamin to folate, 5 mg of folic acid were dispersed in 4 mL of DI water with a minimum amount of 0.45% potassium hydroxide added to convert the folic acid to potassium folate. This solution was then diluted with DI water to yield a total volume of 5 mL (5 g) to form a solution of 1.0 mg/mL folic acid as potassium folate. Water-soluble vitamin stock solutions were stored at -20 °C when not in use. Working standards containing vitamins in 0.015% formic acid (mobile phase A) were prepared on the day of use from these stock solutions.

Fat-Soluble Vitamin Standards

The fat-soluble vitamins were prepared in acetonitrile by weighing 10 mg of DL- α -tocopherol acetate and 2.0 mg of retinol palmitate, respectively, in separate 20 mL glass vials. Acetonitrile was added to yield a 1 mg/mL solution of DL- α -tocopherol acetate and a 0.2 mg/mL solution of retinol palmitate. Retinol palmitate requires several minutes of vortex mixing to dissolve. These solutions were stored at 4 °C in the dark. Stock solutions were allowed to equilibrate at room temperature and mixed using a vortex mixer to ensure that the oils were thoroughly dissolved before being used to make working standards. Retinol palmitate is photosensitive and should be protected from light.

Calibration standards of retinol palmitate and DL- α -tocopherol acetate were not prepared in a matrix of 0.015% formic acid. Instead, due to solubility limitations, working standards of these vitamins were prepared in mobile phase B from stock solutions prepared in acetonitrile and determined separately from the water-soluble vitamins. Retinol palmitate is difficult to prepare as an aqueous solution. Many fat-soluble vitamins are available as water miscible or soluble formulations; however, commercially available standards were used to evaluate this method. In this case, solubility of the fat-

soluble vitamins, when added to a water-soluble vitamin mixture, was limited. For this reason, a separate standard curve was prepared for the fat-soluble vitamins in mobile phase B.

Sample Preparation

Fruit-flavored vitamin-enhanced water samples were prepared by diluting 500 μ L sample beverage with 500 μ L of mobile phase A. For reference, the ingredients in these enhanced water samples are listed in Table 1.

Precautions

Sample filtration is not recommended due to adsorption of the fat-soluble vitamins to plastic surfaces. Filtration was shown to remove these vitamins from solution.

The solubility of the fat-soluble vitamins in methanol-acetonitrile mixtures was assessed. The best solubility of Vitamin A was found in acetonitrile. Mixtures with methanol decreased the apparent solubility of Vitamin A, as retinol palmitate.

The autosampler tray should be maintained at 4 °C and the use of the tray shake option is recommended to ensure sample homogeneity during the runs.

RESULTS AND DISCUSSION

Separation and Detection

Figure 1 demonstrates separation of water- and fat-soluble vitamins in a single run using an Acclaim Polar Advantage II column. Sodium citrate and citric acid, which are common ingredients to enhance tartness, were added to the mixture to demonstrate the separation between nicotinamide and citrate. As shown in Figure 1, citrate and the vitamins are well separated. Vitamins are a structurally diverse group of compounds with different absorbance maxima. For example; retinol palmitate has a strong absorption at 350 nm, while D-pantothenic acid absorbs best in the UV range between 200 and 225 nm. Given the wide UV absorbance range for the vitamins commonly added to enhanced waters, wavelengths of 210, 280, and 350 nm were chosen for detection. In Figure 1, the chromatogram collected at 210 nm captures each of the vitamins except for Vitamin A. Additionally, many smaller acids, including formic acid, absorb at 210 nm. For this reason, the baseline absorbance shifts during the gradient as the amount of formic acid in the mobile phase changes.

