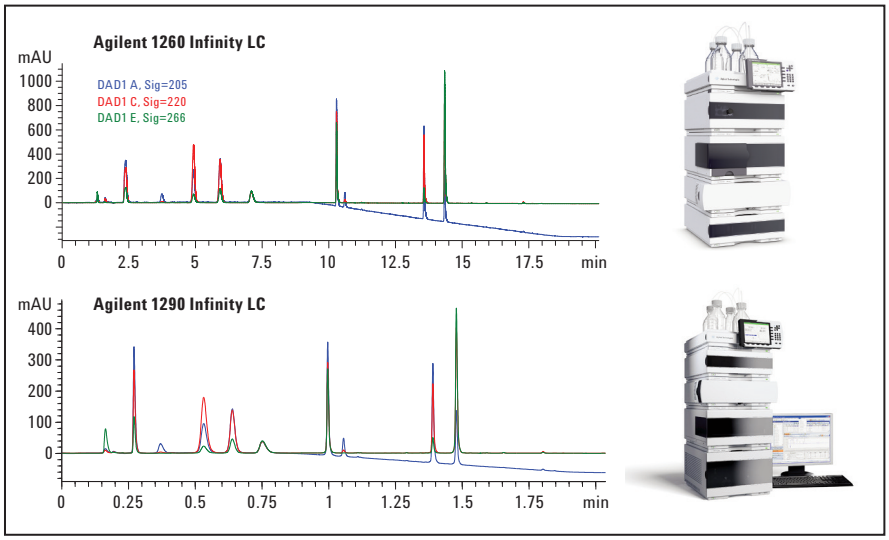
Agilent Application Solution

Analysis of water-soluble vitamins from multivitamin tablets for nutrition labeling

Application Note

Food



Author

Siji Joseph,

Agilent Technologies, Inc.

Bangalore, India

Abstract

In this Application Note we describe an application solution to carry out qualitative and quantitative analysis of water soluble vitamins. We developed a single and robust reverse phase high performance liquid chromatographic (RP-HPLC) method for simultaneous determination of 10 different vitamins unlike a number of different traditional methods which quantify the components individually. Separation and quantification was achieved by an Agilent 1260 Infinity LC system using an Agilent Poroshell EC-C18 column. Detection was carried out in the range of 200 to 640 nm using a photodiode array detector (DAD). As each vitamin has a different absorbance maximum, eight separate wavelengths were selected for acquisition. This method has been partially validated and was found to be appropriate to perform routine nutrition labeling analysis for multivitamin tablets. The limit of detection (LOD), limit of quantitation (LOQ) and linearity of each vitamin were established. The method was effectively transferred to a short Ultra High Pressure Liquid Chromatographic (UHPLC) method using an Agilent 1290 Infinity LC system with the help of the Agilent Method Translator.

Agilent Technologies

Introduction

Vitamins are a range of small organic molecules which are vital nutrients required in trace levels and have specific roles to maintain normal health and growth. As these vitamins are not synthesized naturally in human body a balanced diet is mandatory to keep the amount of vitamins at the required level. However, at times dietary habits can create a deficiency of these vitamins. For these conditions, multivitamins tablets are available in the market for the adequate supply of vitamins. However, as the lack of vitamins creates illness; vitamins in excess levels are also equally harmful to health. Labeling the vitamins in these multivitamin tablets is a mandatory requirement by The Food and Drug Administration (FDA). This clearly emphasizes the importance of having efficient assay methods to quantify vitamins towards the fulfillment FDA nutrition labeling requirements.

Several different traditional analytical methods are available for individual vitamins or for a small group of vitamins. Most of these methods involve tedious, long sample preparation and are non specific. Simultaneous reliable analysis of multiple vitamins is challenging, because of their differences in chemical properties. Wide range of concentrations from lower micrograms to higher milligrams of vitamins in vitamin supplements makes the task even more challenging. Stability, matrix complexity and solubility issues of some vitamins are additional bottlenecks.

Here, we describe an approximatelly 20 minute long, single, reliable, robust method for the simultaneous determination of 10 water soluble vitamins with UV detection.

Method

Instruments and Software

An Agilent 1260 Infinity LC System consisting of the following modules was used:

- Agilent 1260 Series Quaternary Pump and Vacuum Degasser (G1311B)
- Agilent 1260 Series High-Performance Autosampler (G1367E)
- Agilent 1260 Series Thermostatted Column Compartment (G1316C)
- Agilent 1260 Series Diode Array Detector (G4212B) with Max-Light flow cell (60 mm path length) (G4212-60007)
- Poroshell 120 EC-C18 column 3.0 mm × 150 mm, 2.7 µm (p/n 693975-302)
- The UHPLC analysis was developed and performed using the Agilent 1290 Infinity LC system,
- Agilent 1290 Infinity Binary Pump with integrated vacuum degasser (G4220 A) and 100 µL Jet Weaver mixer
- Agilent 1290 Infinity High Performance Autosampler (G4226A)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1290 Infinity Diode Array Detector (G4212A) with Max-Light flow cell (1.0 µL volume, 10 mm path length) (G4212-60008)
- Poroshell 120 EC-C18 columns with internal diameters of 2.1 mm and lengths of 75 mm,packed with 2.7-µm particles (p/n 697775-902)

Both systems were controlled using the Agilent ChemStation B.04.02.

Reagents and materials

All the chemicals and solvents used were HPLC grade and highly purified water from a Milli Q water purification system (Millipore Elix 10 model, USA) was used. Acetonitrile ‘gradient grade’ was purchased from Lab-Scan (Bangkok, Thailand) and dibasic potassium phosphate was purchased from Fluka (Germany). O-Phosphoric acid was purchased from Fluka (Switzerland) and sodium hydroxide was purchased from Sigma (Germany). Standards of ascorbic acid (C), nicotinic acid (B3), calcium pantothenate (B5), pyridoxine (B6), niacinamide (B3), thiamine (B), folic acid(B9), biotin(B7),cyanocobalamine (B12), and riboflavin (B2) were purchased from Aldrich (India).

