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Rapid Separation of Vitamin K_1 Isomers and Vitamin K_2 in Dietary Supplements Using UltraPerformance Convergence Chromatography with a C_{18} Column

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APPLICATION BENEFITS

- Fast and reliable separation of vitamin K_1 *trans* and *cis* isomers and MK-4 in less than three minutes.
- Separation is achieved on a C_{18} column; no special C_{30} column is needed.
- The use of carbon dioxide as the primary mobile phase minimizes organic solvent waste.

WATERS SOLUTIONS

ACQUITY UPC^2® System with the ACQUITY UPC^2 PDA Detector

ACQUITY UPC^2 HSS C_{18} SB Column

Empower® 3 CDS Software

KEY WORDS

Vitamin K_1 isomers, phylloquinone, menaquinone, menatetrenone, MK-4, UPC^2

INTRODUCTION

Vitamin K_1 (phylloquinone) is an essential human nutrient produced in plants, especially green leafy vegetables. The vitamin K_1 in natural products exists mainly as the *trans* form, while the vitamin K_1 used in food supplementation is often synthetic K_1, which may contain appreciable amounts of the *cis* form. The *trans*-vitamin K_1 is bioactive, while the *cis*-K_1 is not. It is highly desirable to separate the *trans*- and the *cis*-vitamin K_1 isomers to truly evaluate the nutritional value of the supplement ingredient. Available HPLC methods for the separation of vitamin K_1 isomers require C_{30} columns. Their typical run time is about 20 minutes, and chlorinated solvents are used in some of the methods.[1-3]

UltraPerformance Convergence Chromatography™ (UPC^2) is a separation technique that leverages the unique properties (*i.e.*, low viscosity and high diffusivity) of compressed CO_2 at or near its supercritical state, as well as sub-2 micron particle packed columns to significantly improve the separation efficiency, speed, and selectivity.[4] This application note demonstrates a fast separation of vitamin K_1 *trans* and *cis* isomers and menatetrenone (MK-4), a common form of vitamin K_2, by UPC^2 in less than three minutes on an ACQUITY UPC^2 HSS C_{18} SB Column. Figure 1 shows the structures of vitamin K_1 isomers and MK-4. Comparing to current LC-based vitamin K_1 *trans* and *cis* isomers analysis methods, this UPC^2 method is faster, simpler (no need to use a C_{30} column), and it uses less organic solvent.

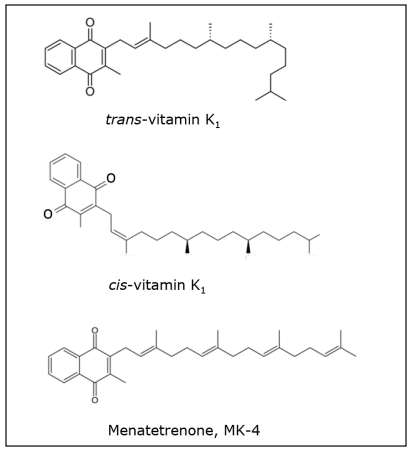

Figure 1. Structures of trans- and cis-vitamin K_1 and menatetrenone.

EXPERIMENTAL

Sample preparation

Vitamin K_1 (Sigma-Aldrich) and MK-4 (Sigma-Aldrich) were weighed and dissolved in iso-octane (ReagentPlus, Sigma-Aldrich) to obtain a stock solution at 1 mg/mL. Intermediate and working standard solutions were obtained by serial dilution of the stock solution with iso-octane. Vitamin K_1 supplement tablets were purchased from a local store and were ground into a powder and extracted with iso-octane. The supernatant was filtered with a 0.45-µm PTFE syringe filter and diluted before injection.

