

Analysis of Fat Soluble Vitamins Using a Core Enhanced Technology Accucore HPLC Column

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Key Words

Accucore RP-MS, fused core, superficially porous, fat soluble vitamins

Abstract

This application note demonstrates the use of the Thermo Scientific Accucore RP-MS HPLC column for the analysis of fat soluble vitamins.

Introduction

Accucore™ HPLC columns use Core Enhanced Technology™ to facilitate fast and high efficiency separations. The 2.6 µm diameter particles are not totally porous, but rather have a solid core and a porous outer layer. The optimised phase bonding creates a series of high coverage, robust phases. The tightly controlled 2.6 µm diameter of Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials.

Vitamins are biologically active compounds that act as controlling agents for an organism's normal health and growth. The level of vitamins in food may be as low as a few µg/100 g. Vitamins often are accompanied by an excess of compounds with similar chemical properties. Thus, not only quantification but also identification is mandatory for the detection of vitamins in food. Vitamins generally are labile compounds that should not be exposed to high temperatures, light, or oxygen. HPLC separates and detects these compounds at room temperature and blocks oxygen and light.



Experimental Details

| Consumables | Part Number |
|--|----------------|
| Fisher Scientific HPLC grade acetonitrile | A/0626/17 |
| Fisher Scientific HPLC grade methanol | M/4056/17 |
| NSC Mass Spec Certified 2 mL clear vial with blue bonded PTFE silicone cap | MSCERT4000-34W |

Sample Preparation

Individual 1 mg/mL standards of vitamin A, vitamin K, vitamin D3, vitamin E and vitamin E acetate were prepared in methanol. Dilutions of these standards were made in methanol to give a working standard with a 10 µg/mL concentration of each compound.

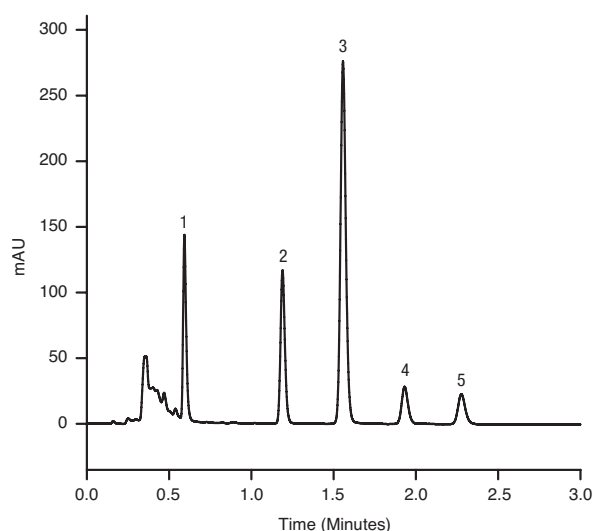
| Separation Conditions | Part Number | |
|-----------------------|-------------------------------------|--------------|
| Column: | Accucore RP-MS 2.6 µm, 100 x 2.1 mm | 17626-102130 |
| Measured pressure: | 152 bar | |
| Mobile phase: | 75:25 (v/v) acetonitrile:methanol | |
| Column temperature | 30 °C | |
| Injection volume: | 2 µL | |
| Flow rate: | 0.4 mL/min | |
| UV detection: | 280 nm | |

Results

Figure 1 shows a chromatogram for the separation of the five fat soluble vitamins, it can be seen that a good separation of all five vitamins has been achieved in three minutes. Table 1 gives the retention time data for replicate injections (n=6) and shows good reproducibility of the separation.

Conclusion

Accucore RP-MS successfully separates fat soluble vitamins with good peak shape and reproducibility in three minutes. It is therefore a good choice for the rapid analysis of fat soluble vitamins.



| | Tr (min) | % RSD | Asymmetry | % RSD |
|--------------------------|----------|-------|-----------|-------|
| Vitamin A | 0.60 | 0.31 | 1.34 | 1.33 |
| Vitamin K | 1.20 | 0.50 | 1.16 | 1.69 |
| Vitamin D3 | 1.58 | 0.62 | 1.14 | 1.49 |
| Vitamin E | 1.95 | 0.63 | 1.15 | 1.05 |
| Vitamin E acetate | 1.95 | 0.60 | 1.15 | 1.37 |

Table 1: Method precision
Measured using retention time and asymmetry of peak 5 and derived from six replicate injections

Figure 1: The separation of five fat soluble vitamins on Accucore RP-MS Compounds: 1. Vitamin A 2. Vitamin K 3. Vitamin D3 4. Vitamin E 5. Vitamin E acetate

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