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Nota applicativa

The Rapid, Simultaneous Analysis of 12 Water-Soluble Vitamin Compounds

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Abstract

This application note explains how to utilize the power of Waters ACQUITY UltraPerformance Liquid Chromatography (UPLC), along with fast mass spectrometric acquisition rates, to provide a rapid and selective method to simultaneously analyze 12 water-soluble vitamin compounds in one four-minute run.

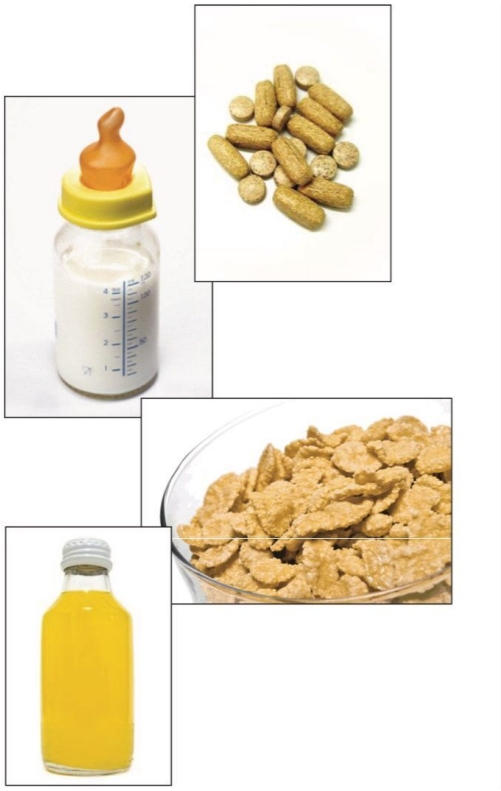
Introduction

As a consequence of global dietary insufficiencies, food manufacturers routinely fortify many food and beverage products to enhance their nutritional value to consumers. Once vitamin compounds have been added to items for consumption, this information should be clearly indicated on the related packaging, in line with strict legal requirements, such as European Regulation (EC) No. 1925/2006[1] regarding the addition of vitamins and minerals to foods; European Directive 2002/46/EC[2] covering dietary supplements; or Title 21 of the U.S. Code of Federal Regulations (CFR) Part 101 – Food Labeling.[3]

Food manufacturers require rapid, reliable, and cost-effective methods to analyze the nutritional content of their products to ensure that their label claims can be substantiated. Vitamins are often present, even after fortification, at very low levels in challenging matrices, making accurate analyses very demanding.

Currently water-soluble vitamin compounds are analyzed individually, or in small groups, using a wide range of different analytical methods, such as microbiological assays, colorimetric analysis, titrimetric procedures, fluorimetric analysis, and HPLC methodologies.[4] This means that analytical laboratories encounter a substantial financial outlay on several different types of instrumentation to facilitate multi-vitamin analysis, as well as a significant investment in personnel time and effort if a series of analyses are to be carried out. The ability to analyze water-soluble vitamin compounds simultaneously in one rapid and selective solution provides businesses with the potential for improved productivity and increased revenue production, resulting from enhanced efficiency, faster sample turnover, and reduced labor and training costs.

This application note describes a quick and selective solution for the simultaneous analysis of 12 water-soluble vitamin compounds commonly used in supplements, or to fortify foods and beverages. This would be particularly suitable for analysts wishing to gain sensitivity, selectivity, and faster sample turn-around time compared with UV detectors for water-soluble vitamin analysis.



Experimental

Throughout the preparation and analyses, all solutions were protected from exposure to light and stored at <5 °C.

LC conditions

LC system:	ACQUITY UPLC System
Column:	ACQUITY UPLC HSS T3, 2.1 x 50 mm, 1.8 µm
Column temp:	40 ˚C
Sample temp:	4 ˚C

Flow rate:	0.6 mL/min.
Mobile phase A:	990 mL water: 10 mL 1M ammonium formate (aq): 1 mL formic acid
Mobile phase B:	990 mL methanol: 10 mL 1M ammonium formate (aq): 1 mL formic acid
Weak needle wash:	0.1% formic acid in water
Strong needle wash:	0.1% formic acid in methanol
Total runtime:	4 min
Injection volume:	20 µL, full-loop injection
Needle type:	Stainless steel (Critical Clean) needle

Gradient:

Time	Composition
0.00 min	99% A
2.00 min	99% A
3.00 min	45% A
3.10 min	99% A
4.00 min	99% A

MS conditions

MS system:	ACQUITY TQ Detector
Ionization mode:	ESI +
Capillary voltage:	1 kV
Desolvation gas:	Nitrogen, 800 L/Hr, 450 ˚C
Cone gas:	Nitrogen, 10 L/Hr
Source temp:	120 ˚C
Acquisition mode:	Multiple Reaction Monitoring (MRM)
Collision gas:	Argon at 3.5 x 10^{-3} mBar

The advantages of the ACQUITY TQD mass spectrometer compared with the core detectors are the selectivity and sensitivity gains that can be achieved in complex matrices, such as food samples. When the MS is used in MRM mode, specific ions are selected that represent the vitamin of interest. This also means that runtimes may be reduced as peaks do not have to be separated by retention time as is necessary with detection techniques such as UV or fluorescence. The MRM transitions for the compounds under analysis are shown in Table 1, along with additional MS parameters and expected retention times.

Vitamin compound	RT	MRM transition	Cone voltage (V)	Collision energy (eV)
Ascorbic acid (C)	0.40	177 > 141	24	8
Thiamine (B1)	0.48	265 > 122	24	17
Nicotinic acid (B3)	0.54	124 > 80	38	20
Pyridoxal (B6)	0.67	168 > 150	27	15
Pyridoxine (B6)	0.89	170 > 152	28	14
Nicotinamide (B3)	0.93	123 > 80	40	20
Calcium pantothenate (B5)	2.77	242 > 153	30	15
Cyanocobalamin (B12)	3.03	678 > 147	36	34
Folic acid (B9)	3.03	442 > 295	23	17
Riboflavin-5'-phosphate (B2)	3.08	457 > 439	41	18
Biotin (B7)	3.15	245 > 227	28	13
Riboflavin (B2)	3.20	377 > 243	42	22

Table 1. MS/MS ion transitions monitored, MS parameters and expected retention times.

Acquisition and processing methods

These data were acquired using Waters' MassLynx Software, v. 4.1.

Incorporated into MassLynx, the IntelliStart Technology automates optimization of MS parameters for the sample, removing the requirement for experienced MS operators to develop the MS method. IntelliStart also monitors the health of the LC-MS system, reducing the time for operator-intensive troubleshooting and upkeep. Automated system check protocols can be scheduled to take place, even outside work hours, and a simple visual indication of the system's health is provided for the operator.

Data processing was carried out using TargetLynx Application Manager, a quantitation package that enables

automated data processing and reporting.

Similar acquisitions and processing can also be carried out using Empower Software.

Results and Discussion

The analysis of 12 water-soluble vitamin compounds was achieved using ACQUITY UPLC with ACQUITY TQD in MRM mode. The compound-specific transitions in MRM mode enabled a rapid and selective solution to be developed, with the elution of all compounds of interest within 4 minutes.

The extracted ion chromatograms for all 12 water-soluble vitamin solvent standards are shown in Figure 1 for concentrations representative of the lowest levels found in food and beverages. They illustrate that the solution is both rapid and selective, with very good peak shapes for this type of analysis.

