



Analysis of Water-Soluble Vitamins by HPLC

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Vitamins are compounds, which are necessary to maintain a healthy and properly functioning human organism. A few milligrams a day are enough to regulate the utilization of nutrients, such as carbohydrates, fats, proteins and minerals. Usually vitamins are supplied in food.

Figure 1 shows the HPLC chromatogram of the eleven water-soluble vitamins pyridoxamine (B₆), thiamine (B₁), niacinamide, pyridoxal (B₆), pyridoxine (B₆), aminobenzoic acid, pantothenic acid, folic acid, riboflavin (B₂), biotin and thiotic acid using gradient analysis on a reversed phase column and UV detection. The autosampler temperature was set to 4 °C to avoid decomposition of the samples.

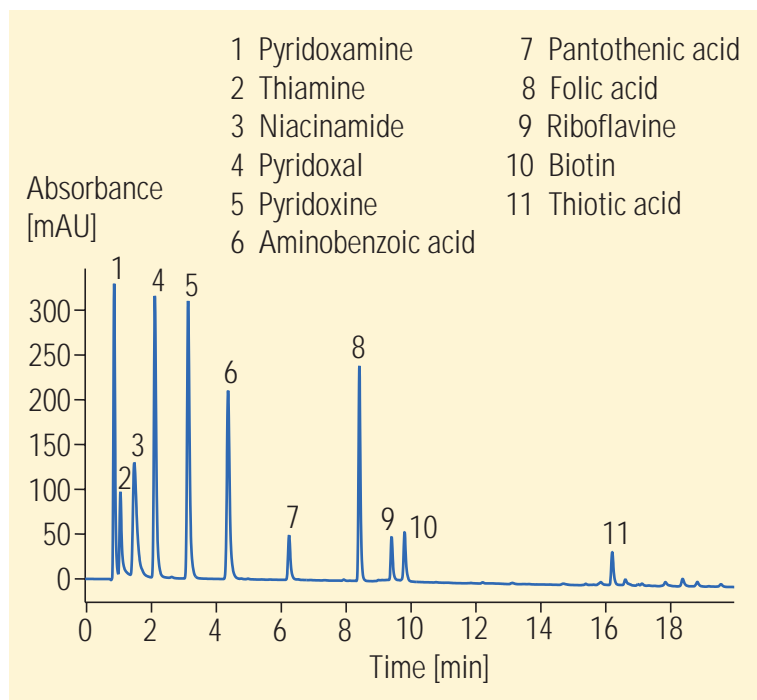


Figure 1
Analysis of water-soluble vitamins

Conditions

Column

4.6 x 75 mm Zorbax SB-C18, 3.5 µm

Mobile phase

A = 0.05M KH₂PO₄ in water (pH = 2.5), B = acetonitrile

Flow rate 1.0 ml/min

Gradient

at 0 min 0.6 % B
at 0.5 min 0.6 % B
at 4 min 6 % B
at 12 min 30 % B
at 17 min 60 % B

Column wash

at 19 min 60 % B
at 20 min 0.6 % B

UV detector

variable wavelength detector
204 nm, standard cell

Column compartment temperature

15 °C

Stop time 20 min

Post time 10 min

Injection volume 5 µl



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HPLC Performance

Compound	LOD for S/N=2 (mg/l)*	Precision of RT (RSD of 10 runs) (250 mg/l)*	Precision of Area (RSD of 10 runs) (250 mg/l)*
Pyridoxamine	0.3	0.09	0.17
Thiamine	0.7	0.23	0.90
Niacinamide	0.7	0.22	0.28
Pyridoxale	0.3	0.36	0.28
Pyridoxine	0.3	0.39	0.13
Aminobenzoic acid	0.4	0.13	0.14
Pantothenic acid	2.5	0.07	0.34
Folic acid	0.7	0.08	0.15
Riboflavin	0.7	0.05	1.04
Biotin	6.0	0.05	0.33
Thiotic acid	6.0	0.04	1.50

* Injection volume: 5 µl

The performance of the HPLC method is shown in the table above.

The HPLC method presented here shows an easy but reliable and precise analysis of the water-soluble vitamins pyridoxamine (B₆), thiamine (B₁), niacinamide, pyridoxal (B₆), pyridoxine (B₆), aminobenzoic acid, pantothenic acid, folic acid, riboflavin (B₂), biotin and thiotic acid. The values for LOD, precision of RT and precision of area show the good performance of the analysis.

Equipment

Agilent 1100 Series

- Quaternary pump (includes vacuum degasser)
- Thermostatted autosampler
- Thermostatted column compartment
- Variable wavelength detector, standard flow cell 10-mm path length, 13-µl cell volume

Alternative:

- Binary pump
- Vacuum degasser
- Diode array detector standard flow cell 10-mm path length, 13-µl cell volume
- Agilent ChemStation + 3D software

Columns

- Zorbax SB-C18, 3.5 µm, 4.6 x 75 mm (Agilent part number 866953-902)
- Recommended: Guard cartridges Zorbax SB-C18, 5 µm, 4.6 x 12.5 mm (Agilent part number 820950-920, 4/pk)

Note:

Since the method was specifically developed on the Agilent 1100 Series system you might not be able to reproduce this analysis on an older system or even on a new system with lower performance. To avoid sample decomposition it is necessary to use a cooled autosampler, for example, the Agilent 1100 Series thermostatted autosampler.

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