Conditions

UPC² conditions

System:	ACQUITY UPC² with ACQUITY UPC² PDA Detector
Software:	Empower 3
Detection:	UV at 243 nm (compensation reference 400 to 500 nm, res. 6 nm)
Column:	ACQUITY UPC² HSS C_{18} SB 3.0 x 100 mm, 1.8 µm
Column temp.:	50 °C
Sample temp.:	10 °C
Injection volume:	20 µL (Full loop)
Flow rate:	3.00 mL/min
Mobile phase A:	Compressed CO_2
Mobile phase B:	Acetonitrile/methanol mixture (50/50 v/v)
Run time:	4 min
ABPR pressure:	1500 psi
Gradient:	0.5% B for 2 min, ramp to 20% B in 1.5 min, hold at 20% B for 0.5 min

RESULTS AND DISCUSSION

Vitamin K_1 *cis* and *trans* isomers and MK-4 were baseline separated in less than three minutes by UPC² using a single UPC² HSS C_{18} SB Column (3.0 x 100 mm, 1.8 µm). The *cis* form eluted first, followed by the *trans* form, then the MK-4, as shown in Figure 2. The USP resolution between the critical pair, the *cis*- and the *trans*-K_1, was 1.7 (Table 1). In the gradient program, the initial two-minute isocratic elution at 0.5% B was necessary for the baseline separation of the *cis*- and the *trans*-vitamin K_1. Precise control of the mobile phase B delivery volume at 0.5% is critical for the critical pair separation. The ACQUITY UPC² System is the only SFC system on the market that can provide this level of precision control. Following the isocratic hold, a generic gradient from 0.5% to 20% B was used in the study. This gradient range could be modified in applications depending on the retention of the actual vitamin K_2 homologues of interest. MK-4 was included in this study because it is a common form of vitamin K_2, and it is structurally the closest vitamin K_2 to K_1. Other forms of vitamin K_2, such as MK-7, have longer side chains, and tend to be retained longer at column. They can therefore be easily separated from vitamin K_1. The total run time was four minutes, which was at least five times faster than the typical run time for HPLC methods using C_{30} columns. The organic solvent consumption was less than 1 mL per injection, which is only a fraction of the typical 15 to 30 mL of solvent used in LC methods.

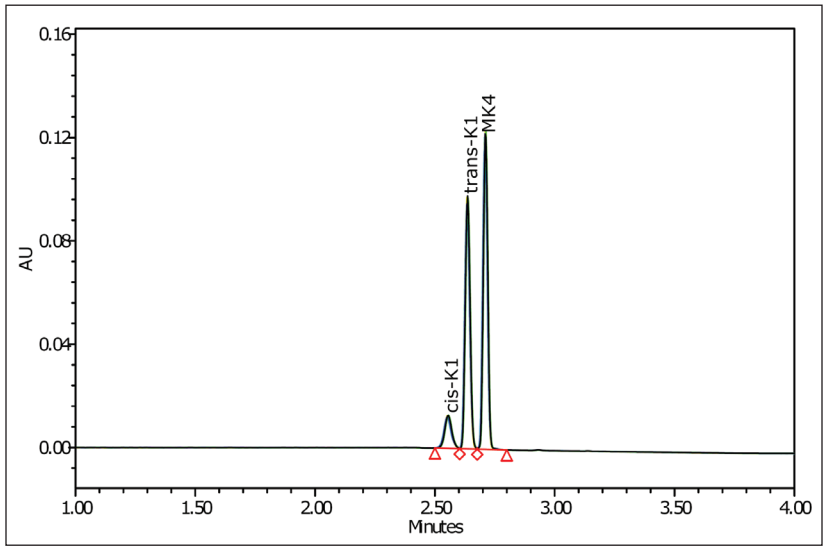

Figure 2. Chromatogram overlay of vitamin K_1 isomers and MK-4 standard mixture (n=10).

	RT (min)	RT RSD	Peak area RSD	Resolution	Resolution RSD
cis-vitamin K1	2.553	0.08%	0.6%	–	–
trans-vitamin K1	2.636	0.05%	0.2%	1.7	1.1%
MK-4	2.710	0.05%	0.2%	2.0	0.9%

Table 1. Results of replicate analysis of vitamin K standard mixture (n=10).

Ten replicate analyses of a standard mixture demonstrated excellent repeatability (Table 1). The limits of quantitation (LOQ), estimated at a signal-to-noise ratio at 10, were 0.06, 0.06, and 0.04 µg/mL for the *cis*-vitamin K_1, the *trans*-vitamin K_1 and the MK-4, respectively (Table 2). Excellent linearity (R^2>0.998) was obtained for these compounds (Table 2). Analysis of a commercial vitamin K supplement product also showed excellent repeatability and resolution (Figure3). In this product, the *cis*-K_1 was found to account for 11.2% of the total vitamin K_1 (Table 3).