Chromatographic parameters

Chromatographic parameters used for reverse phase liquid chromatography and UHPLC are tabulated in Table 1.

Water soluble vitamin standards

Vitamin standards of ascorbic acid, nicotinic acid, calcium pantothenate, pyridoxine, niacinamide, thiamine, folic acid, biotin, cyanocobalamine, and riboflavin were prepared individually by accurately weighing about 50 mg of the vitamin powder and transferring it to a 25mL volumetric standards flask. Milli Q water was added to form a stock solution of 2.0 mg/mL (2000 ppm). Sonication was used when required. Solubility of folic acid was poor in water as a free acid. To convert folic acid to folate, the standard was first dissolved in 20 mL of Milli Q water and a minimum amount of 0.25 M sodium hydroxide was added to convert folic acid to sodium folate. Milli Q water was added to create a 2.0 mg/mL solution. A similar approach was followed for biotin and riboflavin while making the standard solution. Water-soluble vitamin stock solutions were stored at + 4.0 °C when not in use. About 200 µL of each standard were precisely mixed to get a 2000 µL spike mix of water soluble vitamins at concentration of 200 ppm each. Linearity levels were prepared by subsequent dilution of this 200 ppm standard spike mix using mobile phase A as diluent.

Sample preparation

Three multivitamin tablets of a leading brand from Europe were individually dissolved in about 200 mL water and sonicated. A minimum amount of 0.25 M NaOH was added to solve solubility issues for some vitamins. The sample solution was then filtered through a 0.25 µm Agilent Econofilter syringe filter membrane and used for nutrition labeling analysis and recovery analysis.

Parameter	Agilent 1260 Infinity LC	Agilent 1290 Infinity LC
Column oven	35 °C	35 °C
Acquisition rate	20 Hz	80 Hz
Data acquisition	205, 214, 220, 232, 266, 268, 280 nm	205, 214, 220, 232, 266, 268, 280 nm
Flow cell	60 mm path	10 mm path
Injection volume	5 µL (Needle with wash, flush port active for 5 seconds)	1 µL (Needle with wash, flush port active for 3 seconds)
Sample thermostat	5 °C	5 °C
Mobile phase A	25 mM HK_2PO_4, pH 7.0	25 mM HK_2PO_4, pH 7.0
Mobile phase B	Acetonitrile	Acetonitrile
Gradient	At 0 min → 1%B At 5 min → 1%B At 15 min → 30%B At 20 min → 30%B At 20.1 min→ 1%B	At 0 min → 1%B At 0.56 min → 1%B At 1.66 min → 30%B At 2.2 min → 30%B At 2.22 min → 1%B
Post run time	5 minutes	1 minute
Flow rate	0.45 mL/min	1.0 mL/min

Table 1
Chromatographic parameters used for the Agilent 1260 Infinity LC and Agilent 1290 Infinity LC systems.

Precautions

Vitamins are known to be highly sensitive to light and heat. To extend the stability in solution form, all the prepared solutions were stored in a refrigerator, when not in use. The thermosttated autosampler tray was maintained at 4 °C during the analysis.

Procedure

A 5 µL of mobile phase A was injected as blank and followed by each linearity level in six replicates. Area and retention time (RT) information of each level were used to calculate standard deviation (SD) and relative standard deviation (RSD) values. LOD and LOQ were established from the lower linearity level injections. Average area of each linearity level was plotted against the concentration to plot linearity curve.

Six critical method parameters were changed to evaluate the robustness of the method. A standard spike mix concentration of 5 ppm was injected in six replicates and the data was used for the robustness study.

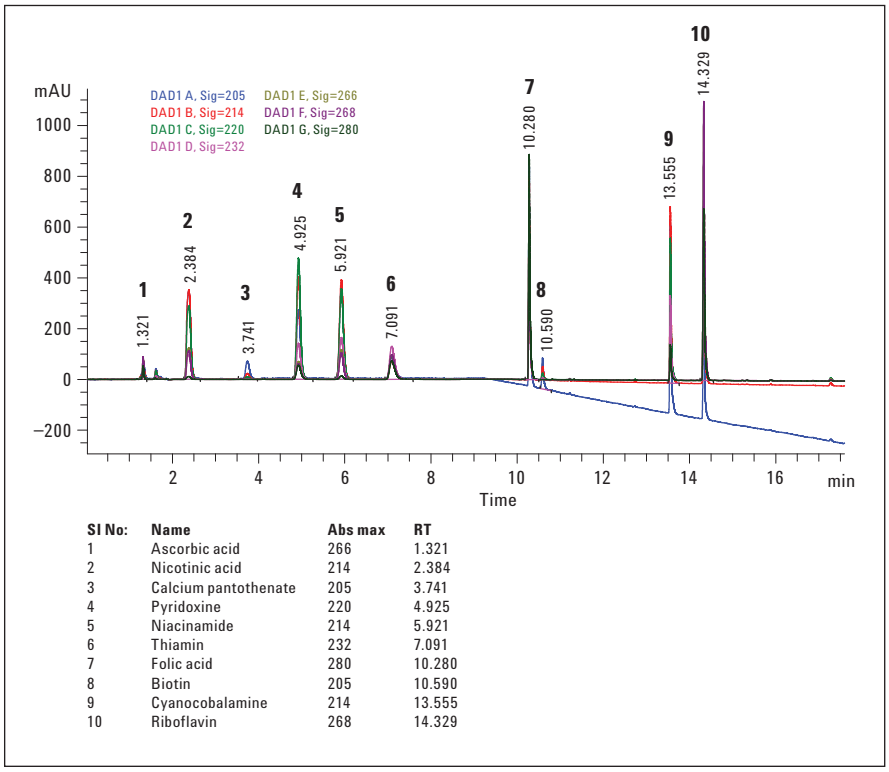
All three prepared multivitamin tablet sample were injected with and without spiking 5 ppm standard mix in three replicates each. Area and RT data were used for recovery as well as nutrition labeling calculations.

