

Determination of B-vitamins in Energy Drinks by CE/MS/MS

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Abstract

Vitamins are essential nutrients found in foods, and are vital to maintaining optimal health. This Application Note describes a sensitive and reliable method for the determination of seven B-vitamins in energy drinks, using CE/MS/MS. The best separation of B1, B2, B3a, B3b, B5, B6, B12, and cetirizine (used as internal standard) was obtained using 50 mM formic acid (pH 2.5) as the background electrolyte (BGE). With a 60 cm fused silica capillary, it was possible to achieve a run time of under eight minutes. The proposed method is simple, fast, and presented a linear calibration with correlation coefficients greater than 0.995. Limits of detection (LODs) and limits of quantification (LOQs), based on the signal-to-noise ratio (S/N), were in the range of 0.01–0.19 and 0.04–0.63 ppm, respectively. The method was successfully used to determine B-vitamins in commercial samples of energy drinks.

Introduction

Vitamins are indispensable compounds for the human organism. However, not all vitamins can be synthesized by the organism itself, so they need to be supplemented¹. Vitamins are so crucial for good health that there is a wide range of fortified foods and supplements on the market. However, some vitamins, such as B-vitamins, are sensitive to temperature and light; therefore, there are concerns about the real amounts of vitamins present in fortified foods. These concerns have led to the need for specific techniques to analyze these vitamins. Traditionally, methods for vitamin analysis were based on microbiological assays and immunoassays. These assays are usually labor-intensive, time-consuming, and generally do not allow simultaneous determination of multivitamins. Liquid chromatography (LC) is also a common technique for determining vitamins in many food products. However, water-soluble vitamins, such as B-vitamins, are very polar and have relatively poor retention on reversed-phase columns.

Capillary electrophoresis (CE) is useful for the separation and determination of ions or ionizable compounds such as B-vitamins, which are fully ionic at low pH. Therefore, CE (compared to LC) offers advantages, including short analysis time, low reagent cost, and minimal sample requirements. It is also considered an environmentally friendly analytical method because it produces less waste^{2,3}. Analytical separation using CE coupled to mass

spectrometry (MS) detection can provide significant advantages by combining the high separation efficiency of CE with the identification power of MS. The aim of this study was to develop and validate a method for the determination of B-vitamins in energy drinks using CE/MS/MS⁴. Figure 1 shows the molecular structures of the B-vitamins analyzed in this study, along with their strongest basic pKa values.

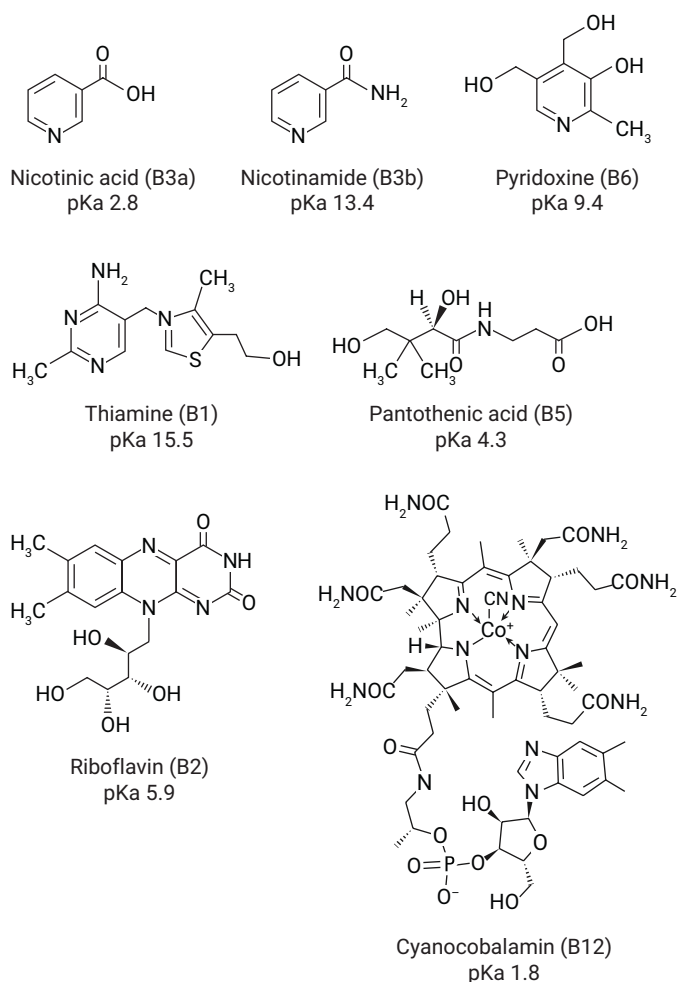


Figure 1. Molecular structures of the B-vitamins analyzed and their strongest basic pKa values. The pKa values were calculated at www.chemicalize.org (accessed April 2018).

