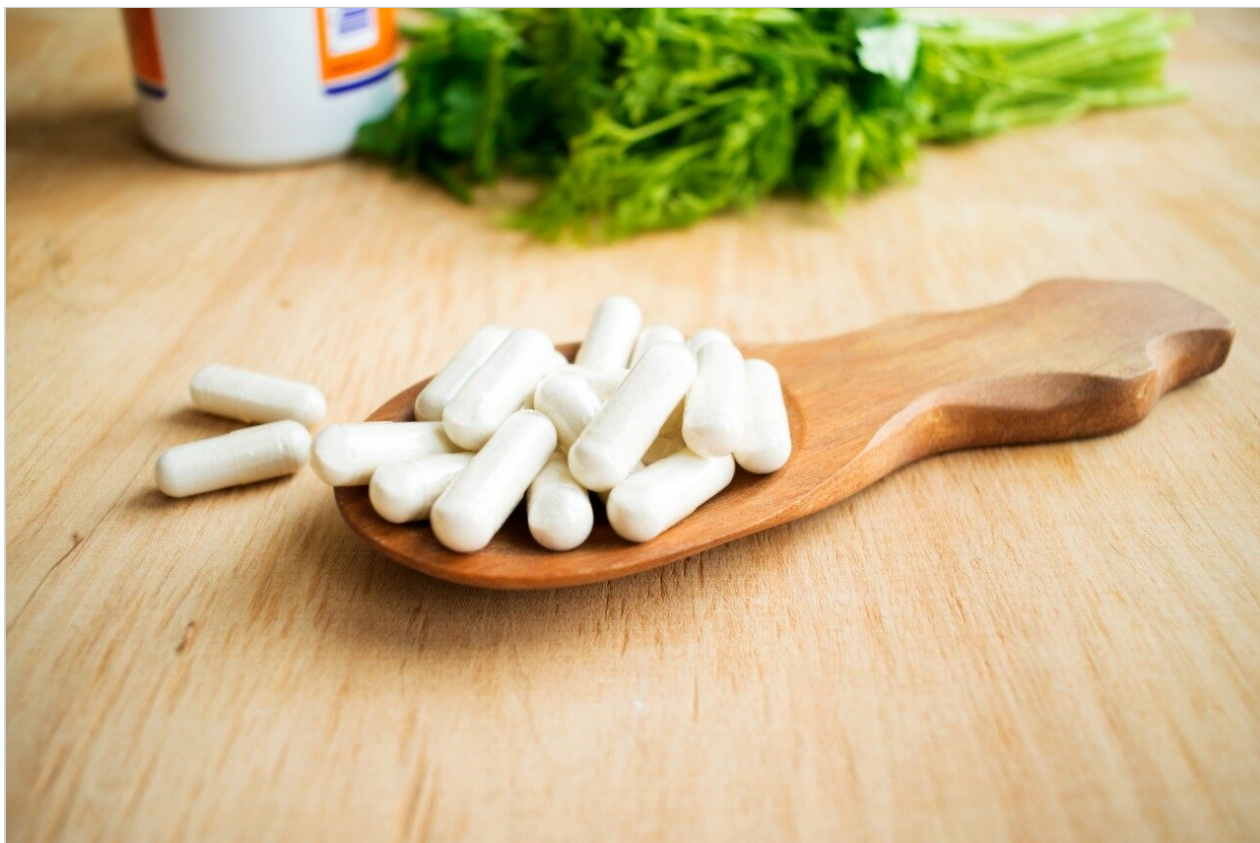


アプリケーションノート

Rapid Separation of Vitamin K₁ Isomers and Vitamin K₂ in Dietary Supplements Using UltraPerformance Convergence Chromatography with a C₁₈ Column

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Abstract

In this application note presents the use of UltraPerformance Convergence Chromatography for a fast separation of vitamin K₁ *trans* and *cis* isomers and menatetrenone (MK-4), a common form of vitamin K₂, on an ACQUITY UPC² HSS C₁₈ SB Column.