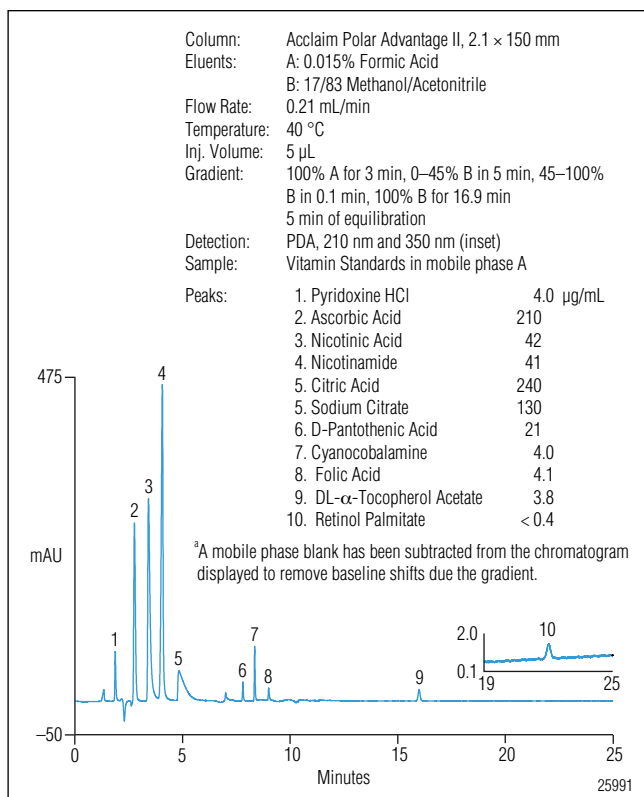


Figure 1. Separation of vitamins on the Acclaim Polar Advantage II column.^a (citric acid and citrate both contribute to peak 5.)

The chromatograms shown here have had a mobile phase blank subtracted from the raw data to correct the baseline shifts. This action can be automated in Chromeleon by acquiring a system blank before samples and using post-acquisition steps within the acquisition program to subtract the blank, or it can be done manually with the Arithmetic Combination option.

Linearity, Limit of Detection, and Limit of Quantitation

The linearity, LOD, LOQ, and the precision data for this gradient method were determined for the determination of vitamins (Table 2). With the exception of ascorbic acid, the LOD was determined by the concentration of the analyte that provides a peak height that is three times the measured noise ($S/N = 3$) and the LOQ was determined as the concentration of the analyte that provides a peak height that is ten times the measured noise ($S/N = 10$). The figures of merit for Vitamin B6 are presented for two wavelengths. Detection at 210 nm is convenient; however, it also maximizes the potential for detecting interfering components. Vitamin B6 sensitivity and linearity are equivalent when detecting at 210 or 280 nm. Either wavelength is appropriate, which provides flexibility in optimizing the method for a particular sample. Folic acid can be detected at 210 nm; however, detection is more sensitive at 280 nm.

Table 2. Linearity, LOD, and LOQ of Vitamins Analyzed

Analyte	Detection Wavelength (nm)	Correlation Coefficient (r^2)	Range (µg/mL)	LOD (ng/mL)	LOQ (ng/mL)	Retention Time Precision (RSD)* n=7	Peak Area Precision (RSD)* n=7
Pyridoxine HCl	210	0.9994	1.0–20	20	60	0.10	0.28
Pyridoxine HCl	280	0.9997	1.0–20	20	60	0.10	0.75
Ascorbic Acid	210	0.9992	15–300	5000	5000	0.07	3.47
Nicotinic Acid	210	0.9995	5.0–100	84	250	0.07	0.40
Nicotinamide	210	0.9995	5.0–100	35	100	0.08	0.54
D-Pantothenic Acid	210	0.9993	5.0–100	213	640	0.23	0.75
Cyanocobalamine	210	0.9995	1.0–20	14	40	0.09	0.83
Folic Acid	280	0.9987	0.25–2.5	20	60	0.16	0.97
DL-α-Tocopherol Acetate	210	0.9985	1.2–25	83	250	0.14	0.77
Retinol Palmitate	350	0.9996	0.63–12	125	400	0.17	0.54

*Analyte concentrations for precision injections: Pyridine HCl (5 µg/mL), Ascorbic Acid (77 µg/mL), Nicotinic Acid, Nicotinamide, and Pantothenic Acid (25 µg/mL each), Cyanocobalamine (5 µg/mL), Folic Acid (1.3 µg/mL), DL-α-Tocopherol Acetate (6.2 mg/mL), and Retinol Palmitate (1.3 µg/mL)

For ascorbic acid, the value listed in Table 2 is the amount that could be consistently measured when dissolved in a 0.015% formic acid solution without additional preservatives. Ascorbic acid (AA) exists in solution in equilibrium with the oxidation product dehydroascorbic acid (DHAA). While both compounds are biologically active as Vitamin C, DHAA does not have a strong UV absorption and therefore is difficult to quantify. The oxidation reaction is reversible and is minimized at low pH or by adding a reducing agent to prevent oxidation of ascorbic acid by dissolved oxygen.⁴ Peak areas for AA concentrations that were <5 µg/mL of were not reproducible due to the rapid oxidation of the sample at low concentrations. Preservation of AA at an acidic pH was not feasible due the presence of folic acid, which precipitates at low pH. While folic acid is classified as a water-soluble vitamin, it is weakly soluble as the protonated organic acid.

