Separation of a Mixture of Vitamin K Isomers Using a Solid Core HPLC Column at Sub-ambient Temperature

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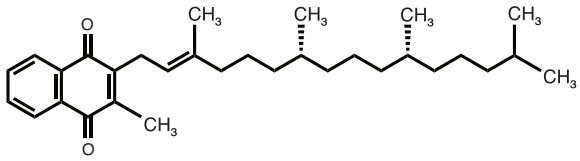
Key Words
Accucore, Core Enhanced Technology, vitamin K, HPLC, vitamins

Abstract
A high speed, high resolution separation of vitamin K1 and K2 was achieved on a Thermo Scientific™ Accucore™ C30 column. The high shape selectivity of this phase enabled the separation of the *cis/trans* isomers of vitamin K1. The separation of the isomers is temperature dependent and is optimum at around 15 °C.

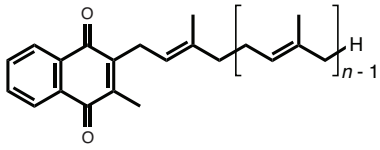
Introduction
Accucore HPLC columns use Core Enhanced Technology™ to facilitate fast and high efficiency separations. The 2.6 µm diameter particles are not totally porous, but rather have a solid core and a porous outer layer. The optimised phase bonding creates a series of high coverage, robust phases.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

Accucore C30 offers high shape selectivity for hydrophobic, long chain, structurally related isomers, for example carotenoids and steroids. It is also an excellent alternative to normal-phase columns for lipid analysis. The optimized bonding density of the long alkyl chains facilitated by a wider pore diameter particle result in a phase that is stable even in highly aqueous mobile phases.

Vitamin K is a group of structurally similar, fat-soluble vitamins that are needed for the post-translational modification of certain proteins, mostly required for blood coagulation but also involved in metabolic pathways in bone and other tissue. They are 2-methyl-1,4-naphthoquinone derivatives. This group includes vitamin K1 and vitamin K2. Plants synthesize vitamin K1 whilst bacteria can produce a number of different vitamin K2 homologues and also convert vitamin K1 into vitamin K2.



Vitamin K1
Present as both cis and trans isomer, synthesized in plants.



Vitamin K2
Multiple homologues with different number of isoprenoid residues in the chain (MK-4 to MK-14). Menaquinone-MK4 is the most common form in animals and has been used in this study. MK 5-14 are formed chiefly from bacterial metabolism.

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Experimental Details

Consumables	Part Number
Fisher Scientific HPLC grade water	W/0106/17
Fisher Scientific HPLC grade methanol	M/4056/17
Fisher Scientific HPLC grade acetonitrile	A/0626/17
Thermo Scientific Premium 8 mm thread, 2 mL clear screw vial, seal, cap (convenience pack)	60180-600
Vitamin K1 and vitamin K2 standards were obtained from a commercial supplier	

Separation Preparation
Working standards were prepared to 200 µg/mL in acetonitrile

Separation Conditions		Part Number
Instrumentation:	Thermo Scientific Accela UHPLC system	
Column:	Accucore C30 2.6 µm, 100 x 2.1 mm	27826-102130
Mobile phase:	4% water: 96% methanol isocratic method	
Column temperature:	15 °C	
Injection details:	2 µL partial loop	
Injection wash solvent:	20:80 (v/v) water / acetonitrile	
UV detector wavelength:	250 nm	
Backpressure:	220 Bar at 350 µL/min	

Results

Vitamin K2 elutes first followed at twice the retention, by the cis and trans isomers of vitamin K1. The larger of this pair corresponds to the trans isomer.

The resolution between this critical pair can be controlled by adjusting the column temperature. At temperatures above 20 °C the two isomers become unresolved.