Benefits

- Fast and reliable separation of vitamin K₁ *trans* and *cis* isomers and MK-4 in less than three minutes.
- Separation is achieved on a C₁₈ column; no special C₃₀ column is needed.
- The use of carbon dioxide as the primary mobile phase minimizes organic solvent waste.

Introduction

Vitamin K₁ (phylloquinone) is an essential human nutrient produced in plants, especially green leafy vegetables. The vitamin K₁ in natural products exists mainly as the *trans* form, while the vitamin K₁ used in food supplementation is often synthetic K₁, which may contain appreciable amounts of the *cis* form. The *trans*-vitamin K₁ is bioactive, while the *cis*-K₁ is not. It is highly desirable to separate the *trans*- and the *cis*-vitamin K₁ isomers to truly evaluate the nutritional value of the supplement ingredient. Available HPLC methods for the separation of vitamin K₁ isomers require C₃₀ columns. Their typical run time is about 20 minutes, and chlorinated solvents are used in some of the methods.¹⁻³

UltraPerformance Convergence Chromatography (UPC²) is a separation technique that leverages the unique properties (i.e., low viscosity and high diffusivity) of compressed CO₂ 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 K₁ *trans* and *cis* isomers and menatetrenone (MK-4), a common form of vitamin K₂, by UPC² in less than three minutes on an ACQUITY UPC² HSS C₁₈ SB Column. Figure 1 shows the structures of vitamin K₁ isomers and MK-4. Comparing to current LC-based vitamin K₁ *trans* and *cis* isomers analysis methods, this UPC² method is faster, simpler (no need to use a C₃₀ column), and it uses less organic solvent.