Parameters	*cis*-vitamin K_1	*trans*-vitamin K_1	MK-4
Range (µg/mL)	0.03 to 1.5	0.02 to 8.5	0.02 to 10
Regression (R^2)	0.9980	0.9997	0.9999
Slopes (mV sec mL/µg)	17.7	16.3	16.0
LOQ (µg/mL)	0.06	0.06	0.04

Table 2. LOQ and linearity.

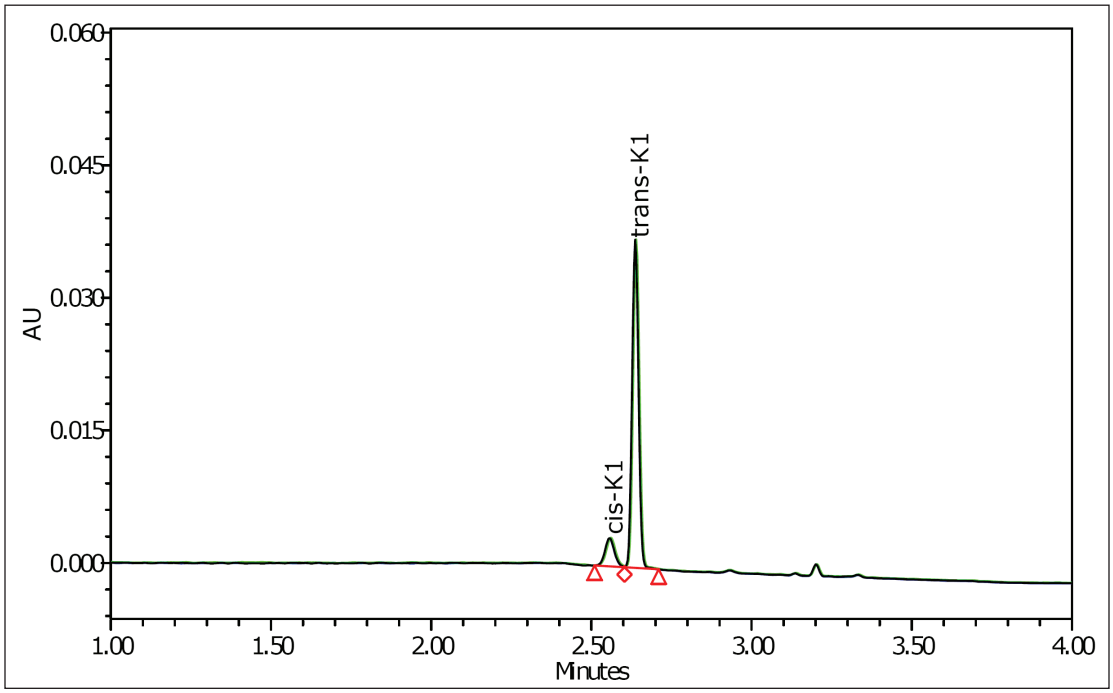

Figure 3. Chromatogram overlay of replicate analysis of vitamin K tablet (n=3).

	RT		Conc.		
	Mean (Min)	RSD (%)	Mean (µg/mL)	RSD (%)	% of total K_1 Conc.
cis-vitamin K_1	2.558	0.09	0.38	2.1	11.2
trans-vitamin K_1	2.638	0.06	3.20	0.3	88.8

Table 3. Results of replicate analysis of vitamin K supplement tablet (n=3).

CONCLUSIONS

UPC^2 Technology enables a rapid separation of the *cis*- and the *trans*-vitamin K_1 isomers and MK-4 on an ACQUITY UPC^2 HSS C_{18} SB Column in less than three minutes. The analysis time is at least five times faster than the current available HPLC methods, and no special C_{30} column is needed. This UPC^2 method has excellent separation selectivity, resolution, sensitivity, repeatability, and it uses much less solvent than HPLC methods. UPC^2 can potentially be used by food ingredient testing labs for routine vitamin K analysis with significant increases in throughput and decreases in operating cost.

References

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