All separations were performed at 25 °C using 50 mM formic acid, pH 2.5, as the BGE. New fused silica capillaries were preconditioned by flushing 0.1 M NaOH solution (5 minutes), de-ionized water (10 minutes), and BGE (10 minutes). An extra flushing step with BGE for 60 seconds with de-ionized water and 90 seconds with BGE was included between the runs. Samples were introduced hydrodynamically in five seconds at 50 mbar and analyzed with an applied voltage of 29 kV. The mass spectrometer was operated in positive ionization mode, using multiple reaction monitoring (MRM) mode for two specific transitions. Table 1 lists the migration time (t_M), monitored ions, and other MS/MS acquisition parameters used for the identification and quantification of the targeted B-vitamins in energy drinks.

Sample preparation

Samples of energy drinks were bought from local supermarkets. They were then degassed for three minutes in an ultrasonic bath and filtered through a 0.2 μ m polyvinylidene fluoride and polypropylene membrane (Agilent Captiva filter cartridges, p/n A5300002). Next, they were diluted 10 times before being transferred to a 250 μ L polypropylene vial for CE/MS/MS analysis (p/n 5188-2788).

Experimental

CE conditions

Parameter	Value
Instrument	Agilent 7100 CE system
Background electrolyte	50 mM formic acid, pH 2.5
Applied voltage	29 kV
Capillary	Silica capillary 50 μ m id with 60 cm total length (p/n 160-2650-5, 500 cm length, cut to 60 cm)
Injection	5 seconds at 50 mbar
Temperature	25 °C

MS conditions

Parameter	Value
Instrument	Agilent 6430 triple quadrupole LC/MS
Ion mode	ESI, positive ionization
Sheath liquid	0.1 % Formic acid/methanol (50:50 v/v)
Flow rate	5.0 μ L/min
Capillary voltage	2,500 V
Drying gas flow (N_2)	7 L/min
Drying gas temperature	350 °C
Nebulizer pressure	9 psi

Table 1. Migration time (t_M) and MS/MS acquisition parameters used for the identification and quantification of B-vitamins in energy drinks.

Vitamins	t_M (min)	Q1 ^a (m/z)	Q3 ^b (m/z)	CE ^c (V)	FE ^d (V)
Thiamine B1	3.01	265.1	122.1* 81.2	17 37	91
Nicotinamide B3b	3.43	123.1	80.2* 53.3	21 37	111
Pyridoxine B6	3.66	170.1	134.0* 77.2	21 45	91
Ceritizine (IS)	4.61	389.2	201.0* 166.0	20 44	127
Nicotinic acid B3a	5.41	124.0	80.2* 78.2	21 25	121
Cyanocobalamin B12	6.76	678.7	359.1* 147.1	25 41	71
Riboflavin B2	6.87	377.2	243.1* 172.1	25 41	161
Pantothenic acid B5	6.89	220.1	90.2* 72.3	13 21	111

^a = precursor ion (Q1); ^b = fragment ion (Q3); ^c = collision energy; ^d = fragmentor energy

Results and discussion

The BGE, sheath liquid composition, applied potential, and hydrodynamic injection were optimized for separation efficiency and sensitivity. Figure 2 shows a representative MRM electropherogram obtained under optimum conditions for the B-vitamin standards and internal standard in the BGE. The t_M was shorter than 8.0 minutes.

The methodology for B-vitamins analysis using CE/MS/MS was validated in terms of selectivity, linearity, sensitivity, precision, and accuracy. Calibration curves were constructed with standard solutions at 11 levels of concentration using cetirizine as the IS. The correlation coefficients (R^2) of calibration curves were greater than 0.995. Each level of concentration was analyzed in quintuplicate, and the related standard deviations (RSDs) ranged from 0.2 to 6.6 % for run-to-run precision. The LODs and LOQs were determined considering the corresponding concentration to three and ten times, respectively, the baseline noise in a time close to the migration time measured around each vitamin. The proposed method allows B-vitamins to be determined with LODs in the range of 0.01–0.19 ppm using a very low injection volume (calculated approximately 4.5 nL). Table 2 shows the regression equations and other characteristic parameters for the developed method. Standard deviations of residuals were obtained by analysis of variance (ANOVA).