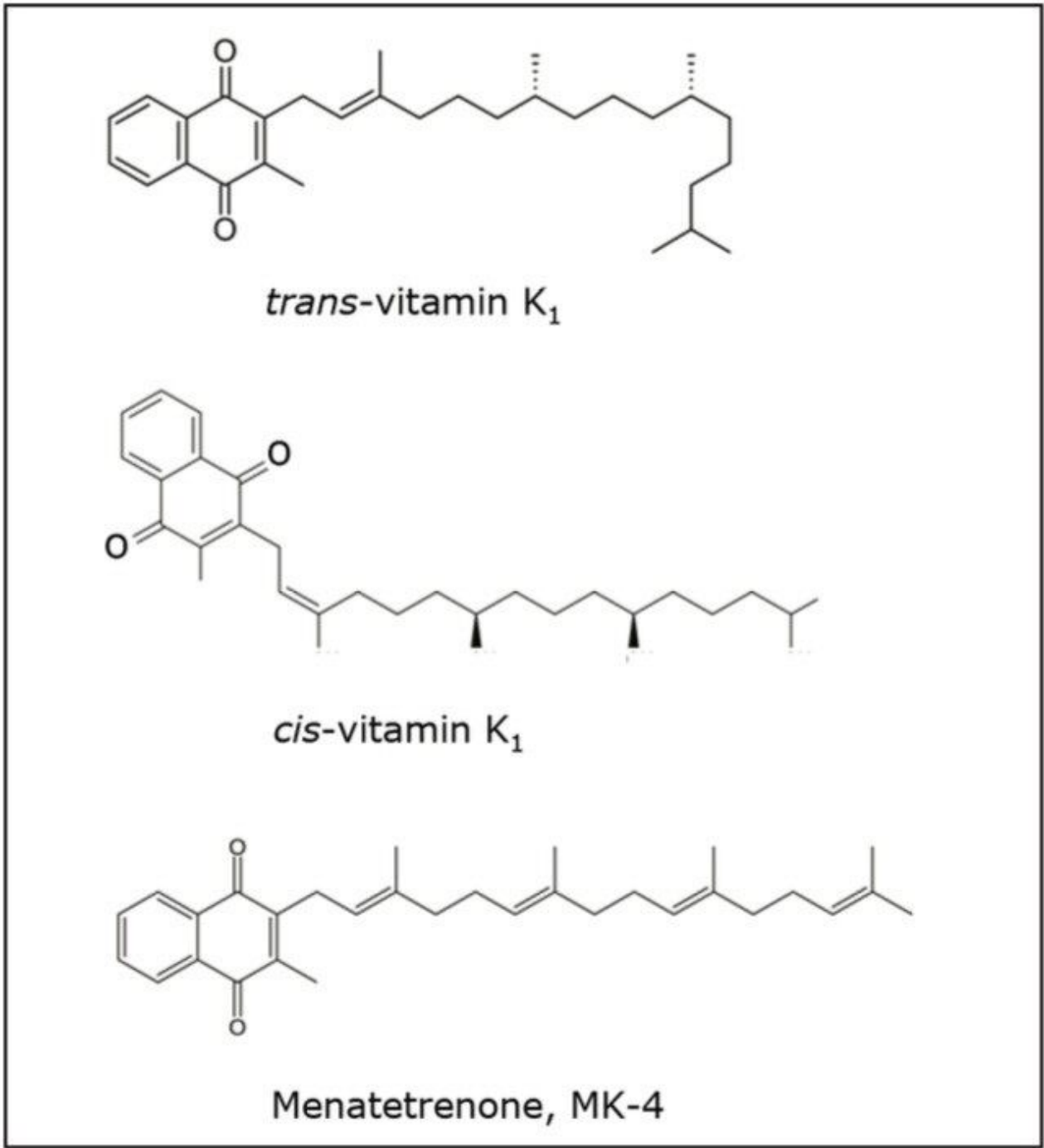


Figure 1. Structures of *trans*- and *cis*-vitamin K₁ and menatetrenone.

Experimental

Sample preparation

Sample preparation Vitamin K₁ (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 K₁ 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- μ m PTFE syringe filter and diluted before injection.

Conditions

UPC₂ conditions

System	ACQUITY UPC ² with ACQUITY UPC ² PDA Detector
Software	Empower 3
Detection	UV at 243 nm (compensation reference 400 to 500 nm, res. 6 nm)
Column	ACQUITY UPC ² HSS C ₁₈ SB 3.0 x 100 mm, 1.8 μ m
Column temp.	50 °C
Sample temp.	10 °C
Injection volume	20 μ L (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 K₁ *cis* and *trans* isomers and MK-4 were baseline separated in less than three minutes by UPC² using a single UPC² HSS C₁₈ 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*-K₁, 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 K₁. 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 K₂ homologues of interest. MK-4 was included in this study because it is a common form of vitamin K₂, and it is structurally the closest vitamin K₂ to K₁. Other forms of vitamin K₂, such as MK-7, have longer side chains, and tend to be retained longer at column. They can therefore be easily separated from vitamin K₁. The total run time was four minutes, which was at least five times faster than the typical run time for HPLC methods using C₃₀ 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.

