Application brief | 000578

Liquid chromatography

Normal phase liquid chromatography assay for vitamin K1 isomers

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Keywords

Fat soluble vitamins, vitamin K1, phytomenadione, phylloquinone, phytonadione, normal phase kit for Vanquish Core system

Goal

Highlight the normal phase capabilities of the Thermo Scientific™ Vanquish™ Core HPLC system for routine, reliable, and highly reproducible LC analysis.

Introduction

Vitamin K1 isomers, also known as phylloquinone and phytomenadione, were separated using a silica HPLC column and a normal phase eluent according to the European Pharmacopoeia monograph ("all-rac-Phytomenadione." Ph. Eur. 10.6, 6202 (01/2022:3011)).

Normal phase liquid chromatography (NP-LC), for which a polar stationary phase and nonpolar mobile phases are used, is an essential analytical technique. NP-LC is the technique of choice for many organic, non-polar or inorganic analytes. HPLC systems primarily used for reversed phase LC usually have tubing, membranes, and plastic parts that are incompatible with the organic solvents used in NP-LC. Solubilized plastic components can contaminate the system. Holes can form in tubing and plastic reservoirs upon frequent exposure to organic solvents. For these reasons, newly purchased Vanquish Core systems are prepared for normal phase upon installation using the normal phase kit (P/N 6036.3972).

Experimental

NP eluents usually produce very low back pressures. The EP-recommended flow rate for vitamin K analysis of 0.4 mL/min results in a back pressure less than 20 bar, which is under the operating specification of the Vanquish Core system. The EP-allowed change of doubling the flow rate was made to speed up the method and increase the back pressure. The introduction of a small diameter capillary between the pump and the autosampler is an alternative solution to increase the backpressure.

For NP-LC, unstable retention times, peak areas, or variable peak tailing indicate a column or mobile phase that is not sufficiently eqilibrated with respect to water. NP-LC is sensitive to water and other polar impurities in the eluent. Because HPLC solvents are not normally held under inert atmosphere, the water content of the eluent and column continually increases due to exposure to the moist air. A special solvent preparation was used to speed up the equilibration. For a 1 L portion of eluent, a 500 mL portion of heptane was stirred with 1 mL of water using a magnetic stir bar for 20 min. The heptane was then decanted into a graduated cylinder, such that all visible water droplets were left behind. The heptane was filled up to 500 mL and then poured into a 1 L eluent bottle along with an additional 500 mL of "unwashed" heptane, 3.33 mL diisopropyl ether, and 0.7 mL octanol. With this eluent, stable retention times and peak areas were achieved in 2 hours instead of 24 hours.

Chemicals and standards	Part number
Isopropanol, Fisher Scientific™ Optima™ LC/MS grade	A461-212
Ammonium formate, Fisher Scientific Certified ACS grade	A666-500
Vitamin K1, Fisher Scientific / Alfa Aesar™	L1057503
n-Heptane, Fisher Scientific Optima LC/MS grade	H/0106/17 or H360-1
1-octanol, 99+% TCI America	O0036500ML
Diisopropyl ether, Fisher Scientific Certified ACS grade	E141-500

The following system was used:

Module	Part number
Vanquish Core system consisting of:	
System Base Vanquish Core	VC-S01-A-02
Vanquish Quaternary Pump CN	VC-P21-A-01
Vanquish Split Sampler CT	VC-A12-A
Vanquish Thermostatted Column Compartment C	VC-C10-A-03
Vanquish Diode Array Detector CG	VC-D11-A
Standard Flow Cell, path length 10 mm (13 µL, SST)	6083.0510
Normal Phase Kit	6036.3972

Chromatographic conditions	
Column	Thermo Scientific™ Hypersil™ Silica 5 µm, 250 × 4.6 mm (P/N 30005-254630)
Mobile phase	Heptane/diisopropylether/octanol (1000:3.3:0.7 (v/v/v))
Flow rate	0.8 mL/min
Column temp.	22 °C
Autosampler temp.	20 °C
Injection volume	50 µL
Sample conc.	8 µg/mL in mobile phase
Detector settings	Wavelength: 254 nm
Rear seal wash	2 mM ammonium formate in isopropanol

The Thermo Scientific™ Chromeleon™ 7.3 Chromatography Data System (CDS) was used for data acquisition and analysis.

Results

Retention time reproducibility and peak area reproducibility both met the EP standard of <1% relative standard deviation (RSD) over six injections. Retention time RSDs were 0.08% for the cis-isomer and 0.11% for the trans-isomer. Peak area RSDs were 0.12% and 0.09% for the cis- and trans-isomers. Tailing was not observed, and peak asymmetries were 0.99 and 1.01. The resolution was 5.1 between the two peaks, which satisfies the EP resolution requirement of 4. These results highlight the exceptional analytical precision and reliability that the robust Vanquish Core HPLC systems deliver for normal phase applications.

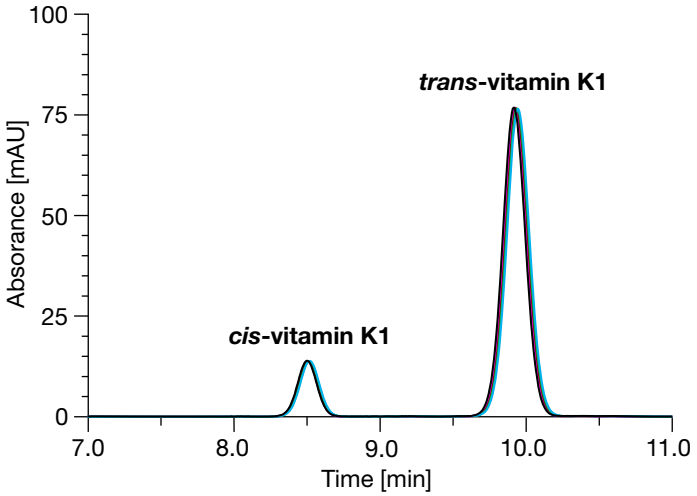

Figure 1. Overlay of six consecutive injections of Vitamin K1

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