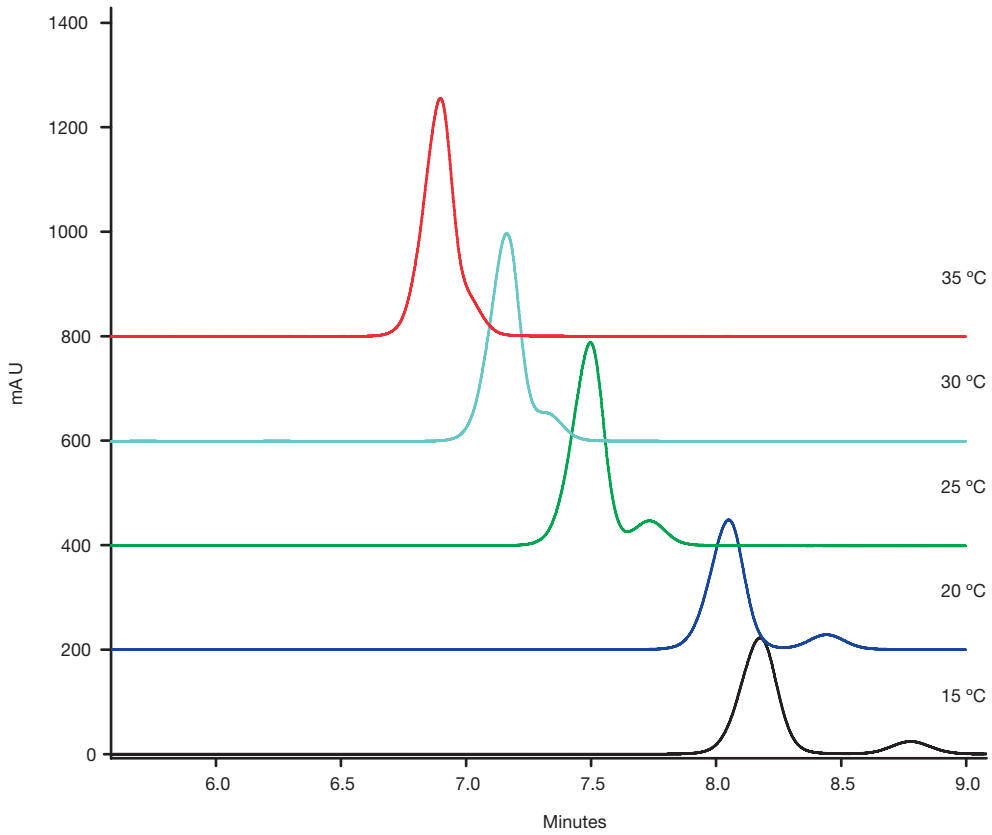

Figure 1: Chromatograms showing the effect of column temperature on the retention time and resolution of the critical pair of vitamin K1 isomers. Lower trace 15 °C, incrementing upwards at 5 °C.

The Accucore HPLC column provides good efficiency over a wider range of flow rates than with fully porous material. This was demonstrated by running the method at six different flow rates and examining the effect on the retention and resolution of the peaks from the vitamin K1 pair of isomers