Functional Beverage Samples

Water- and fat-soluble vitamins are determined in fruit-flavored vitamin-enhanced waters as demonstrated in Figures 2 and 3. Brand A is a vitamin-enhanced water that is sweetened with artificial sweeteners (Figure 2). Figure 3 shows results from the analysis of Brand C, an enhanced beverage sweetened with sugar and containing natural extracts and caffeine. This sample is a more complex matrix than Brand A as shown by the large number of unidentified peaks. In both samples, citrate is resolved from nicotinamide and neither sample contains nicotinic acid. A wavelength of 280 nm was chosen for determining Vitamin B6 due to limited interferences at this wavelength relative to detection at 210 nm. As can be seen in Figure 2 and more dramatically in Figure 3, there is coelution of peaks near the retention time of Vitamin B6. Detection at 280 nm does reduce these interfering peaks.

Precision and Accuracy

As shown in Table 2, retention time precision of the standards is excellent, with RSDs ranging from 0.07 to 0.23. This demonstrates good precision of the gradient using the HPG- 3200M. The compounds that elute during the gradient have only a slight increase in the deviation of the retention times. With the exception of ascorbic acid, peak area precision is excellent with RSDs of <1.0. Due to the equilibrium with DHAA, in the absence of a preservative AA is prone to larger changes in peak area.

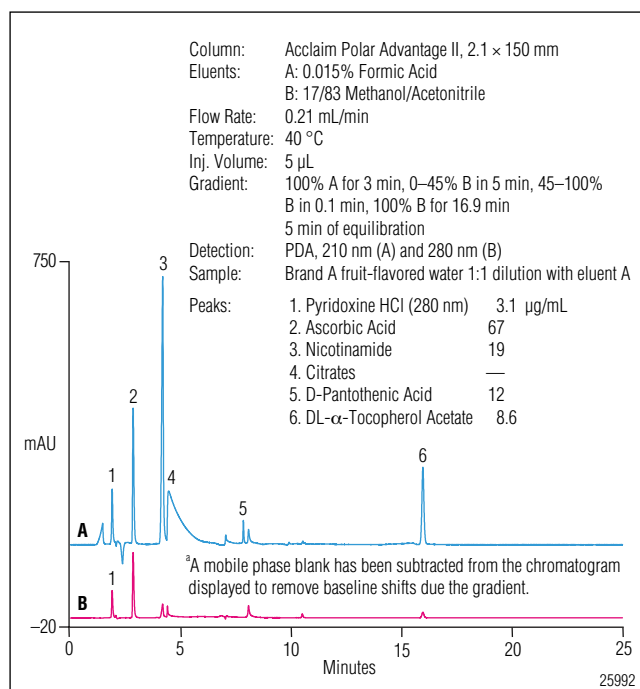


Figure 2. Separation of Brand A, a fruit-flavored, artificially-sweetened, vitamin-enhanced water.^a

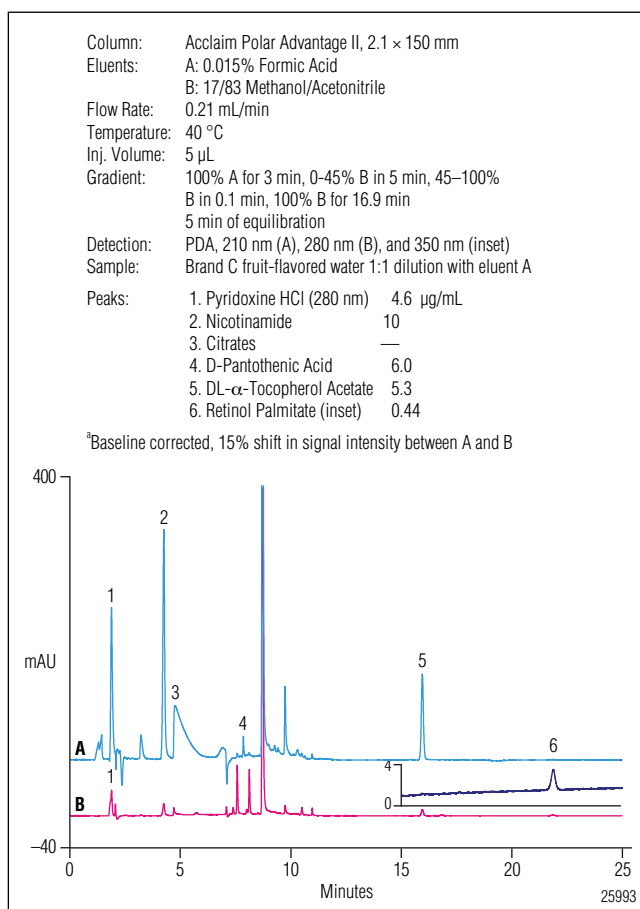


Figure 3. Separation of Brand C, a fruit-flavored, sugar-sweetened, vitamin-enhanced water with added natural extracts and caffeine.^a