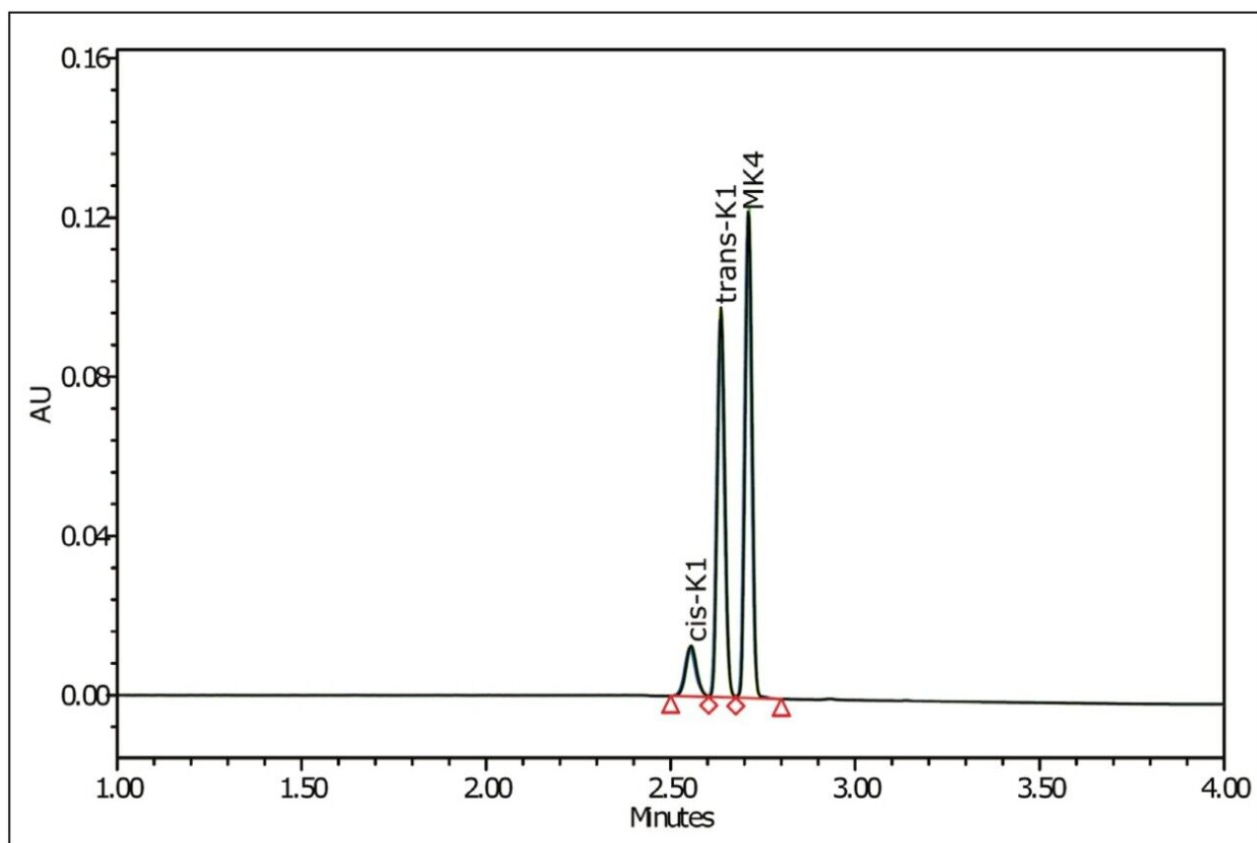


Figure 2. Chromatogram overlay of vitamin K₁ isomers and MK-4 standard mixture (n=10).

	RT	RTRSD	Peak area		Resolution	
	(min)		RSD	Resolution	RSD	
<i>cis</i> -vitamin K ₁	2.553	0.08%	0.6%	–	–	
<i>trans</i> -vitamin K ₁	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 K₁, the *trans*-vitamin K₁ 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 (Figure 3). In this product, the *cis*-K₁ was found to account for 11.2% of the total vitamin K₁ (Table 3).

Parameters	<i>cis</i> -vitamin K ₁	<i>trans</i> -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.

