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Application Note

► Rapid analysis of water-soluble vitamins

Category	Food
Matrix	Vitamin supplement tablets
Method	HPLC
Keywords	Water-soluble and B-complex vitamins
Analytes	Ascorbic acid (vitamin C), thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), pyridoxine (vitamin B6)
ID	**VFD0001N**, 10/07, updated 04/14

Summary

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.

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: thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3) and pyridoxine (vitamin B6). A specially modified C18 phase was used with an acidic mobile phase to provide fast resolution of all vitamins present.

Experimental

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

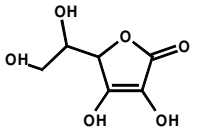
Preparation of Standard Solution

All standard solutions were prepared with 0.5% oxalic acid in double-distilled water. A preliminary standard (V1) was prepared 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. In a second 100 ml volume flask, 70 mg ascorbic acid and 20 mg niacin 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:

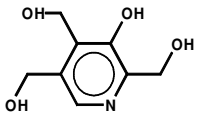
thiamine	0.010 µg/µl
pyridoxine	0.020 µg/µl
riboflavin	0.010 µg/µl
ascorbic acid	0.70 µg/µl
niacin	0.20 µg/µl

Chemical Structures

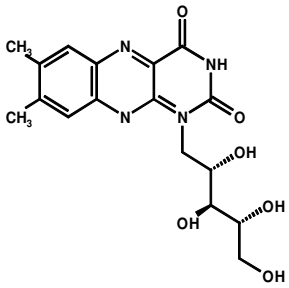
Since each vitamin has a slightly different optimal absorption wavelength (Fig. 1), a wavelength switching program was used to increase the sensitivity of quantification for each vitamin.



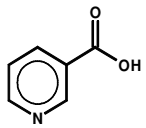
ascorbic acid (vitamin C)



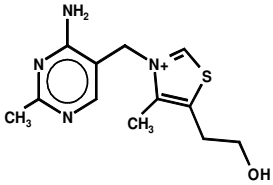
pyridoxine (vitamin B6)



riboflavin (vitamin B2)



niacin (vitamin B3)



thiamine (vitamin B1)

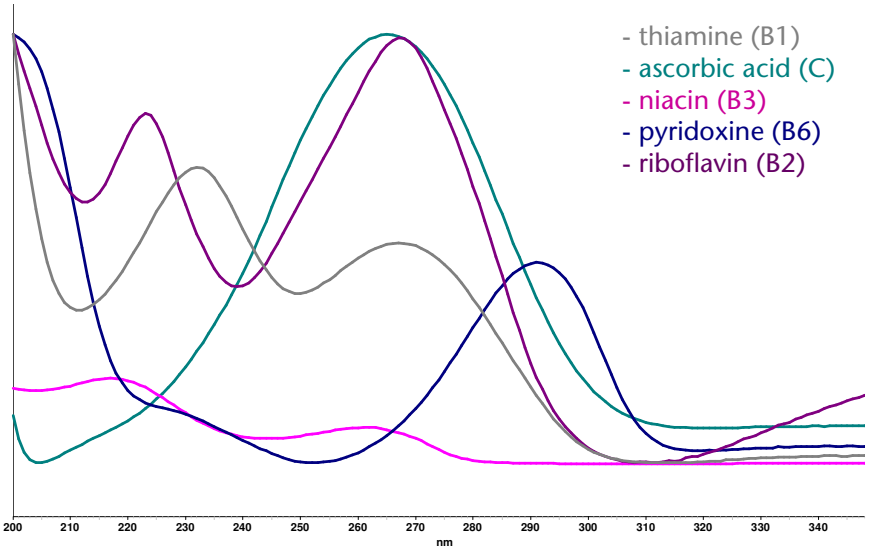


Fig. 1

Wavelength spectrum of five water-soluble vitamins.

