Rapid, Sensitive and Cost-effective Detection of B Vitamins in Foods by UHPLC/MS/MS

Application Note

Food Testing

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Abstract

A fast UHPLC/MS/MS method has been developed on the Agilent 1290 Infinity LC System coupled to the Agilent 6460 Triple Quadrupole LC/MS System with Agilent Jet Stream technology for the extraction and determination of five B vitamins in foods. The method has a sensitivity of at least 1 µg/kg for all six vitamins.

Introduction

The B vitamins play important roles in cell metabolism and in promoting human health. They are water soluble and not stored very well by the body, necessitating daily intake through diet or nutritional supplements. Deficiencies of one or more of the B vitamins may occur fairly easily, especially during times of fasting or weight-loss diets or with diets that include substantial amounts of refined and processed food, sugar, or alcohol. The germ and bran of cereal grains are good sources of these vitamins, as are some beans, peas, and nuts. Milk and many leafy green vegetables may also supply small amounts of B vitamins. While most of the B vitamins are not harmful when ingested at high amounts, B3 and B6 can, in fact, be toxic at high concentrations, leading to neuropathy and excessive blood vessel dilation.

To ensure safe levels of vitamins in foods and nutritional supplements, the European Union (EU) has put in place Regulation No. 1925/2006, specifing which vitamins can be added to foods, which foods can and cannot be fortified with vitamins, and minimum and maximum levels for vitamins in foods. The B vitamins are included in this regulation, and food processors must, therefore, have the means to assure that the vitamin B levels in their foods meet the minimum and maximum requirements.

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The B vitamins are typically analyzed using high performance liquid chromatography (HPLC), and the EU has posted standard HPLC methods for the B vitamins. However, these methods detect and quantitate each B vitamin individually, thus requiring a lot of time and effort to analyze all of them.

This application note describes a fast and sensitive method for quantitation of five of the B vitamins simultaneously, using the 1290 Infinity LC System coupled to a 6460 Triple Quadrupole LC/MS System in multiple reaction monitoring (MRM) mode. The total run time is 10 minutes, with a sensitivity of at least 1 µg/L (1 ppb) for all compounds. Since only one analysis is required, rather than the five ISO HPLC methods used previously, this approach is also very cost-effective when multiple vitamins are required to be determined in a given food sample. This method has also been accredited by the United Kingdom Accreditation Service (UKAS) for use with dairy products, meat, vegetables, fruit, cereals and beverages (ISO 17025).

Experimental

Standards

Calibration standards were prepared in 0.1 M HCl.

Instruments

This method was developed on the 1290 Infinity LC System coupled to the 6460 Triple Quadrupole LC/MS System with Jet Stream technology. The instrument conditions are listed in Table 1.

Table 1. LC and MS Instrument Conditions

LC conditions		
Analytical column	Agilent ZORBAX RRHT SB-Aq, 3.0 × 100 mm, 1.8 µm (p/n 861954-314)	
Column temperature	30 °C	
Injection volume	20 µL	
Mobile phase	A = Water, 20 mM ammonium formate 0.1% formic acid B = Methanol, 20 mM ammonium formate 0.1% formic acid	
Run time	10.0 minutes	
Gradient program	Time (min)	% Solvent B
	0	10
	7	50
	8	100
	9	100
	10	10
Mass spec conditions		
Ion mode	ESI with Agilent Jet Stream technology	
Drying gas temperature	200 °C	
Drying gas flow	8 mL/min	
Nebulizer pressure	45 psi	
Sheath gas temperature	400 °C	
Sheath gas flow	12 L/min	
Nozzle voltage	1,250 V	
Capillary voltage	4,000 V	
MRM acquisition	+VE	
Delta EMV	+500	

Sample Preparation

The sample preparation varies significantly, depending on the foodstuff being analyzed. Beverages are diluted and then injected. The dilution factor is dependent on the concentration of the vitamin in the beverage and is determined empirically. A mild acid hydrolysis in an autoclave is used to recover the vitamins from most solid food stuffs. After neutralization with sodium acetate solution and cooling, incubation with selected enzymes such as acid phosphatase and β-glucosidase takes place.