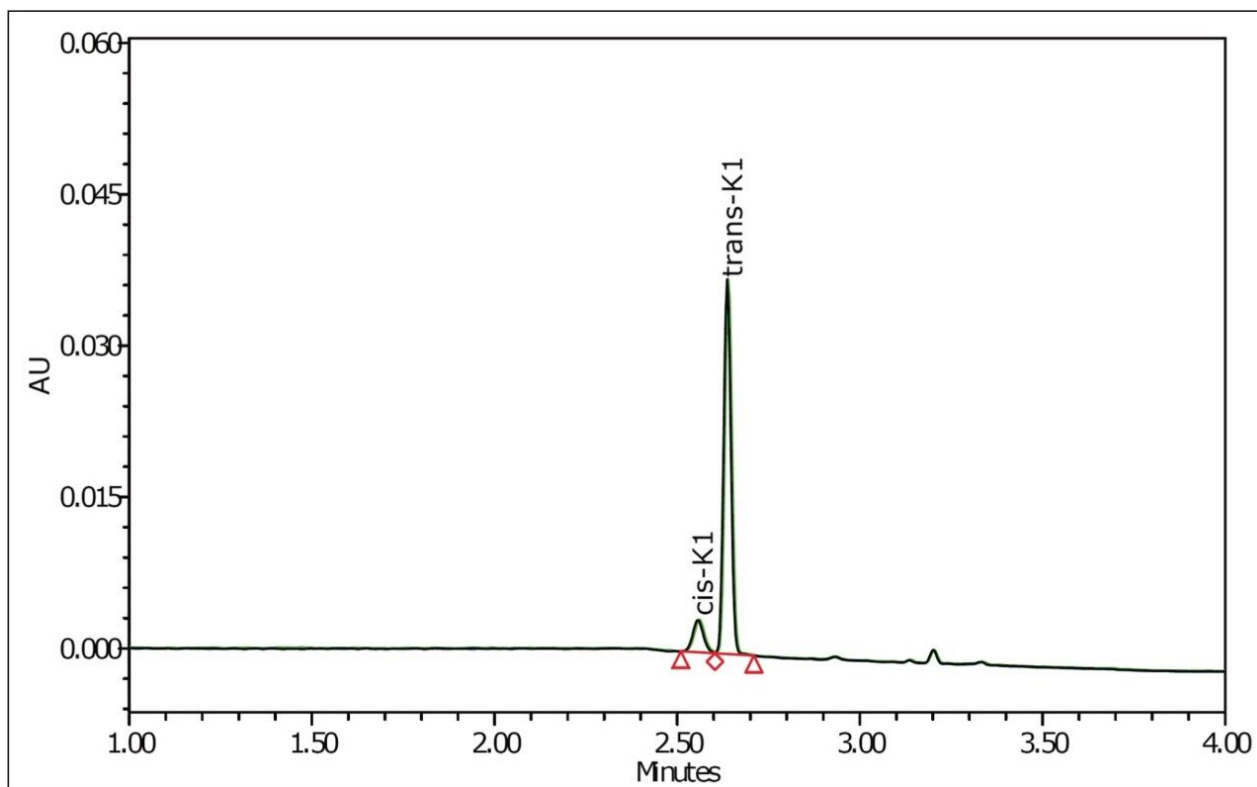


Figure 3. Chromatogram overlay of replicate analysis of vitamin K tablet (n=3).

	RT		Conc.		% of total K ₁ Conc.
	Mean (Min)	RSD (%)	Mean (µg/mL)	RSD (%)	
<i>cis</i> -vitamin K ₁	2.558	0.09	0.38	2.1	11.2
<i>trans</i> -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).

Conclusion

UPC² Technology enables a rapid separation of the *cis*- and the *trans*-vitamin K₁ isomers and MK-4 on an ACQUITY UPC² HSS C₁₈ 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 C₃₀ column is needed. This UPC² method has excellent separation selectivity, resolution, sensitivity, repeatability, and it uses much less solvent than HPLC methods. UPC² 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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4. Aubin A. Analysis of fat-soluble vitamin capsules using UltraPerformance Convergence Chromatography. Waters Application Note No. 720004394en. June, 2012.

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Viridis HSS C₁₈ SB Column, 100Å, 1.8 µm, 3 mm X 100 mm, 1/pkg <<https://www.waters.com/waters/partDetail.htm?partNumber=186006623>>

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