

Rapid analysis of water-soluble vitamins using Smartline HPLC

Abstract

Efficient baseline resolution of vitamin C and four B-complex vitamins in micronutrient tablets was achieved on a C18 phase with unique bonding technology, especially developed for use in an aqueous mobile phase. Outstanding method performance was ensured by use of Smartline HPLC and the SmartMix static mixer.

Introduction

Vitamins are biologically active compounds which act as controlling agents for an organism's normal health and growth. While the level of vitamins in food may be as low as a few micrograms per 100 g, these vitamins are often accompanied by an excess of compounds with similar chemical behavior. Analysis of water-soluble vitamins using traditional reversed-phase HPLC is not possible however because the highly polar compounds are not retained on conventional silica C18 columns. For instance, thiamine and ascorbic acid (vitamin C) show almost no retention on standard C18 material. Reversed-phase analytical methods employing ion-pair reagents have been offered as a potential solution to this problem, but these methods tend to suffer from column-to-column reproducibility problems due to the somewhat unpredictable way ion-pairing reagents interact with the silica surface and bonded phase.

In this study we focus on the HPLC analysis of micronutrient tablets containing vitamin C and four B-complex vitamins: vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B3 (nicotinamide) and vitamin B6 (pyridoxine). A specially modified C18 phase was used with an acidic mobile phase to provide fast resolution of all vitamins present.

Sample Preparation

Water soluble vitamins can be extracted from simple matrices such as vitamin tablets (after homogenization) with water in an ultrasonic bath. Only 250 mg from the total sample are transferred into a 50 ml volume flask. Approximately 40 ml of 0.5% oxalic acid solution was added and the sample stirred. After 20 min treatment in an ultrasonic bath, the sample solution must be cooled down and the volume adjusted to 50 ml with 0.5% oxalic acid. Before injection the sample was filtered through a 0.45 µm syringe filter.

Experimental

All standard solutions were prepared with 0.5% oxalic acid in double-distilled water. A preliminary standard of three water-soluble vitamins was created by weighing out 10 mg of thiamine, 20 mg of pyridoxine and 10 mg riboflavin into a 100 ml volume flask. The flask was brought up to 100 ml with 0.5% oxalic acid (V1). In a second 100 ml volume flask, 70 mg ascorbic acid and 20 mg nicotinamide were diluted with 70 ml of 0.5% oxalic acid. The final standard was created by adding 10 ml of the V1 solution to the second flask and adjusting the volume to 100 ml with 0.5% oxalic acid. The final concentration of each vitamin in the standard was as follows:

thiamine	0.010 µg/µl
pyridoxine	0.020 µg/µl
riboflavin	0.010 µg/µl
ascorbic acid	0.70 µg/µl
nicotinamide	0.20 µg/µl

HPLC parameters

Column:	ProntoSil 120 5µm C18 AQ 150 x 3 mm (part number 15CF184PSJ)
Eluent A:	50 mM H ₃ PO ₄ (adjusted to pH 2.5)
Eluent B:	ACN
Gradient:	0-2 min 99% A; 2-8.5 min 30% A; 8.5-11 min 30% A; 11.02-15 min 99% A
Flow:	0.6 ml/min
Pressure:	77 bar
Detection:	UV at 268 nm or wavelength program (see Fig. 1)
Inj. Vol.:	10 µl
Temperature:	40 °C

Instrumentation

KNAUER Smartline HPLC system equipped with Autosampler 3900, Pump 1000 with 10 ml pump head, SmartMix static mixer, Manager 5000 with low pressure gradient and degasser modules, Column Oven 4000 and UV Detector 2600 with analytical flow cell.

Results

Since each vitamin has a slightly different optimal absorption wavelength (Fig. 1), a wavelength switching program was used to increase the sensitivity of quantification for each vitamin. Figure 2 depicts the chromatogram obtained for the micronutrient tablet sample. Analysis results are listed in Table 1.

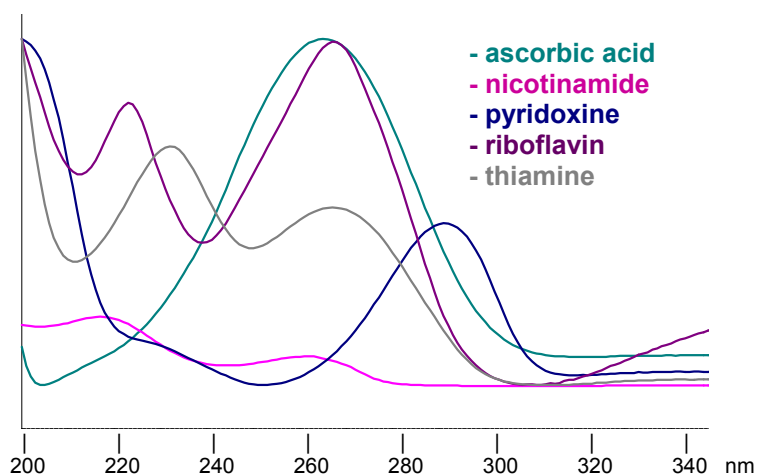


Fig. 1 Wavelength spectrum of 5 water-soluble vitamins.