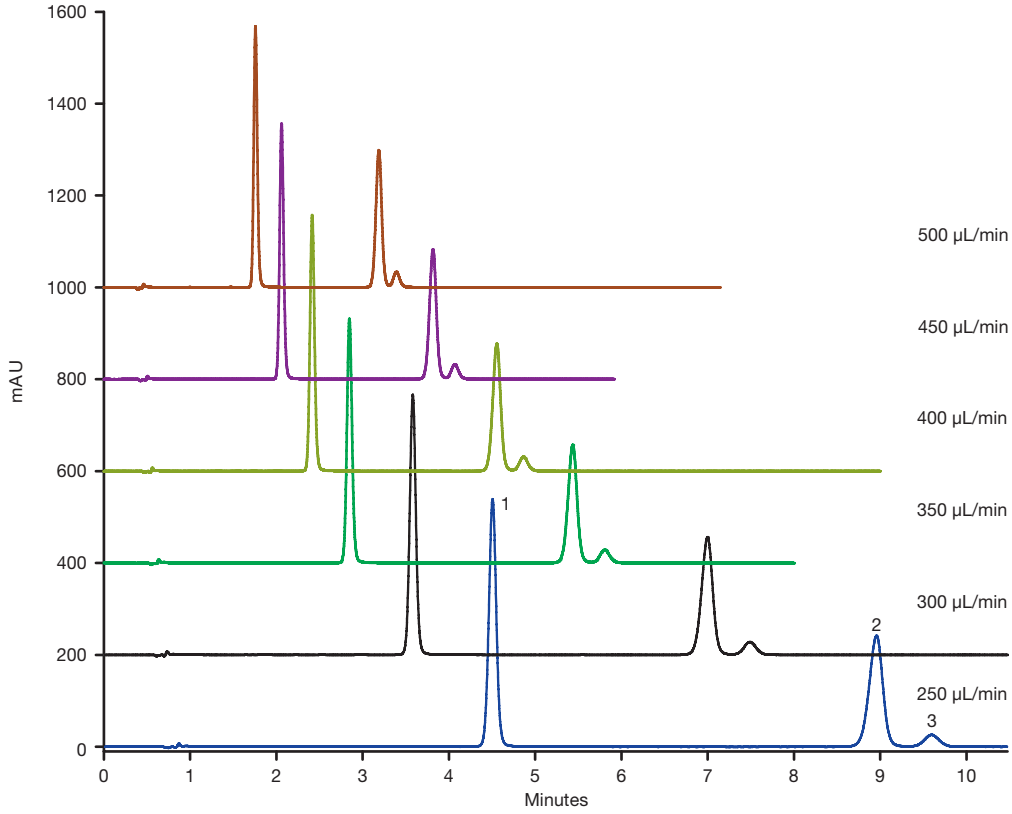

Figure 2: Chromatograms showing the effect of flow rate on the retention time and resolution of the vitamin K1 and K2 mixed standard. Lower trace 250 µL/min, incrementing upwards at 50 µL/min intervals.
1. vitamin K2 2. vitamin K1 (trans isomer) 3. vitamin K1 (cis isomer)

Flow rate (µL/min)	Resolution K1 isomers	Column backpressure (bar)
250	2.012	163
300	1.895	193
350	1.782	221
400	1.690	248
450	1.588	273
500	1.488	295

Table 1: resolution and backpressure achieved at different flow rates

Baseline resolution is achieved even at the elevated flow rates, and with backpressures compatible with conventional HPLC equipment. The analysis can be completed in under 4 minutes when using the 500 µL/min method.

A flow rate of 350 µL/min with column temperature of 15 °C was selected and replicate injections made to demonstrate the reproducibility of the method.

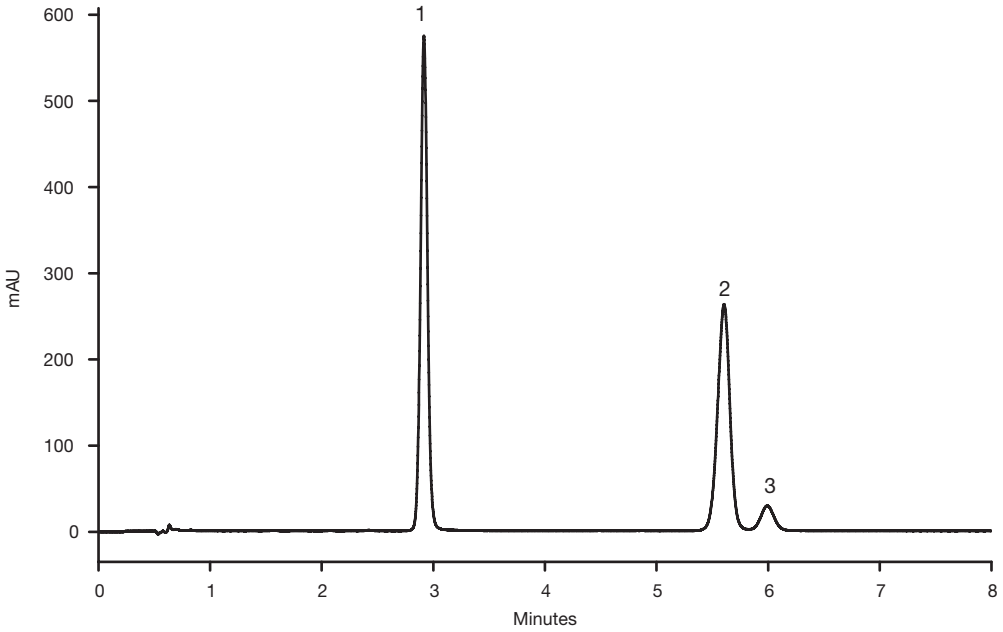

Figure 3: Chromatogram showing the separation of vitamin K compounds using the final method conditions
1. vitamin K2 2. vitamin K1 (trans isomer) 3. vitamin K1 (cis isomer)

	Vitamin K2 retention time (min)	Vitamin K1 (trans) retention time (min)	Vitamin K1 (cis) retention time (min)	Resolution between Vitamin K1 isomers
Mean	2.93	5.63	6.02	1.79
CV	0.1%	0.1%	0.1%	0.2%

Table 2: Results of replicate analysis (n=6) showing retention time and resolution achieved

Conclusion

The use of an Accucore C30 column enables the analysis of vitamin K compounds in less than 6 minutes and at standard HPLC backpressures. Sub-ambient temperature control improves resolution of isomeric forms of vitamin K1. Faster methods are possible by increasing the flow rate whilst maintaining acceptable critical pair resolution.

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