The method was effectively transferred to UHPLC using Agilent's method translator. LOD, LOQ and linearity of each vitamin were evaluated and precision of the method was established by Area and RT RSD.

Results and Discussion

Separation and detection

Several trials were undertaken with a standard spike mix under gradient and isocratic conditions with various mobile phases. We observed that using 25 mM K_2HPO_4 at pH 7 in a gradient separation displayed the best resolution. Figure 1 demonstrates the excellent separation of 10 water soluble vitamins in 20 minutes using an Agilent Poroshell 120 EC-C18, 150 mm × 3.0 mm, 2.7 µm column. Since all these vitamins are structurally diverse in nature, spectra and absorbance maxima of each vitamin are different. Ascorbic acid has a strong absorption at 266 nm while folic acid best absorbs at 280 nm. Biotin and calcium pantothenate were found to have poor UV absorbance so a wavelength of 205 nm was selected for the analysis. For nicotinic acid, niacinamide and cyanocobalamine, 214 nm was found to be the best absorbing wavelength, while absorbance maxima of pyridoxine was about 220 nm. For the analysis, thiamine, 232 nm was selected and for riboflavin, 268 nm was chosen. In Figure 1, chromatograms collected at seven different wavelengths are overlaid. Peak height variation of each vitamin is clearly seen in different acquisition traces. Baseline absorbance shifts during the gradient run as the amount of buffer in the mobile phase changes. This is more prominent in lower wavelengths, and explains the observed base line drift at 205 nm. An advanced feature of ChemStation software allowed checking the peak purity of each individual peak and the specificity of the method was evaluated. Precision, linear range, accuracy, specificity, recovery and robustness studies were taken care to validate the method.



Sl No:	Name	Abs max	RT
1	Ascorbic acid	266	1.321
2	Nicotinic acid	214	2.384
3	Calcium pantothenate	205	3.741
4	Pyridoxine	220	4.925
5	Niacinamide	214	5.921
6	Thiamin	232	7.091
7	Folic acid	280	10.280
8	Biotin	205	10.590
9	Cyanocobalamine	214	13.555
10	Riboflavin	268	14.329

Figure 1
Separation of 10 water soluble vitamins using a 15 cm Agilent Poroshell 120 EC-C18 column. Chromatograms collected at seven different wavelengths are overlaid.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The analyte concentration that provides a signal to noise ratio (S/N) of > 3 was considered as LOD and analyte concentration with S/N > 10 was considered as LOQ. Observed LOQ, LOQ and S/N results of each vitamin are tabulated in Table 1. As an example, the LOQ level of folic acid overlaid with two blank (before and after) chromatograms are shown in Figure 2.

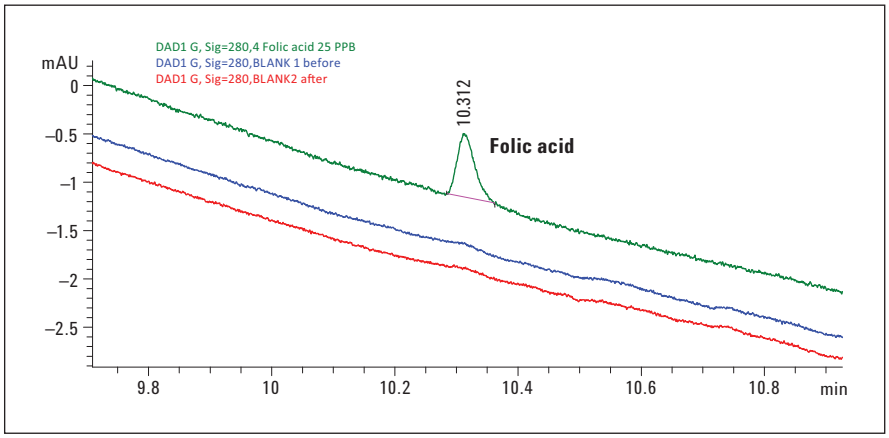


Figure 2
A 0.025 ppm (25 ppb) of folic acid (LOQ level) overlaid with two blank (before and after) chromatograms.

Linearity

A linearity curve for each vitamin was constructed from the LOQ level to a maximum concentration and results are tabulated in Table 2. Each linearity solution was injected six times and the average area was used to construct linearity curve. The observed linearity range covers vitamin content in most multivitamin tablets. The linearity curve for Biotin is displayed in Figure 3. All the vitamins except ascorbic acid provided an excellent regression coefficient. Ascorbic acid in solution undergoes reversible equilibration to its oxidized form of dehydro ascorbic acid by dissolved oxygen [1,2,3]. This reaction can be minimized by reducing the pH of the mobile phase to acidic range. Due to the presence of folic acid which precipitates at lower pH, maintaining an acidic pH was not practical. The area response reproducibility of ascorbic acid was not consistent and this was reflected by poor R^2 values of ascorbic acid. Along with LOD and LOQ values linearity results were also included in the Table 2.