References 1 and 2 provide more information on sample preparation approaches for these vitamins in food. These references illustrate that vitamin extraction involves the release of different conjugates from a food sample, requiring a lab to find a balance between the effort required to extract a high percentage of all of the active compounds and some consideration for what is bioavailable. The extraction is also easier to design and use, if the subsequent method of analysis can then address all potential target compounds for each given vitamin, as is the case with this LC/MS method.

The standard operating procedures (SOPs) around Campden's sample preparation are part of the ISO-17025 accreditation for vitamin analysis with LC/MS/MS. The exact details of the extraction SOPs are proprietary. However, these procedures have been validated using various reference materials, including mixed meat/vegetable processed meals.

Analysis Parameters

The Triple Quadrupole LC/MS System analysis parameters are shown in Table 2.

Table 2. MRM Acquisition Parameters for the B Vitamins

Retention time (min)	Compound	Transition (*m/z*)	Collision energy (V)
1.2	Pyridoxamine	169→151.9	8
		169→134	20
		169→106	32
1.5	Thiamin	265.4→81	32
		265.4→144	8
1.8	Pyridoxal	168→150	8
		168→94	24
		168→67	28
		168→53.1	36
1.85	Pyridoxine	170→152	8
		170→134	20
		170→78	36
		170→65.7	40
2.05	Niacinamide	124→53.3	36
		124→80.6	20
2.55	Pantothenic acid	220→90	8
		220→202	8
3.2	Nicotinic acid	123→78	24
		123→51.7	44
5.75	Riboflavin	377→242.9	20
		377→198	40
		377→172	44
		377→69.2	40

Results and Discussion

One of the challenges for vitamin B analysis is that some of the vitamins are actually composed of multiple compounds. The fact that this MS method does not depend on getting a particular vitamin into one particular compound form is key to its utility. Table 3 lists the five B vitamins analyzed using this method, and the compounds that make up each vitamin. The chromatography used in this method produces good peak shapes and retention for all the vitamin compounds to ensure selective and sensitive MS/MS detection by MRM (Figure 1). The use of MS/MS means that complete separation between all compounds is unnecessary.

Table 3. The B Vitamins Analyzed and Their Component Compounds

Vitamin	Compound(s)
B1	Thiamin
B2	Riboflavin
B3	Niacinamide, nicotinic acid
B5	Pantothenic acid
B6	Pyridoxine, pyridoxamine and pyridoxal

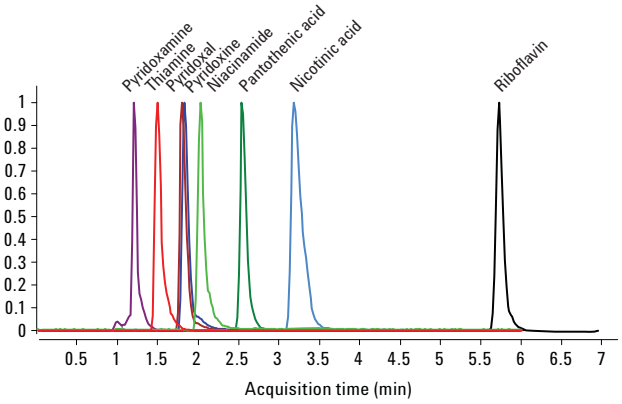

Figure 1. Normalized chromatogram of the separation of vitamins B1, B2, B3, B5, and B6.

Compared with photometric detection, MS/MS detection confers considerable additional qualitative verification of the result through the structurally significant mass transitions of the various vitamins. This advantage is further leveraged by employing additional qualifying mass transitions per compound which can be used to flag any suspect result if the ratio of peak areas (quantifier versus qualifier) is outside a validated expected ratio. Figure 2 illustrates typical MRM results obtained for each vitamin in the calibration solution. These ratios can also be displayed for manual inspection either as chromatographic overlay or as an MRM composite spectrum, as shown in the examples of low level detection of vitamins in beer and breakfast cereal in Figures 3 and 4.