Three brands of vitamin-enhanced water were analyzed over three days to evaluate the precision of the method. Representative data from Brands A, B, and C are presented in Table 3. Summarized data for between day precision for all three samples are in Table 4. Between-day retention time precision ranges from 0.04% and 0.23%, which is equivalent to the precision of the standards. Interday peak area precision ranges from 0.37% to 9.5%. The increased imprecision observed in the folic acid results is due to both the presence of a closely eluting peak present in the Brand B sample and to the low amounts of folic acid present in the sample. While this is a challenging matrix and there is a low concentration of folic acid, it is still easily quantified.

Recoveries (Table 5) for the water soluble vitamins ranged from 93% to 119%. As with precision, the extremes in recoveries were for ascorbic acid and folic acid. Despite the challenges in quantifying these two vitamins, the recoveries are good, proving method accuracy. In this recovery experiment, samples were spiked with folic acid at the lower end of the calibration curve. This concentration of vitamin, while within the linear range for standards, is susceptible to integration error in the beverage matrix due to both potential interfering peaks, and a sloping baseline during the gradient.

Direct spiking of the acetonitrile stocks of Vitamins A and E into vitamin-enhanced waters led to the formation of an unstable suspension. Therefore, a control sample was used to indirectly measure recovery. Use of plastic sample vials exacerbates the removal of fat-soluble vitamins from solution and should be avoided. Recoveries for fat-soluble vitamins were determined by comparison of spiked samples to a control sample of retinol palmitate and DL- α -tocopherol acetate in 0.015% formic acid leading to recovery values ranging between 101%–110%.

As an additional check, the determined values of Vitamin A and Vitamin E were compared to the label claim. Brand A claims to provide 10% of the DV of Vitamin E per serving and Brand B claims 20% of the DV of Vitamin E. In this study, 15% and 25% of Vitamin E were determined in Brands A and B, respectively. Brand C claims 10% each of vitamins A and E. The determined amounts were 10% each, based on FDA guidelines for nutritional labels.⁵ In the absence of a formulated aqueous-soluble mixture of the lipophilic vitamins, these results agree well with the label claim and show suitability of the method to determine these vitamins in water.

CONCLUSION

An Acclaim PolarAdvantage II column was used to determine water- and fat-soluble vitamins in a single injection. The described gradient method uses the full range of eluent compatibility of the Acclaim PA II with 100% aqueous to 100% organic mobile phases. Additionally, these mobile phases are MS compatible, allowing for a complementary detection method. This single-method determination of both water- and fat-soluble vitamins was shown to have good precision, linearity, recovery, and LOQs, making it an excellent method for determination of vitamins in complex aqueous matrixes such as vitamin-enhanced flavored waters.

LIST OF SUPPLIERS

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03842 USA. Tel: 800-766-7000, www.fishersci.com
Sigma-Aldrich, P.O. Box 14508, St. Louis, MO 63178
USA. Tel: 800-325-3010. www.sigma-aldrich.com
AccuStandard, Inc. 125 Market Street, New Haven,
CT 06513 USA Tel: 203-786-5290.
www.AccuStandard.com

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Table 3. Sample Analysis Precision, n=3

