

Fast analysis of water-soluble vitamins

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This application note describes a fast LC method for the analysis of water-soluble vitamins using a Thermo Scientific Hypersil GOLD aQ column.

Key Words:

Accela

Hypersil GOLD aQ

Polar retention

Selectivity

Small particles

Speed

Introduction

Water-soluble vitamins represent a group of various, essential dietary compounds, which are classified by their biochemical activities, not their physico-chemical properties. A single vitamin generally is formed by several vitamers (various chemical species, each of which displays the same biological activity). As a result of their different chemical properties, it can be difficult to reliably and simultaneously analyse vitamins.

The method in this application note describes the use of a 1.9 μm particle polar endcapped C18 stationary phase to allow the fast and efficient chromatographic determination of nine water-soluble vitamins.

Materials and Method

Chromatographic conditions

Column: Hypersil GOLD aQ™ 1.9 μm ,
100 x 2.1 mm.

Part number: 25302-102130.

Mobile phase: A – 50 mM KH_2PO_4
(pH 3.5): MeOH (95:5)

B – 50 mM KH_2PO_4
(pH 3.5): MeOH (5:95)

Gradient: 0 to 100% B in 3.8 min.

Flow rate: 0.5 mL/min.

Temperature: 30 °C.

Detection: UV at 205, 254 and 280 nm.

Injection volume: 0.5 μL .

Standards: 1) Ascorbic acid, 2) Pyridoxine,
3) Nicotinamide, 4) Panthotenic acid,
5) Folic acid, 6) Cyanocobalamin,
7) Thiamine, 8) Biotin, 9) Riboflavin.

Standard stock solution concentrations: 1 mg/mL.
Standards were dissolved in methanol, except for pyridoxine, panthotenic acid and riboflavin, which were

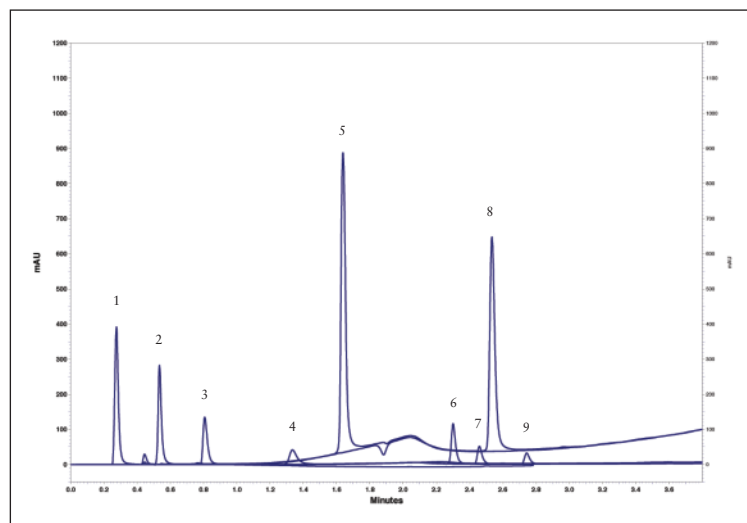


Figure1: Injection of water soluble vitamins on a Hypersil GOLD aQ 1.9 μm , 100 x 2.1 mm. Peak assignment: 1) Ascorbic acid, 2) Thiamine, 3) Pyridoxine, 4) Nicotinamide, 5) Panthotenic acid, 6) Folic acid, 7) Cyanocobalamin, 8) Biotin, 9) Riboflavin.

dissolved in water. Folic acid and biotin were prepared in water + 50 μL 1 M NaOH.

Standard working solution concentrations: 100 $\mu\text{L/mL}$.
All standards were diluted in water.

Results and discussion

Most water-soluble vitamins are assayed in reversed-phase HPLC with UV detection. However, since many water-soluble vitamins are polar, their analysis requires high separation selectivities and high retention capabilities.

These characteristics can be achieved thanks to the polar endcapping technology used in Hypersil GOLD aQ, which combines C18 retention with additional controlled interaction mechanisms, responsible for superior retention of polar analytes.

The polar endcapping also helps to prevent phase collapse, permitting the use of highly aqueous mobile phase at the start of the gradient and so allowing retention of ascorbic acid away from the solvent front.

The separation selectivity offered by Hypersil GOLD aQ 1.9 μm , 100 x 2.1 mm, is presented in Figure 1. Compared to columns packed with 5 μm or 3 μm particles, the use of 1.9 μm particle size allows operation at higher flow rates without loss in chromatographic efficiency. This means that faster analysis can be performed and full separation of the nine vitamins was achieved in less than 3 minutes.



Conclusions

Hypersil GOLD aQ, with its C18 bonding and polar endcapping technology provided suitable interaction mechanisms to separate a range of water-soluble vitamins, which have different degree of polarity. The use of 1.9 μm particle size allows fast analysis. It was possible to achieve full separation of the nine vitamins in just under 3 min.

References

- [1] L.F. Russell, Quantitative determination of water-soluble vitamins. In: L.M.L. Nollet ed. Food analysis by HPLC. New York, Marcel Dekker, 2000, 403.

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