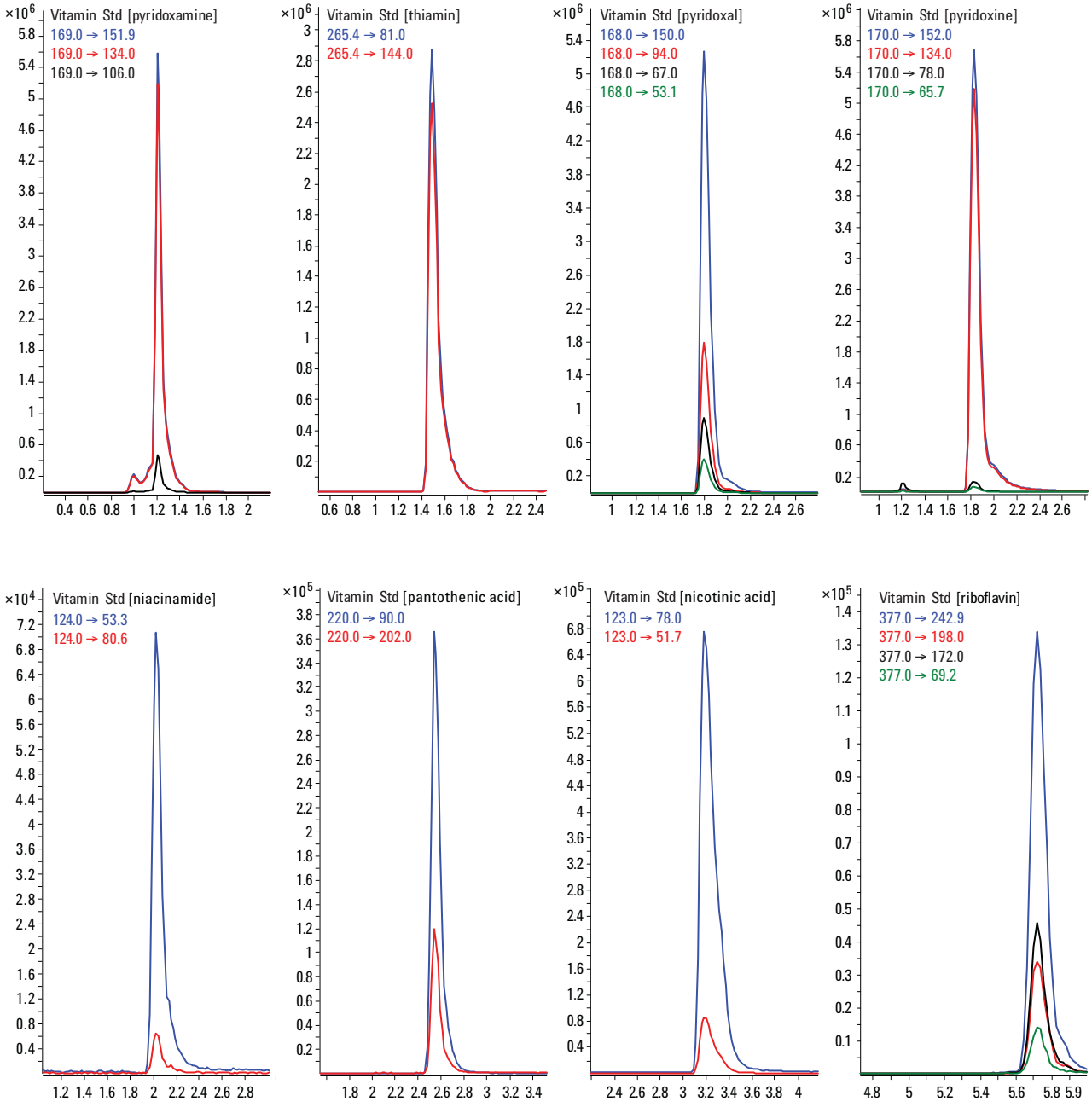

Figure 2. Quantifier and qualifier transitions from a standard which can be used to assess reliability of sample results.