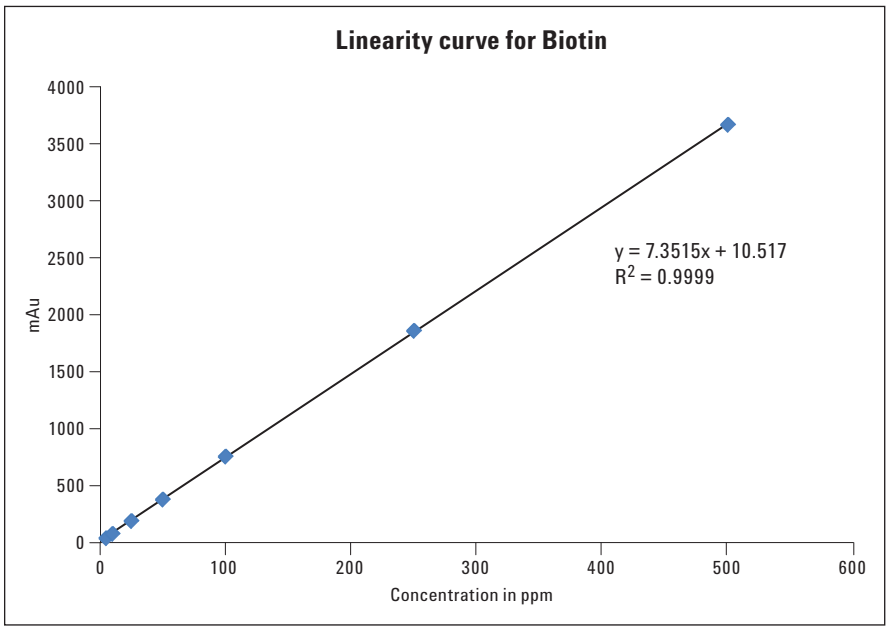


Figure 3
Linearity curve of biotin from 5 ng/mL to 500 ng/mL showing excellent coefficient value.

		LOD		LOQ				
Sl no:	Name:	ng/µL (ppm)	S/N	ng/µL (ppm)	S/N	Linearity range (ppm) On-column	R^2 value	Levels, replicates = 6
1	Ascorbic acid	1	4.8	2.5	9.7	2.5–500	0.9827	8
2	Nicotinic acid	0.025	3.1	0.05	10.9	0.05–250	1	12
3	Calcium pantothenate	1	5.0	2.5	12.6	2.5–500	0.9998	8
4	Pyridoxine	0.025	4.4	0.05	9.9	0.05–250	0.9994	12
5	Niacinamide	0.025	4.8	0.05	9.8	0.05–250	1	12
6	Thiamin	0.025	3.1	0.1	9.9	0.1–250	1	11
7	Folic acid	0.01	4.7	0.025	10.3	0.025–250	0.9985	13
8	Biotin	2.5	4.2	5	9.7	5–500	0.9999	7
9	Cyanocobalamine	0.01	6.4	0.025	15.2	0.025–250	0.9994	13
10	Riboflavin	0.005	8.1	0.01	12.4	0.01–250	0.9985	14

Table 2
LOD, LOQ, S/N and linearity results of all 10 vitamins.

Precision of retention time and area

RT RSD values for all 10 vitamins across the linearity levels were calculated and the highest value observed was 0.65%. With the exception of ascorbic acid, area RSD for all vitamins across the linearity levels was found to be excellent. Due to it's instability, the area of ascorbic acid could not be measured consistently over a period of time. The minimum area and RT RSD values ensure the acceptable reproducibility of the method and thus the precision of the system. Graphical representation of area and RT RSD values of few vitamins are shown in Figures 4 and 5 respectively.

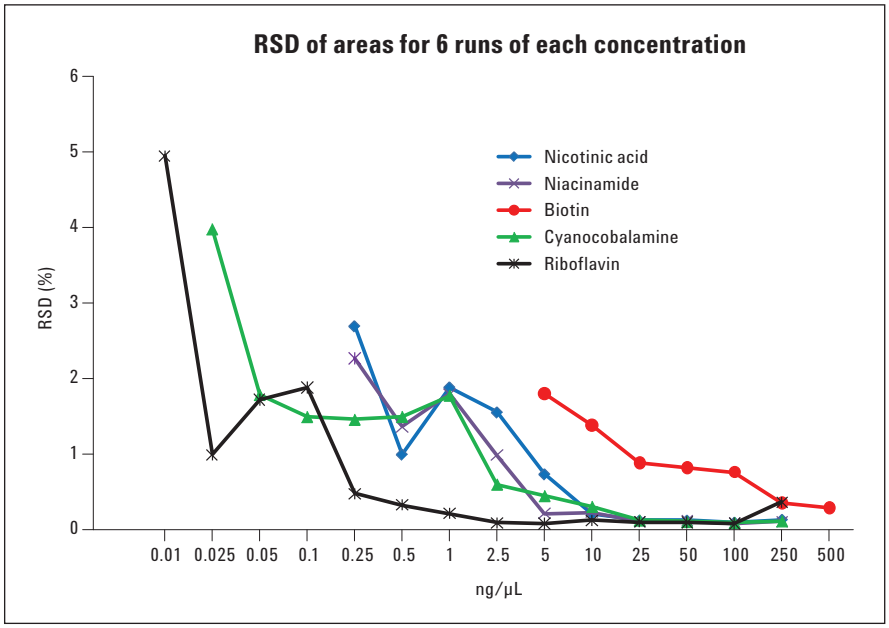


Figure 4
Precision of areas of different concentrations of various vitamins; six replicate injections for each concentration.

