

# Simultaneous analysis of 15 water-soluble vitamins in beverages and dietary supplements with multi-mode ODS column using LC-MS/MS

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## 1. Introduction

Recent years have seen a market growth of vitamin enriched-products. The manufactures must manage and analyze their products in order that the nutrient function claims are indicated properly. The water-soluble vitamins are high polar compounds, so they are hard to be retained to reverse-phased column such as ODS, which is typically used in LC-MS analysis. It needs addition of ion pair reagent in mobile phase to retain them, but it causes decrease of sensitivity. In this work, we developed a LC-MS/MS method for the simultaneous analysis of 15 water-soluble vitamins with multi-mode ODS column (anion exchange, cation exchange, ODS) without addition of ion pair reagent. Furthermore, we tried the quantification of water-soluble vitamins in energy drink and multi-vitamin supplement.

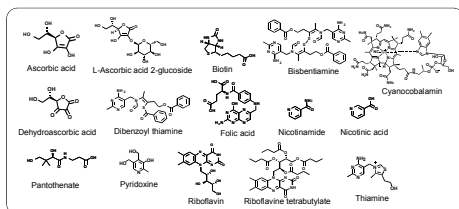


Figure 1 Structure of 15 water-soluble vitamins

## 2. Methods and Materials

Water-soluble vitamins were determined by LC-MS/MS using Nexera UHPLC system coupled to an LCMS-8040 triple quadrupole mass spectrometer. Chromatographic separations were carried out using multi-mode column. Scherzo SM-C18 (2.0 mm i.d. x 150 mm, 3µm) maintained at 40 Celsius. Vitamins were separated using a gradient elution with a flow rate of 0.2mL/min, solvent A: 5 mM ammonium formate aqueous solution with formic acid 0.05% (v/v) and solvent B: acetonitrile with formic acid 0.3% (v/v). The linear gradient was formed as follows: 0min (0% B), 4 min (5% B), 10 min (50% B), 11-18min (100% B) and 19-30min (0% B).

MRM transitions and responses were optimized for individual compounds in both positive and negative ionization. MRM transition are shown in Table 1. The LCMS-8040 acquired positive and negative data using a polarity switching speed of 15msec with a pause time of 1msec.



High Speed Mass Spectrometer  
 > Polarity Switching Time: 15msec  
 > Scanning Speed: Max. 15,000u/sec

Figure 1 LCMS-8040 triple quadrupole mass spectrometer

Table 1 MRM Transitions of 15 water-soluble vitamins

Compound	Polarity	MRM Transition (m/z)	Compound	Polarity	MRM Transition (m/z)
Thiamine	+	265 > 122 <sup>a</sup> , 265 > 144	Cyanocobalamin	+	679 <sup>b</sup> > 147 <sup>b</sup> , 679 <sup>b</sup> > 359
Dehydroascorbic acid	-	219 <sup>c</sup> > 172 <sup>c</sup> , 219 <sup>c</sup> > 113	Riboflavin	+	373 > 242 <sup>d</sup> , 373 > 172
Pyridoxine	+	170 > 134 <sup>e</sup> , 170 > 152	Biotin	+	245 > 227 <sup>f</sup> , 245 > 97
Ascorbic acid	-	175 > 119 <sup>g</sup> , 175 > 87	Folic acid	+	442 > 299 <sup>h</sup> , 442 > 176
Nicotinic acid	+	124 > 78 <sup>i</sup> , 124 > 80	Bisbentiamine	+	771 > 123 <sup>j</sup> , 771 > 124
L-Ascorbic acid 2-glucoside	-	337 > 174 <sup>k</sup> , 337 > 277	Dibenzoyl thiamine	+	491 > 122 <sup>l</sup> , 491 > 105
Nicotinamide	+	123 > 82 <sup>m</sup> , 123 > 78	Riboflavin tetrabutylate	+	657 > 587 <sup>n</sup> , 657 > 517
Pantothenate	+	220 > 99 <sup>o</sup> , 220 > 202			

Notes: <sup>a</sup> For dehydroascorbic acid and cyanocobalamin, the [M + HCOO]<sup>-</sup> ion and the doubly charged ion ([M + 2H]<sup>2+</sup>) were selected as precursor ions, respectively.  
<sup>b</sup> Most abundant product ions were used for quantification.