2 mg/kg Pantothenic acid in beer

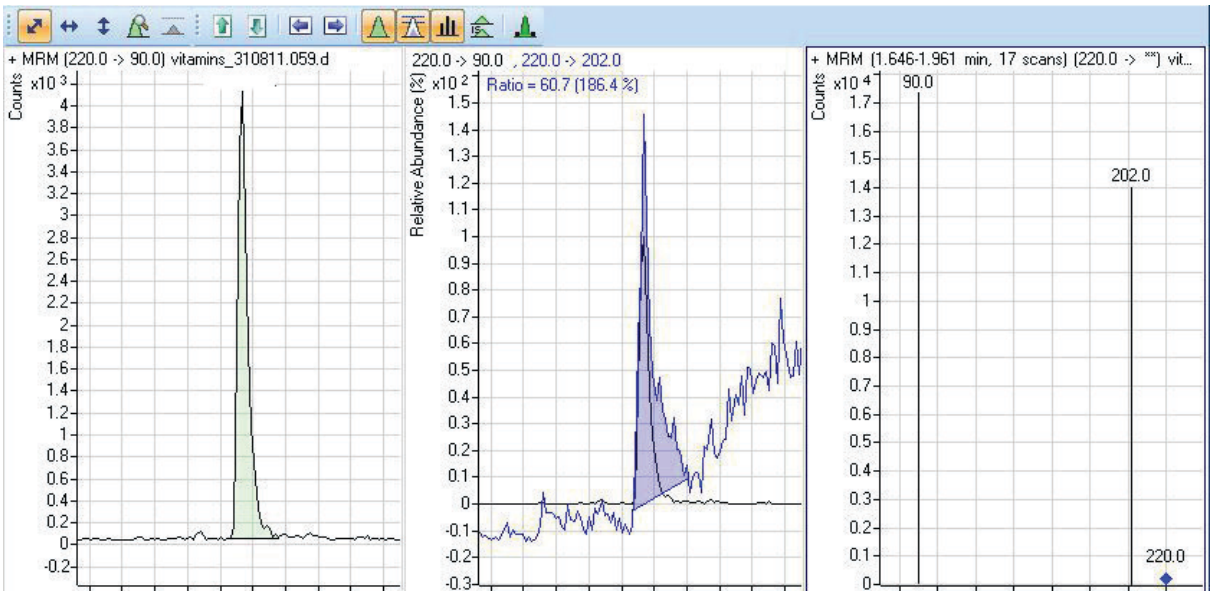

Figure 3. Typical results obtained for vitamin B analysis in beer, in this case for vitamin B5.

25 µg/kg Pyridoxamine in cereal product

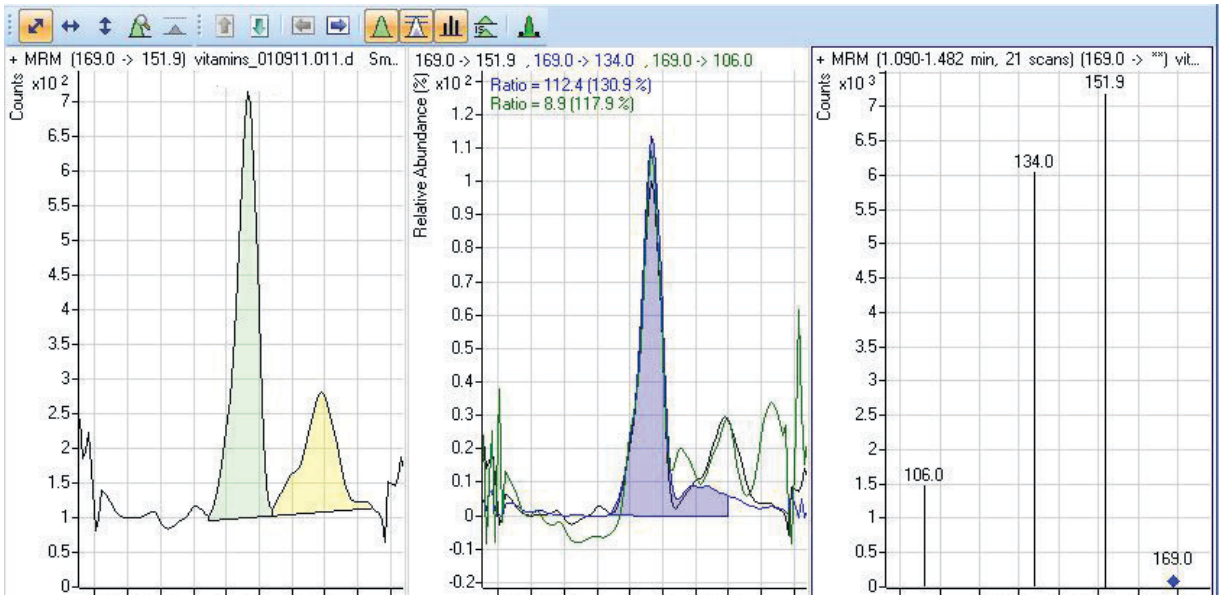

Figure 4. Analysis of pyridoxamine in breakfast cereal at 25 ppb.