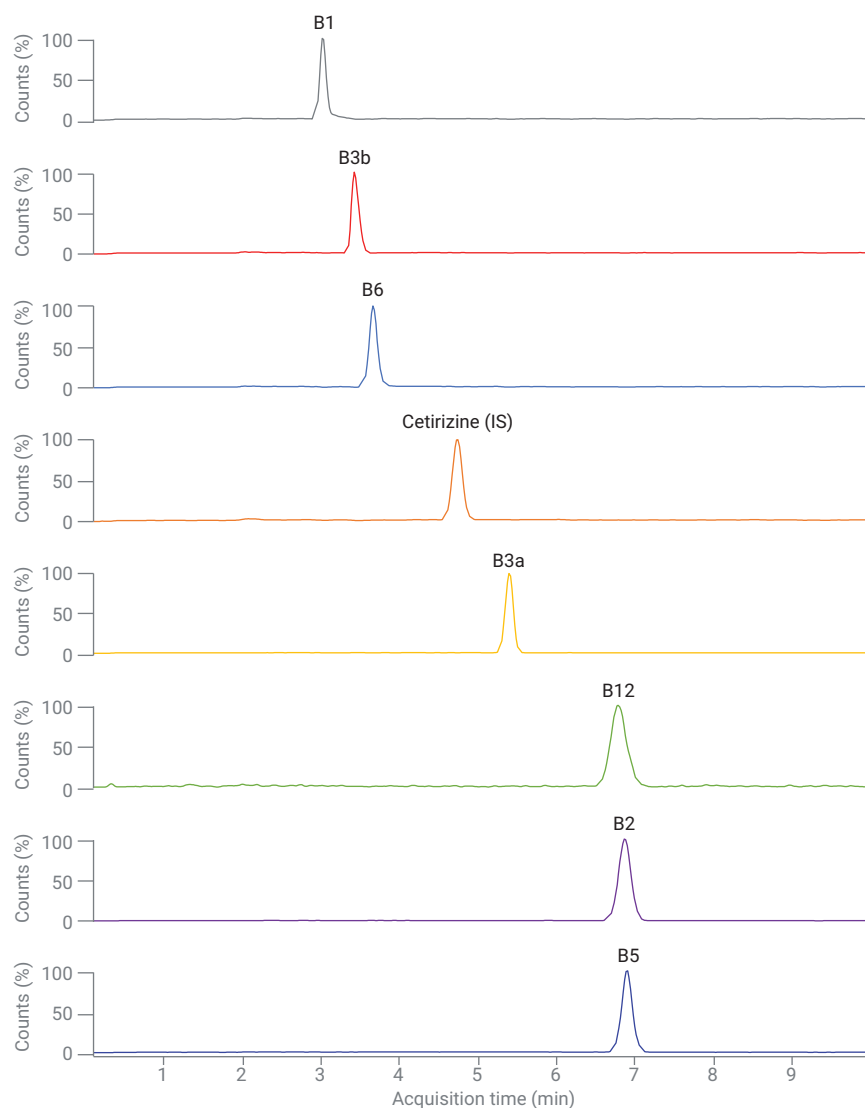


Figure 2. Normalized MRM electropherogram under optimum conditions for the B-vitamins and internal standard in BGE at 10.8 ppm for B1, B2, B6, and IS, at 108 ppm for B3a and B3b, and at 45 ppm for B5 and B12.

Table 2. Figures of merit for the B-vitamin analysis in energy drinks by CE/MS/MS.








Vitamin	Linear range (ppm)	$y = ax+b$	R^2	$S_{x/y}$	LOD (ppm)	LOQ (ppm)
B1	0.3–10.8	$y = 0.019x + 0.021$	0.995	0.015	0.03	0.09
B3a	3.0–108	$y = 0.925x + 1.442$	0.997	0.147	0.09	0.30
B6	0.3–10.8	$y = 0.018x + 0.011$	0.995	0.015	0.01	0.04
B3b	3.0–108	$y = 0.017x + 0.016$	0.995	0.127	0.08	0.25
B12	1.25–50	$y = 0.095x - 0.017$	0.997	0.298	0.19	0.63
B2	0.3–10.8	$y = 0.351x + 0.104$	0.997	0.285	0.01	0.05
B5	1.25–50	$y = 0.254x - 0.095$	0.996	0.770	0.08	0.25

a = Intercept; b = slope; R^2 = determination coefficient; $S_{y/x}$ = standard deviation of residuals; LOD = limit of detection; LOQ = limit of quantitation

The consistency of the proposed method was evaluated by applying it to real samples to determine B-vitamins in 11 energy drinks sold at a Brazilian market, using standard additions to avoid any matrix effects. The RSD was lower than 8.3 %. No significant difference was observed between the values found and those values declared by the manufactures on the labels. Table 3 summarizes these results, where the values of vitamins B3a and B3b were added together and expressed as total vitamin B3.