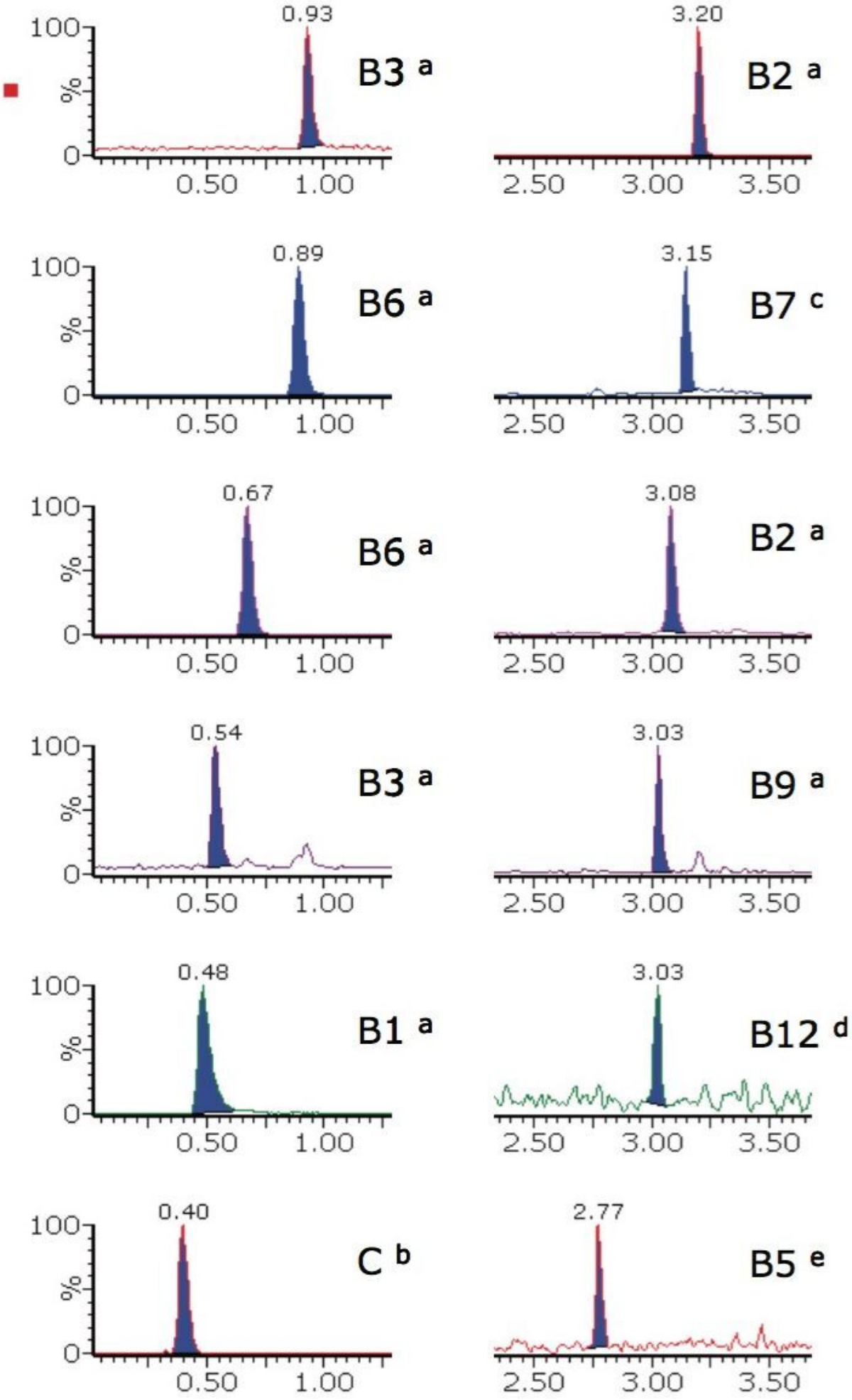


Figure 1. Extracted ion chromatograms showing 12 different water-soluble vitamin solvent standard solutions.

- IntelliStart Technology, which is designed to reduce the burden of complicated operation, new user training, and time-intensive troubleshooting and upkeep.
- A smaller instrument footprint, which provides any laboratory with space-saving advantages.

This system solution, which combines the chromatographic speed of ACQUITY UPLC, with the selectivity of the ACQUITY TQD mass spectrometer, has enabled the amalgamation of 12 different water-soluble vitamin compounds into one single analysis. This offers the revenue-conscious laboratory increased efficiency due to analytical time saving, improved profit through increased sample turn over, and better productivity. In addition, cost savings can be achieved by lowering the use of laboratory consumables, as well as reducing the environmental impact through decreased solvent usage.

This solution provides an ideal basis for a streamlined approach to water-soluble vitamin analysis as it removes the need for multiple methods and offers the analyst the ability to analyze all B-complex vitamins and vitamin C in one rapid and simple procedure.

Figure 5. ACQUITY UPLC System with ACQUITY TQD Mass Spectrometer.

References

1. Website: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:40 4:0026:0038:EN:PDF
2. Website: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:18 3:0051:0057:EN:PDF
3. Website: http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&sid=342a1d06a

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4. Website: http://findarticles.com/p/articles/mi_7098/is_1_88/ai_n28321865/pg_2/

Featured Products

ACQUITY UPLC System <https://www.waters.com/514207>

MassLynx MS Software <https://www.waters.com/513662>

TargetLynx <https://www.waters.com/513791>

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