Day	Vitamin	Peak Area Precision (RSD)	Retention Time (min)	Retention Time Precision (RSD)	Amount (µg/mL)
Brand A					
1	Pyridoxine HCl	0.32	1.88	0.14	3.1
	Ascorbic Acid	6.1	2.83	0.16	67
	Nicotinamide	0.65	4.17	0.07	19
	D-Pantothenic Acid	0.95	7.83	0.10	12
	Vitamin E	0.74	15.95	0.12	8.6
2	Pyridoxine HCl	1.4	1.88	0.08	3.2
	Ascorbic Acid	0.30	2.83	0.09	72
	Nicotinamide	1.4	4.16	0.10	19
	D-Pantothenic Acid	2.0	7.82	0.12	13
	Vitamin E	0.88	15.94	0.14	8.8
3	Pyridoxine HCl	0.75	1.86	0.06	3.2
	Ascorbic Acid	9.9	2.84	0.04	64
	Nicotinamide	0.57	4.19	0.04	19
	D-Pantothenic Acid	0.91	7.84	0.23	13
	Vitamin E	1.7	15.92	0.10	8.7
Brand B					
1	Pyridoxine HCl	0.89	1.83	0.10	3.2
	Ascorbic Acid	1.2	2.84	0.10	72
	Nicotinamide	0.59	4.27	0.06	9.4
	D-Pantothenic Acid	1.7	7.83	0.18	5.1
	Folic Acid	1.3	9.12	0.19	0.25
	Vitamin E	1.3	15.94	0.12	15
2	Pyridoxine HCl	0.98	1.82	0.11	3.2
	Ascorbic Acid	1.2	2.84	0.07	72
	Nicotinamide	1.0	4.26	0.06	9.4
	D-Pantothenic Acid	1.1	7.82	0.19	5.0
	Folic Acid	2.3	9.10	0.08	0.24
	Vitamin E	0.65	15.93	0.14	15
3	Pyridoxine HCl	0.47	1.81	0.06	3.2
	Ascorbic Acid	0.42	2.85	0.04	61
	Nicotinamide	0.53	4.25	0.05	9.7
	D-Pantothenic Acid	3.46	7.83	0.20	4.8
	Folic Acid	1.5	9.15	0.08	0.21
	Vitamin E	1.1	15.90	0.11	14
Brand C					
1	Pyridoxine HCl	0.47	1.86	0.13	2.2
	Nicotinamide	1.27	4.24	0.02	10
	D-Pantothenic Acid	0.79	7.82	0.17	6.0
	Vitamin E (dl-alpha-tocopherol acetate)	1.21	15.94	0.14	5.3
	Vitamin A (retinol palmitate)	1.56	21.86	0.19	0.44
2	Pyridoxine HCl	0.55	1.87	0.11	2.3
	Nicotinamide	0.62	4.24	<0.01	10
	D-Pantothenic Acid	0.58	7.83	0.16	6.0
	Vitamin E (dl-alpha-tocopherol acetate)	1.59	15.95	0.12	5.4
	Vitamin A (retinol palmitate)	2.07	21.88	0.12	0.43
3	Pyridoxine HCl	1.26	1.84	0.10	2.4
	Nicotinamide	0.42	4.23	0.06	10
	D-Pantothenic Acid	0.66	7.84	0.18	5.9
	Vitamin E (dl-alpha-tocopherol acetate)	2.20	15.92	0.14	5.3
	Vitamin A (retinol palmitate)	3.87	21.81	0.12	0.46

Sample	Vitamin	Interday Average Amount (µg/mL)	Interday Precision (RSD)
Brand A	Pyridoxine HCl	3.2	1.16
	Ascorbic Acid	68	6.52
	Nicotinamide	19	1.02
	D-Pantothenic Acid	13	3.38
	Vitamin E	8.7	1.01
Brand B	Pyridoxine HCl	3.2	0.72
	Ascorbic Acid	71	9.3
	Nicotinamide	9.5	1.75
	D-Pantothenic Acid	5.0	2.73
	Folic Acid	0.24	9.52
	Vitamin E	15	0.37
	Retinol Palmitate	0.44	4.11
Brand C	Pyridoxine HCl	2.3	4.58
	Nicotinamide	10	0.76
	D-Pantothenic Acid	6.0	0.61
	Vitamin E	5.3	1.39
	Retinol Palmitate	0.44	4.11

Sample	Vitamin	Amount found in sample (µg/mL)	Amount added (µg/mL)	% Recovery
Brand A	Pyridoxine HCl	3.1	7.6	103
	Ascorbic Acid	66	250	119
	Nicotinic acid	<LOD	7.5	99
	Nicotinamide	19	7.5	106
	D-Pantothenic Acid	12	5.0	110
	Cyanocobalamine	<LOD	1.0	93
	Folic Acid	<LOD	0.25	118
	Vitamin E	8.6	7.9	*
	Retinol Palmitate	<LOD	1.0	*
	Brand B	Pyridoxine HCl	3.2	7.6
Ascorbic Acid		72	250	118
Nicotinic Acid		<LOD	7.5	101
Nicotinamide		9.4	7.5	115
D-Pantothenic Acid		5.1	5.0	102
Cyanocobalamine		<LOD	1.0	93
Folic Acid		0.25	0.25	106
Vitamin E		15	7.9	*
Retinol Palmitate		<LOD	1.0	*
Brand C		Pyridoxine HCl	2.2	7.6
	Ascorbic Acid	<LOD	250	114
	Nicotinic acid	<LOD	7.5	94
	Nicotinamide	10	7.5	100
	D-Pantothenic Acid	6.0	5.0	104
	Cyanocobalamine	<LOD	1.0	94
	Folic Acid	<LOD	0.25	115
	Vitamin E	5.3	7.9	*
	Retinol Palmitate	0.44	1.0	*

*see text for discussion on recovery values for these vitamins

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LPN 2145 PDF 04/09
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