## 3. Result

### 3-1. Method development

Scherzo SM-C18, a multi-mode ODS column contains anion, cation, and C18 ligands (Figure 2). It was expected that this column provides multiple modes of separation (i.e. reversed phase, anion exchange and cation exchange), both basic and acidic substances can be retained.

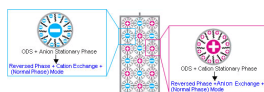


Figure 2 Structure of multi-mode ODS column

Chromatographic condition was investigated with this column. Since weakly acidic conditions promote dissociation of anionic ligands on this column, basic substances such as thiamine and pyridoxine exhibit stronger retention due to ionic interactions. Furthermore, acidic compounds such as nicotinic acid and ascorbic acid are ionic under weakly acidic conditions. As a result, they interact with cationic ligands to remain on the columns. Figure 3 illustrates that ionic compounds such as pyridoxine and nicotinic acid elute later under a lower concentration of formic acid. On the other hand, compounds that strongly interact with cationic ligands, such as folic acid, under similar weakly acidic conditions, were difficult to elute. Therefore, an increasing concentration gradient of formic acid was necessary to elute folic acid.

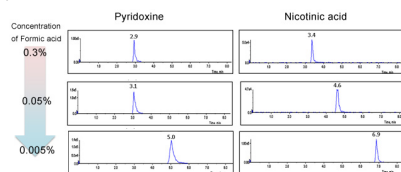


Figure 3 Influence by different concentration of formic acid in the mobile phase

The optimization was achieved by adjusting concentrations of formic acid and compositions of organic solvents in the mobile phases. Figure 4 shows a chromatogram of the 15 compounds in standards of water-soluble vitamins.

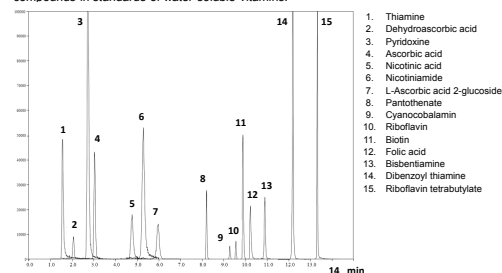


Figure 4 MRM Chromatograms of 15 water-soluble vitamins

### 3-2. Method Validation

Good linearity for the calibration curve of the standard solutions was demonstrated with a correlation coefficient of 0.999 or higher. The concentrations consisted of the following: 2-50 mg/kg for dehydroascorbic acid; 1-40 mg/kg for ascorbic acid and ascorbic acid 2-glucoside, and 2-500 µg/kg for thiamine and riboflavin. Concentrations for the other vitamins were 5-500 µg/kg.

This method has been validated for precision, accuracy and linearity with the analysis of SRM 3280, which provided representative results for supplements. SRM 3280 was spiked with 500 mg/kg of nicotinic acid, ascorbic acid 2-glucoside, bisbentiamine, dibenzoyl thiamine and riboflavin tetrabutylate, which were not contained. As matrix effects (both ion suppression and enhancement) were found, we followed a standard addition method to subtract the matrix effects. All vitamins were detected with good repeatability, accuracy and repeatability (Table 2).