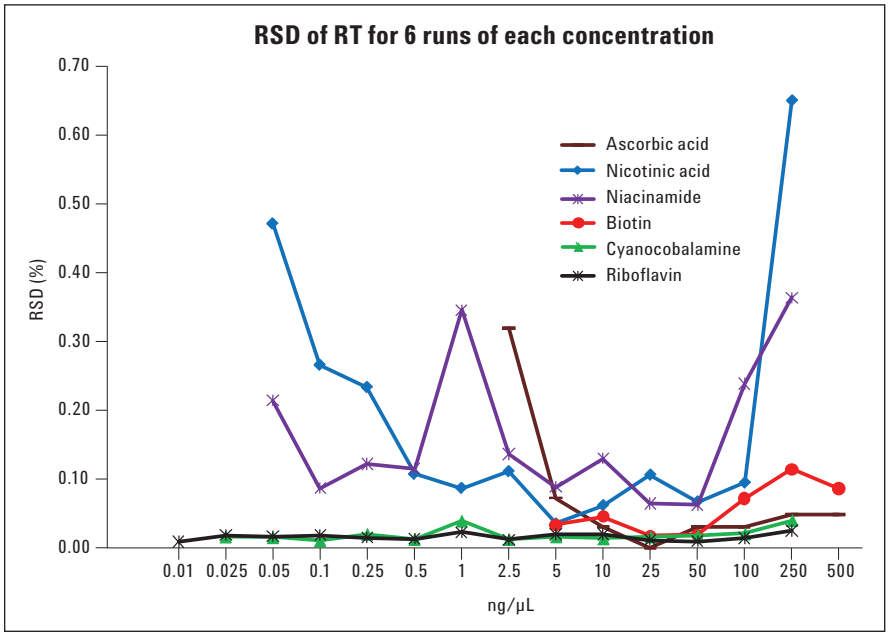


Figure 5
Precision of retention times of various vitamins; six replicate injections for each concentration.

Sl no:	Parameter	Value in original method	Measured deviation	Modified values	Observed RT deviation (Allowed limit = ± 2%)	Observed area deviation (Allowed limit = ± 5%)
1	Column temperature	35 °C	± 5%	33 °C	Passed	Passed
				37 °C	Failed for three mid eluting compounds	Passed
2	Column flow	0.45 mL/min	± 2%	0.44 mL/min	Passed for five last eluting compounds	Passed
				0.46 mL/min	Passed for five last eluting compounds	Passed
3	Injection volume	5 µL	± 5%	4.75 µL	Passed	Passed
				5.25 µL	Passed	Passed
4	Gradient steepness	2.9 (1 to 30 in 10 minutes)	10%	3.2 (1 to 30 in 9 minutes)	Passed for eight first eluting compounds	Passed
				2.6 (1 to 30 in 11 minutes)	Passed for eight first eluting compounds	Passed
5	Detection wavelength	205, 214, 220, 232, 266, 268, 280 nm	± 3 nm	202, 211, 217, 229, 263, 265, 277 nm	Passed	Passed for seven compounds
				208, 217, 223, 235, 269, 271, 283 nm	Passed	Passed for five compounds
6	pH of mobile phase	7.0	± 0.2	6.8	Failed	Failed
				7.2	Failed	Failed

Table 3
Robustness test result summary.

Robustness

To test the robustness of the method, a standard spike mix solution of vitamins with a concentration of 10 ng/mL was used. With an injection volume set value of 5 µL, on column concentration of this standard mix was 50 ng/mL (ppm). Six critical parameters were changed and data collected in 10 replicate injections. Values from the last six replicates were used for the analysis. Allowed deviation for retention time and area was set to ± 2.0% and ± 5% respectively.

Results from robustness study are summarized in Table 3. From those results, it is clear that, selecting exact absorbance maxima of vitamins for detection is important. Area responses for several vitamins were sensitive to increase or decrease in detection wavelength by 3 nm. Impact of column temperature on retention time was measured and was observed that, the deviation was within the limit when the column temperature was lowered by 2 °C (33 °C) compared to the actual method. When the column temperature was raised by 2 °C (37 °C) compared to the actual method, retention time of three mid eluting peaks were deviated more than the allowed limit. Robustness studies with changing column flow confirmed that, retention times of early eluting five highly polar vitamins are sensitive to the column flow rate. A representative chromatogram of vitamin standard mix with actual and varied column flow rates are shown in Figure 6. Studies with change in gradient slope confirmed that retention times of last two peaks are sensitive to gradient slope. *Study with change in pH of mobile phase demonstrates that*

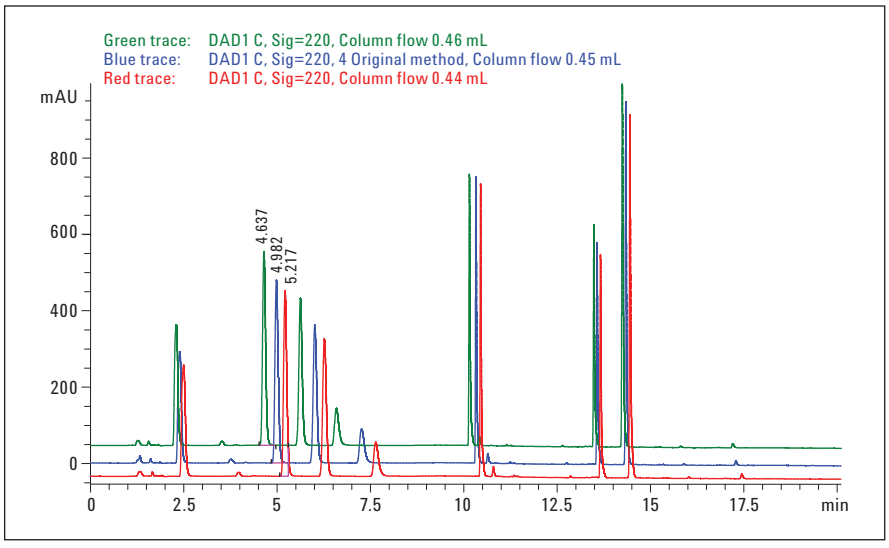


Figure 6
Results from robustness study. Effect of varying column flow.

mobile phase pH is very critical for area and retention time reproducibility and must be carefully controlled. Elution pattern was significantly different, when the pH was changed by +0.2 to the actual value. The variation in elution pattern with change in pH is shown in Figure 7. Robustness results indicate that, the method is reliable for normal usage and to a great extent, the performance remains unaffected by deliberate change in parameters. However, some parameters are critical and must be carefully controlled.