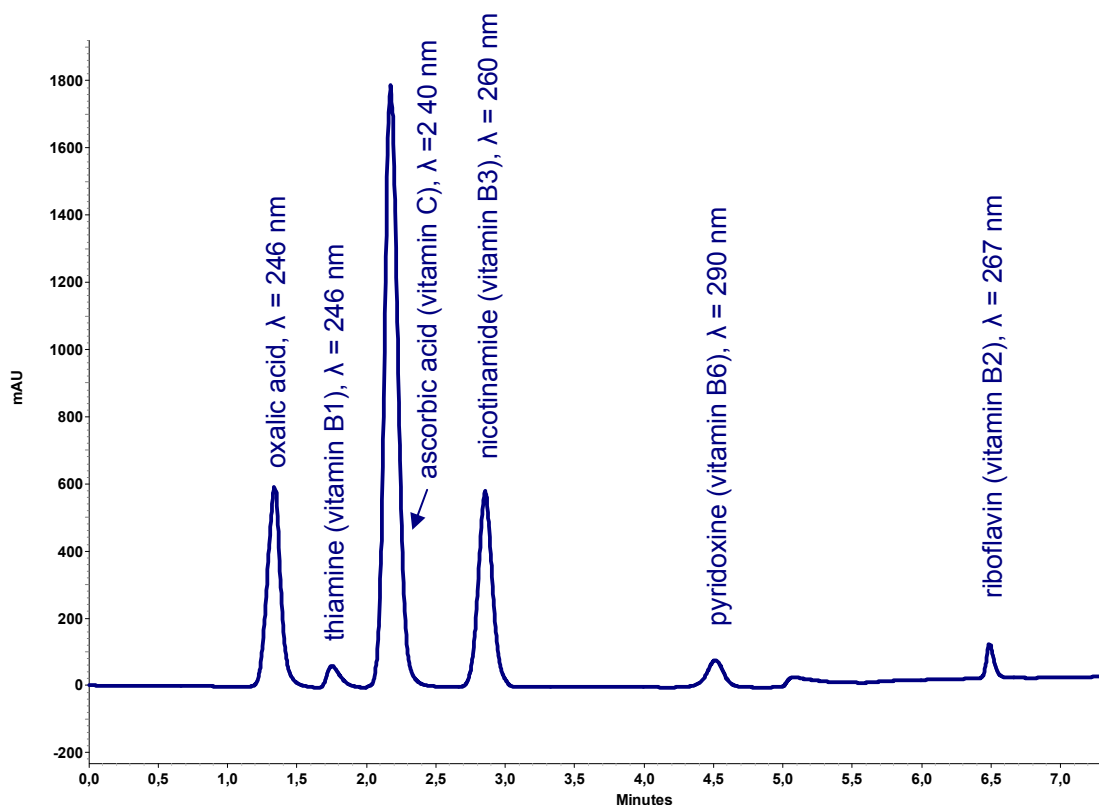


Fig. 2 Separation of five vitamins in a micronutrient tablet.

Table 1

Peak #	Substance	t _R [min]	Area	Asymmetry	ESTD [mg/core]
1	Oxalic acid	1.328	2877790	1.18	-
2	Thiamine (vitamin B1)	1.742	509181	2.04	1.26
3	Ascorbic acid (vitamin C)	2.185	12239574	1.05	63.5
4	Nicotinamide (vitamin B3)	2.877	4208215	1.00	16.2
5	Pyridoxine (vitamin B6)	4.538	695962	0.90	1.71
6	Riboflavin (vitamin B2)	6.436	395154	1.25	1.26

HPLC method performance

Limit of detection (S/N = 3)

Substance	LOD [ng]
Oxalic acid	-
Thiamine (vitamin B1)	17.5
Ascorbic acid (vitamin C)	59
Nicotinamide (vitamin B3)	20
Pyridoxine (vitamin B6)	8
Riboflavin (vitamin B2)	2

Repeatability of

Retention time over 10 runs: < 0.2 % RSD

Areas over 10 runs < 3 % RSD

Conclusion

A fast separation of five vitamins with good peak symmetry is easily accomplished by reversed-phase HPLC using the ProntoSil C18 AQ column and Smartline HPLC. Thanks to the SmartMix static mixer, gradient mixing was performed efficiently while minimizing baseline noise, making lower limits of detection and < 0.2 % RSD in retention times possible.

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