The method is linear over more than three orders of magnitude for the B vitamins, with R^2 values > 0.995 (see Figure 6). Batches of samples of a given commodity that arrive at the lab for analysis are always checked for recovery for the target vitamins by spiking one of the sample extracts within the batch. This approach does not check for extraction recovery (this having been validated for a given commodity as part of ISO-17025 accreditation). It does, however, check for matrix effects coming from a particular set of samples. If the spiked extract shows recovery below 70% (or above 110%) then a standard addition approach is applied (five levels) to those samples. This occurs infrequently (less than 5% of the time). For samples showing better than 70% recovery, the results can be expressed as absolute and/or corrected values.

When analyzing food of known vitamin content, both analysis and extraction can be assessed for recovery. Soft drinks and beer typically give recoveries greater than 85%. Bread products also give recoveries greater than 85%, and breakfast cereal recoveries are typically greater than 75%.

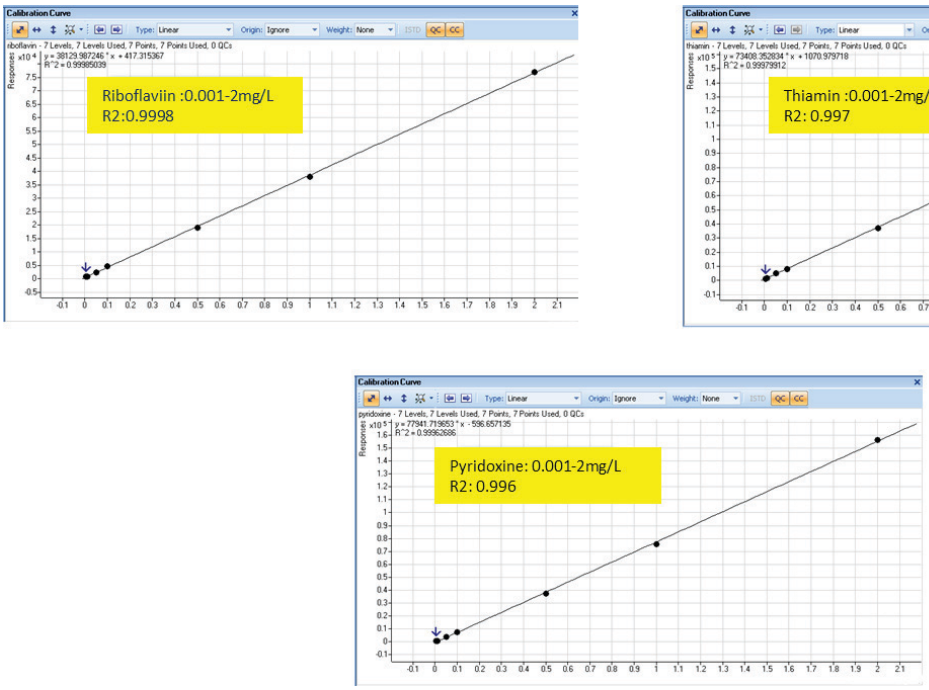

Figure 6. Linearity of seven level calibration curves in 0.1 M hydrochloric acid.

Conclusions

This method developed on the 1290 Infinity LC System coupled to a 6460 Triple Quadrupole LC/MS System for the determination of the B vitamins in various foodstuffs enables the simultaneous quantitation of five B vitamins from one injection. For the Campden Lab, a 10 minute LC/MS/MS approach reduces the equivalent total analysis time by 10 fold, in comparison to the separate HPLC methods previously employed. LC/MS/MS is currently the accredited technique offered by Campden to its customers for these analyses.

References

1. Vitamins in Foods: Analysis, Bioavailability, and Stability, George F.M. Ball, CRC Press, Boca Raton, FL, 2006.
2. Manual of Methods for Determining Micronutrients in Fortified Foods, USAID Micronutrient and Child Blindness Project, September 2010

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