Recovery from sample matrix

As the blank matrix was not available, recovery analysis of water soluble vitamins from multivitamin tablet was carried out by standard addition method. A standard spike mix solution containing 5 ng/µL (ppm) of the individual vitamins was used for this analysis. For recovery analysis, tablets were dissolved in 200 mL water and analyzed. This sample was spiked with the standard mix. The area response change after the addition of the standard mix was measured and the amount corresponding to each vitamin was back calculated using the linearity equation [4,5]. The variation from the known initial added value (5 ng/µL) was reported in percentage as recovery. The recovery analysis was also carried out using a tenfold lower diluted sample matrix. The observed results from recovery studies were tabulated in Table 4. Ascorbic acid was found to be degrading and omitted from recovery calculation. The excellent recovery values confirms the reliability of extraction procedure and the method can be utilized for monitoring and quantifying water soluble vitamins in nutrition labeling analysis.

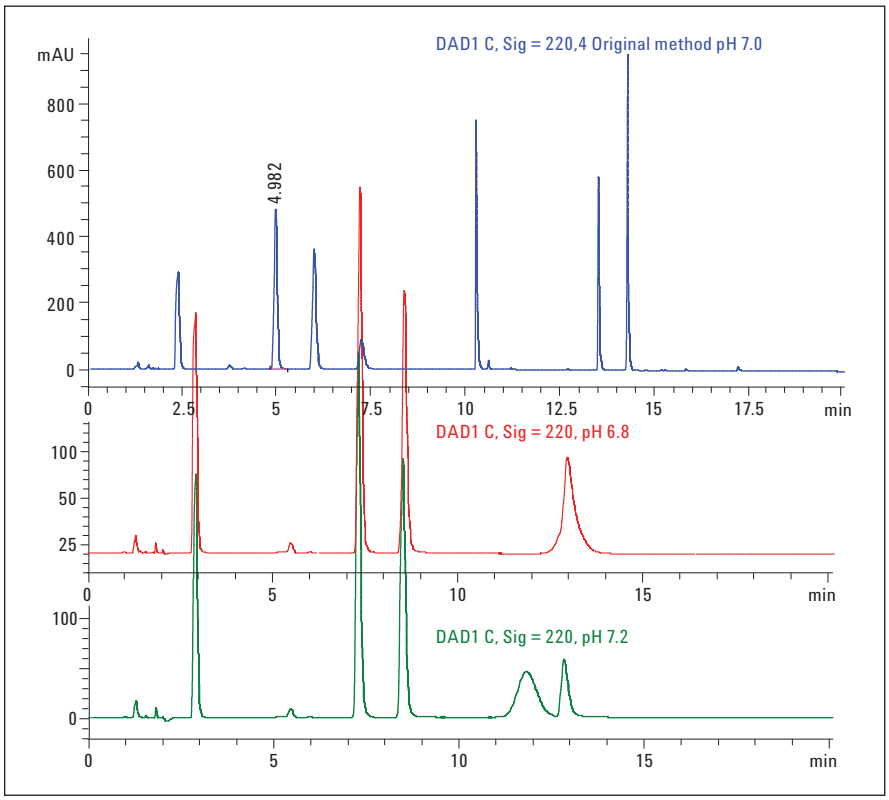


Figure 7
Results from robustness study. Effect of varying pH.

Vitamin	R^2	Linearity equation	Recovery (%)					
			Sample 1	Sample 1 dilute	Sample 2	Sample 2 dilute	Sample 3	Sample 3 dilute
Nicotinic acid	1	y = 44.956x + 9.8116	95.4	96.3	94.2	99.1	97.5	95.1
Calcium pantothenate	0.9998	y = 8.1153x + 13.278	84.2	93.4	92.0	92.0	86.4	80.2
Pyridoxine	0.9994	y = 64.275x – 102.77	125.3	96.3	123.6	98.1	125.7	94.5
Niacinamide	1	y = 51.034x + 6.7257	96.9	100.0	99.6	98.3	100.1	87.0
Thiamine	1	y = 23.202x - 8.8965	100.7	99.7	100.6	102.3	99.2	98.0
Folic acid	0.9985	y = 38.06x + 42.787	80.8	82.2	81.6	85.1	82.3	81.8
Biotin	0.9999	y = 7.3515x + 10.517	82.2	94.8	80.4	96.3	84.0	95.6
Cyanocobalamine	0.9994	y = 31.164x + 31.428	84.9	83.8	85.6	87.3	80.5	83.0
Riboflavin	0.9985	y = 50.904x + 71.323	82.7	82.2	81.6	85.4	84.1	80.6

Table 4
Recovery analysis results.