Note that even in samples where levels of vitamins B1 and B3 were declared as zero by the manufacturer, small concentrations of these vitamins were found. This happens because guarana extract is used in the formulation of many of these samples, and is a natural source of vitamins B1 and B3.

Table 3. Concentration (mg/L) of B-vitamins in energy drink samples (n = 5) as well the RSD (%) values. <LOD = lower than detection limit. Continued on next page.

Sample	Vitamin	Concentration informed (mg/L)	Concentration found (mg/L)	RSD (%)
 A	B1	8.7	16.2 ± 1.0	6.1
	B2	9.4	14.4 ± 1.2	8.3
	B3	101.8	110.7 ± 6.3	5.7
	B5	36.4	37.9 ± 0.3	0.8
	B6	9.4	12.4 ± 0.1	0.8
	B12	17.4 µg/L	<LOD	–
 B	B1	0.4	0.9 ± 0.05	5.5
	B2	6.0	6.5 ± 0.2	3.1
	B3	86.8	76.0 ± 3.2	4.2
	B5	14.4	18.5 ± 1.1	5.9
	B6	8.4	7.1 ± 0.4	5.6
	B12	18 µg/L	<LOD	–
 C	B1	0.4	0.5 ± 0.03	5.8
	B2	6.0	6.6 ± 0.4	6.1
	B3	86.8	64.1 ± 3.3	5.1
	B5	14.4	18.1 ± 0.6	3.3
	B6	8.4	7.2 ± 0.2	2.8
	B12	18 µg/L	<LOD	–
 D	B1	0.4	0.3 ± 0.01	3.3
	B2	6.0	2.3 ± 0.1	4.3
	B3	86.8	59.6 ± 3.5	5.9
	B5	14.4	25.1 ± 1.2	4.8
	B6	8.4	6.3 ± 0.2	3.2
	B12	18 µg/L	<LOD	–
 E	B1	0	0.6 ± 0.0	1.0
	B2	0	0.9 ± 0.0	0.8
	B3	0	6.5 ± 0.1	5.3
	B5	0	2.2 ± 0.1	6.8
	B6	0	1.1 ± 0.0	2.2
	B12	0	<LOD	–
 F	B1	0	0.2 ± 0.0	0.4
	B2	4.83	8.8 ± 0.1	1.2
	B3	59.5	92.3 ± 5.7	6.2
	B5	18.6	29.4 ± 1.8	6.1
	B6	4.83	30.6 ± 0.4	1.5
	B12	8.9 µg/L	<LOD	–
 G	B1	0	0.2 ± 0.0	4.3
	B2	4.83	8.6 ± 0.1	1.6
	B3	59.5	58.8 ± 1.6	2.7
	B5	18.6	31.0 ± 0.2	0.8
	B6	4.83	4.6 ± 0.0	0.1
	B12	8.9 µg/L	<LOD	–

 H	B1	0	0.3 ± 0.0	3.6
	B2	4.7	0.1 ± 0.0	3.9
	B3	54.0	67.5 ± 0.9	1.3
	B5	16.9	20.5 ± 1.2	5.8
	B6	4.7	7.5 ± 0.3	4.0
	B12	3.6 µg/L	<LOD	–
 I	B1	0	0.2 ± 0.0	2.2
	B2	4.7	5.2 ± 0.0	0.9
	B3	54.0	75.9 ± 0.3	0.4
	B5	16.9	14.6 ± 0.0	0.2
	B6	4.7	7.6 ± 0.1	0.8
	B12	0	0.2 ± 0.0	2.2
 J	B1	0	0.2 ± 0.0	3.9
	B2	0	1.4 ± 0.1	5.6
	B3	48.3	79.0 ± 0.8	1.0
	B5	18.6	17.8 ± 0.5	2.9
	B6	5.2	7.8 ± 0.4	4.6
	B12	3.9 µg/L	<LOD	–
 K	B1	0	0.2 ± 0.0	0.7
	B2	5.0	4.5 ± 0.1	1.2
	B3	65.0	12.3 ± 0.2	1.6
	B5	5.0	4.3 ± 0.2	5.4
	B6	5.0	1.6 ± 0.1	6.3
	B12	10.0 µg/L	<LOD	–

Conclusions

We have been able to show that CE/MS/MS is well suited for the analysis of B-vitamins in energy drinks. The proposed method presented a linear response with excellent precision data for replicate injections and LODs lower than 0.19 ppm. In addition, the method is simple and fast, spending less than eight minutes per sample, using a small amount of sample with low reagent consumption.

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