Method Parameters

Column	ProntoSIL 120-5 C18 AQ 150 x 3 mm		
Eluent A	50 mM H_3PO_4 (adjusted to pH 2.5)		
Eluent B	acetonitrile		
Gradient	**Time (min)**	**% A**	**% B**
	0.00	99	1
	2.00	99	1
	8.50	30	70
	11.00	30	70
	11.02	99	1
	15.00	99	1
Flow rate	0.6 ml/min		
Injection volume	10 µl		
Column temperature	40 °C		
System pressure	approx. 77 bar		
Detection	UV at 268 nm or program (see Fig. 1)		
Run time	7 min (15 min incl. regeneration)		

Results

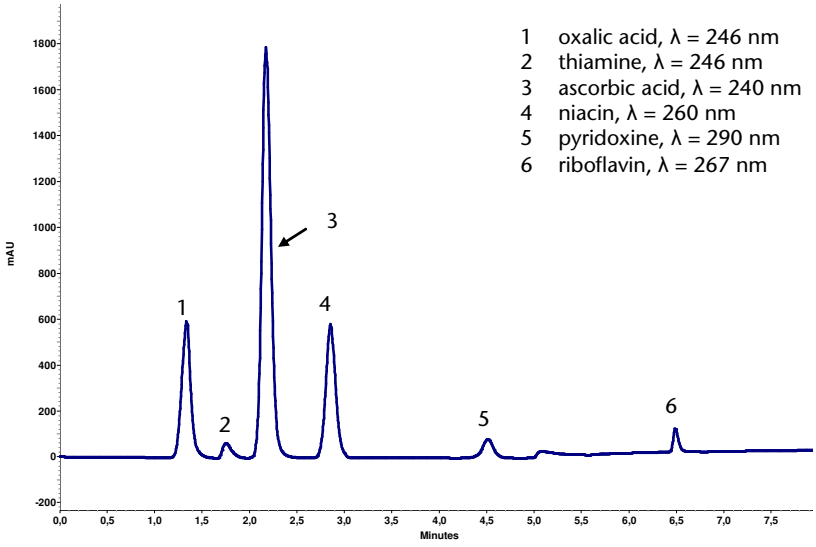


Fig. 2

Separation of five vitamins in a micronutrient tablet

Substance	**t_R (min)**	**mg/tablet**	**LOD (ng)**
oxalic acid	1.328	-	-
thiamine (vitamin B1)	1.742	1.26	17.5
ascorbic acid (vitamin C)	2.185	63.5	59
niacin (vitamin B3)	2.877	16.2	20
pyridoxine (vitamin B6)	4.538	1.71	8
riboflavin (vitamin B2)	6.436	1.26	2

Method Performance

Limit of detection	ng range (S/N = 3)
Linearity (r^2)	0.999885-0.99993
Linearity range	0.1 to 100 ng
Retention time precision*	< 1 % RSD
Peak area precision*	< 3 % RSD

*repeatability calculated over 5 replicate runs

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 AZURA HPLC. Thanks to the static mixer, gradient mixing was performed efficiently while minimizing baseline noise, making lower limits of detection and < 0.2 % RSD in retention times possible.

References

[1] P.Moreno, and V. Salvado, J.Chromatogr. A, 870, 207 (2000)

[2] V. M. Staroverov,V. I. Deineka, M. Grigor'ev, E. F. Prokhoda, M. V. Pokrovskii, and V. V. Ivanov,
Pharm. Chem. Journal, Vol. 38, 3, 172-174 (2004)

[3] L.M. Nollet, Food Analysis by HPLC, New York, 1992

Physical Properties of recommended Column

ProntoSIL C18 AQ was specially developed for use in aqueous mobile phases with < 10% organic content. Produced with a unique bonding technology, this phase is particularly suited to the separation of polar analytes, giving excellent peak shapes and enhanced selectivity

Stationary phase	ProntoSIL 120-5 C18 AQ
USP code	L1
Pore size	120 Å
Pore volume	1.06 ml/g
Specific surface area	323 m^2/g
Particle size	3µm
Form	spherical
Surface area	300 m^2/g
% C	14
Endcapping	yes
Dimensions	150 x 3 mm
Order number	15CF184PSJ

Recommended Instrumentation

This application requires a binary gradient HPLC system (low pressure or high pressure gradient configuration) equipped with degasser, autosampler, column oven, and multi-wavelength UV detector. Other configurations are also available. Please contact KNAUER to configure a system that's perfect for your needs.

Description	Order No.
P 6.1L 10ml VA LPG	APH34EA
DAD 6.1L	ADC11
Column Thermostat CT 2.1	A05852
Autosampler 3950	A50070
UV cell (10 mm), analytical version	AMC38
OpenLAB CDS	A2600-1
OpenLAB CDS 3D Option	A2611-1

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