Nutrition labeling

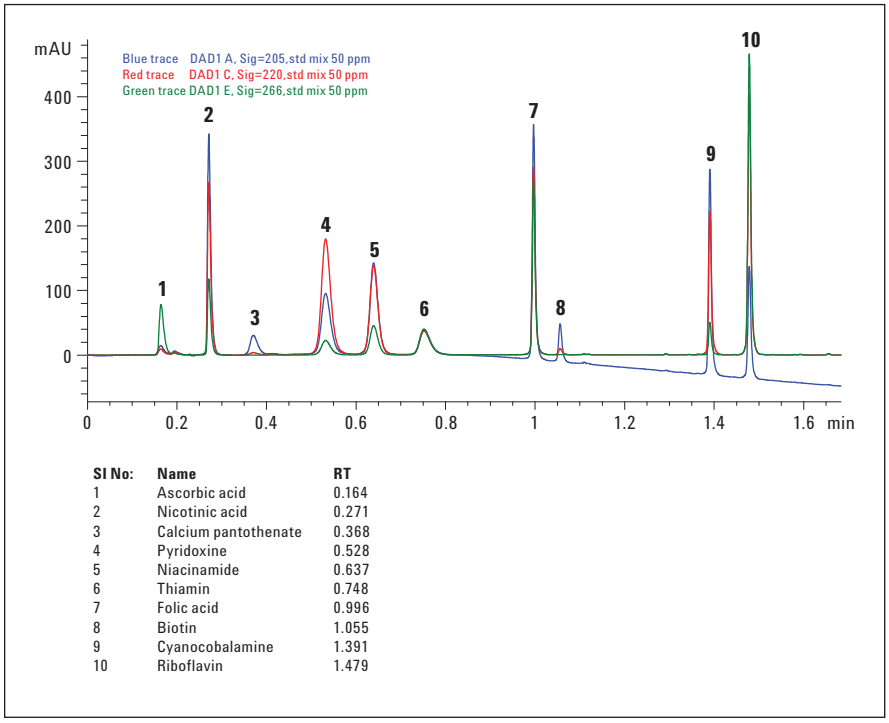
In this study, vitamins present in multivitamin tablets were estimated from a chromatographic method and compared with the concentration claimed on the label. Three replicate analyses were carried out after dissolving tablets in 200 mL water. Since vitamins are present over a wide concentration range, a diluted sample was also analyzed. Ascorbic acid was present in high amounts and the corresponding peaks were saturating the detector. Diluted sample analysis was more accurate for ascorbic acid as this reduced the peak height and area to the detector linearity range. However, peaks corresponding to some vitamins which are present in microgram levels could not be detected in the diluted sample. No interference was observed for any vitamin peaks in the sample analysis chromatogram. The area corresponding to each vitamin peak in the sample and diluted sample analysis were used to perform the nutrition labeling analysis for multivitamin tables. Linearity equations originated from linearity curves were used for the calculation. The extraction process is very simple and can be adopted easily for routine analysis. Nicotinic acid (acid form of B3) is absent in the sample as per the label claim and no peak was observed for nicotinic acid in the sample chromatogram. The results show excellent suitability of the method to quantify vitamins in multivitamin tablets.

		Tablet 1		Tablet 2		Tablet 3	
Vitammin	Amount present as per label	As such	Diluted	As such	Diluted	As such	Diluted
Ascorbic acid (C)	80 mg	Saturated peak	201.7	Saturated peak	203.7	Saturated peak	198.7
Calcium pantothenate (B5)	6 mg	6.7	6.1	6.6	6.5	6.6	6.3
Pyridoxine (B6)	1.4 mg	1.2	1.2	1.2	1.2	1.2	1.2
Niacinamide (B3)	16 mg	15.7	16.0	15.4	16.0	15.2	15.6
Thiamin (B1)	1.1 mg	1.3	1.3	1.3	1.3	1.2	1.2
Folic acid (B9)	200 µg	114.9	117.6	114.3	120.6	115.3	121.0
Biotin (B7)	50 µg	42.4		35.7		38.5	
Cyanocobalamine (B12)	2.5 µg	0.5		0.4		0.4	
Riboflavin (B2)	1.4 mg	1.4		1.4		1.3	

Table 5
Calculated amount of vitamins and label claims for each vitamin from three samples and diluted samples are tabulated.

UHPLC Method

A UHPLC method with diode array detection was established for the separation of water soluble vitamins using the Agilent method translator. This tool enables to easily convert methods from either binary or quaternary pump systems to optimized methods for the Agilent 1290 Infinity LC system. The resulting UHPLC method is fast and saves about 90% percent time and more than 70% of solvent compared to the 20 minute long gradient. It shows excellent resolution and good peak shape (Figure 8). This method can also be used for the quick nutrition labeling analysis of vitamin tablets. The LOD and LOQ levels are also established and linearity of each vitamin was evaluated using the UHPLC method. Precision of the method was confirmed by low RSD values for area and RT. The observed LOD, LOQ are tabulated in Table 6. One example for observed excellent linearity is shown in Figure 9 and RSD values for RT and area for an on-column concentration of 50 ppm with an injection volume of 1 µL are shown in Figure 10. These results verify that the developed UHPLC method is rapid, sensitive and reliable.



Sl No:	Name	RT
1	Ascorbic acid	0.164
2	Nicotinic acid	0.271
3	Calcium pantothenate	0.368
4	Pyridoxine	0.528
5	Niacinamide	0.637
6	Thiamin	0.748
7	Folic acid	0.996
8	Biotin	1.055
9	Cyanocobalamine	1.391
10	Riboflavin	1.479

Figure 8
Separation of 10 water soluble vitamins using a 75 mm Agilent Poroshell 120 EC-C18 column with an UHPLC method. Chromatograms collected at different wavelengths are overlaid.

		LOD		LOQ	
Sl No:	Name:	Concentration (ppm)	S/N	Concentration (ppm)	S/N
1	Nicotinic acid	0.02	4.3	0.05	12.8
2	Calcium pantothenate	0.5	4.2	1.0	11.3
3	Pyridoxine	0.05	5.3	0.1	9.6
4	Niacinamide	0.05	4.3	0.1	11.1
5	Thiamin	0.1	3.1	0.5	11.3
6	Folic acid	0.02	3.1	0.1	16.3
7	Biotin	0.5	5.3	1	16.5
8	Cyanocobalamine	0.002	3.9	0.005	10.2
9	Riboflavin	0.02	3.5	0.05	9.6

Table 6
LOD and LOQ values derived from the UHPLC method.