Table 2 Precision, accuracy, and linearity for SRM 3280<sup>a</sup>

Compound	RSD (%)		Spiked level (mg kg <sup>-1</sup> )	Accuracy (%)	Linearity	
	Intra-day <sup>b</sup>	Inter-day <sup>c</sup>			Correlation coefficient (r)	Range (mg kg <sup>-1</sup> )
Thiamine	6.9	4.1	-	105.7	0.9986	0-1000
Dehydroascorbic acid	4.5	7.2	-	96.7	0.9994	0-3000
Pyridoxine	5.2	4.8	-	100.6	0.9985	0-1000
Ascorbic acid	5.9	7.0	-	96.7	0.9970	0-20000
Nicotinic acid	2.6	3.0	500	100.2	0.9997	0-1000
L-Ascorbic acid 2-glucoside	4.9	7.6	500	93.2	0.9986	0-3000
Nicotinamide	6.9	5.8	-	100.0	0.9987	0-1000
Pantothenate	4.8	5.7	-	104.2	0.9955	0-1000
Cyanocobalamin	5.8	6.7	-	106.7	0.9979	0-0.5
Riboflavin	6.7	6.7	-	97.0	0.9993	0-500
Biotin	4.0	5.8	-	108.1	0.9977	0-20
Folic acid	5.8	5.7	-	97.7	0.9993	0-20
Bisbentiamine	5.0	5.9	500	93.2	0.9989	0-1000
Dibenzoyl thiamine	3.7	3.7	500	98.6	0.9999	0-1000
Riboflavin tetrabutylate	5.6	7.3	500	99.0	0.9999	0-1000

<sup>a</sup> The dilution was conducted as the following: 5-fold for cyanocobalamin; 100-fold for dehydroascorbic acid, biotin, folic acid, and L-ascorbic acid 2-glucoside; 1000-fold for ascorbic acid; 10000-fold for the other vitamins.  
<sup>b</sup> RSD value for intra-day represents the repeatability which was obtained by analyzing six replicates.  
<sup>c</sup> RSD value for inter-day represents the between day repeatability which was obtained by analyzing samples during six consecutive days

### 3-3. Sample determination: comparison our result with the label values and the result by official methods

Furthermore, the method was applied to identify the vitamins in commercial beverages and dietary supplements. Niacin was calculated from the total amounts of nicotinic acid and nicotinamide, while vitamin C was calculated from the total amounts of ascorbic acid and dehydroascorbic acid. The determined levels of all compounds were quite similar to the label values and the levels obtained using official methods.

Table 3 Result of quantification (Dietary supplement, per 1 tablet)

Vitamins	Labeled contents	Determined contents	
		New method	Official method <sup>a</sup>
Thiamine	3 mg	3.2 mg	3.2 mg
Riboflavin	3.3 mg	3.2 mg	3.4 mg
Pyridoxine	3 mg	3.2 mg	2.9 mg
Cyanocobalamin	0.006 mg	0.006 mg	0.009 mg
Vitamin C	80 mg	95 mg	90 mg
Biotin	0.045 mg	0.044 mg	0.046 mg
Niacin	11 mg	12 mg	11 mg
Pantothenate	5.5 mg	5.3 mg	5.1 mg
Folic acid	0.200 mg	0.208 mg	0.199 mg

<sup>a</sup> As official methods, HPLC methods were used to analyze thiamine, riboflavin, and vitamin C, while microbiological assays were used to analyze other vitamins.

Table 4 Result of quantification (Commercial beverage sample A, per 100mL)

Vitamins	Labeled contents	Determined contents	
		New method	Official method <sup>a</sup>
Pyridoxine	0.21 mg	0.20 mg	0.22 mg
Niacin	2.1 mg	2.1 mg	1.9 mg
Vitamin C	12-48 mg	41 mg	33 mg

<sup>a</sup> As official methods, HPLC methods were used to analyze thiamine, riboflavin, and vitamin C, while microbiological assays were used to analyze other vitamins.

Table 5 Result of quantification (Commercial beverage sample B, per 100mL)

Vitamins	Labeled contents	Determined contents	
		New method	Official method <sup>a</sup>
Pyridoxine	0.3 mg	0.3 mg	0.4 mg
Folic acid	15-68 mg	25 mg	13 mg
Vitamin C	35-289 mg	130 mg	

## 4. Conclusions

- Utilizing of multi-mode ODS column permits us to separate all compounds within 15 minutes using simple mobile phase condition.
- This method was applied to identify the vitamins in commercial beverages and dietary supplements.
- These findings suggest that this method is further applicable to control the quality of vitamin products efficiently throughout the production, storage, and distribution processes.

## 5. Reference

- Imtakt Technical Report TR03A (Imtakt Co., Kyoto)
- Tetsuo Tanigawa, et al, High speed data acquisition and polarity switching MS/MS applied to water-soluble vitamin analysis using a novel multi-mode ODS separation (Poster No.MP165), ASMS 2011 in Denver, June 3-11, 2011.