Conclusion

Water soluble vitamins in multivitamin tablet were separated and quantified using Agilent Poroshell 120 EC-C18 column. A robust, 20 minute long, reverse phase LC gradient method was developed using the Agilent 1260 Infinity LC system. Method transfer to a 2 minute UHPLC was effectively carried out using Agilent's method translator. We performed the UHPLC analysis using the Agilent 1290 Infinity LC. The method successfully quantifies vitamins C, B1, B2, B3,B5, B6, B7, B9, and B12 even if present in various concentration ranges in a single injection. Gradient conditions ensured better chromatographic resolution improved sensitivity and lesser matrix interference. The methods were simple, specific, sensitive, rapid and also provide good precision, linearity and recovery values. This method can be applied effectively to the routine analysis of above listed water soluble vitamins in multivitamin tablets with ease.

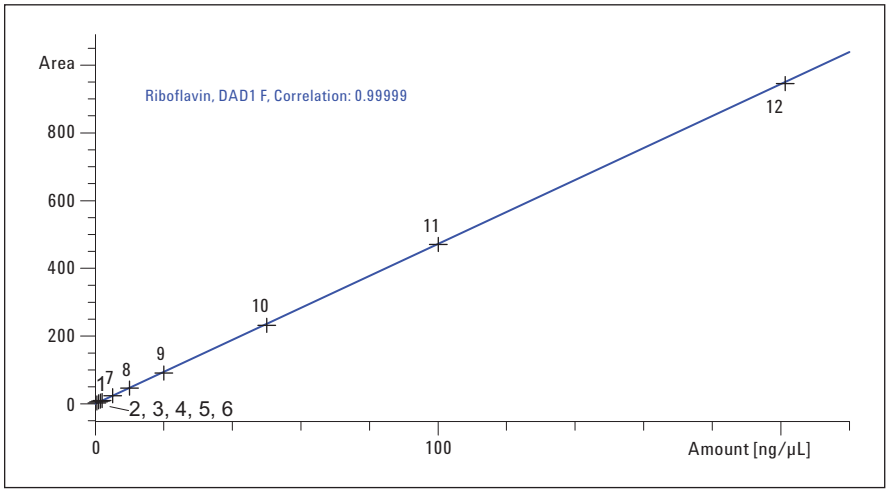


Figure 9
Linearity of Riboflavin from 0.05 ppm to 200 ppm showing a correlation of 0.99999 (12 levels and six replicates). Injection volume is 1 µL.

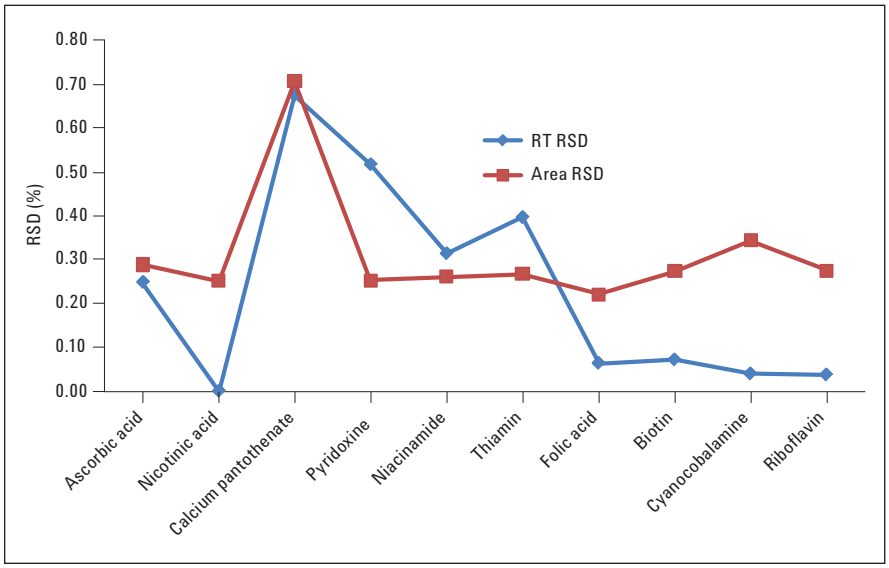


Figure 10
Area and RT RSD values from UHPLC results for all 10 vitamins at an on-column concentration of 50 ppm. Injection volume is 1 µL and six replicates.

References

1.
Katherine E. Sharpless, Sam Margolis, Jeanice Brown Thomas, "Determination of vitamins in food-matrix Standard Reference Materials," National Institute of Standards and Technology, 100 Bureau Drive, Stop 8392, Gaithersburg, MD 20899-8392, USA

2.
A. Jedlicka and J. Klimes, "Determination of Water- and Fat-Soluble Vitamins in Different Matrices Using High-Performance Liquid Chromatography," Chem. Pap., 2005, 59, 202–222.

3.
Sam A. Margolis, David L Duewer, "Measurement of Ascorbic Acid in Human Plasma and Serum: Stability, Intralaboratory Repeatability, and Interlaboratory Reproducibility," Clinical Chemistry, 1996, 42, 1257–1262.

4.
AOAC Official Method 2004.07 Vitamin B6 in Reconstituted Infant Formula Liquid Chromatographic Method First Action 2004.

5.
Duncan Thorburn Burns, Klaus Danzer, and Alan Townshend, "Use of the terms 'recovery' and 'apparent recovery' in analytical procedures," Pure Appl. Chem., Vol. 74, No. 11, pp. 2201–2205, 2002.

www.agilent.com/chem/lc

Published June 1, 2011
Publication Number 5990-7950EN

Agilent Technologies