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Application Note

Analysis of Fat-Soluble Vitamin Capsules using UltraPerformance Convergence Chromatography UPC^2

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Abstract

The use of UltraPerformance Convergence Chromatography (UPC^2) in fat-soluble vitamin analysis provides a single viable technique that is cost-effective, sustainable, and a green technology alternative that lowers the use of organic solvents, provides fast analysis times, and maintains chromatographic data quality. Waters ACQUITY UPC^2 System was able to successfully analyze six different formulations of fat-soluble vitamins.

Benefits

- Fast analysis of a wide range of fat-soluble vitamin formulations.
- Waters ACQUITY UPC^2 System was able to successfully analyze six different formulations of fat-soluble vitamins. A single technique was able to quickly analyze these different formulations.
- This system can greatly streamline fat-soluble vitamin analysis by allowing labs to use a single technique on a single system to analyze a wide range of FSV formulations.
- Each of the fat-soluble vitamin formulations were analyzed rapidly, and components of interest resolved well from excipient materials.
- Isomers of vitamins A, E, and K1 were successfully resolved from each other.

Introduction

The analysis of fat-soluble vitamins (FSV) formulations, often from oil-filled and powder-filled capsules, or pressed tablets, can be a challenging task. Most often, analysis of these formulations employs a normal phase chromatographic method using traditional normal phase solvents (hexane, tertiary butyl alcohol, ethyl acetate, dichloromethane, and others) that can be expensive to procure and dispose. Other analytical chromatographic techniques for these analyses include reversed phase liquid chromatography, gas chromatography, thin layer chromatography, and colorimetric techniques. The use of UltraPerformance Convergence Chromatography (UPC^2) in fat-soluble vitamin analysis provides a single viable technique that is cost-effective, sustainable, and a green technology alternative that lowers the use of organic solvents, provides fast analysis times, and maintains chromatographic data quality. A series of FSV formulations were analyzed using the ACQUITY UPC^2 System. The examined formulations contained vitamin A only, vitamins A + D3, vitamin E, vitamin D3 only, vitamin K1 only, and vitamin K2 only, as shown in Table 1. Results from these experiments show that UPC^2 has the potential to replace many of the separation methods in use today as the sole technique with no compromises.

Active ingredient(s)	Amount per capsule/tablet	Inactive ingredients
Vitamin A	10,000 IU A	Soy oil, gelatin, glycerin, water
Vitamin A & D3	10,000 IU A 2000 IU D3	Soy oil, gelatin, glycerin, water
Vitamin D3	2000 IU D3	Sunflower oil, gelatin, glycerin, water
Vitamin E	400 IU E	Soy oil, gelatin, glycerin, water, FD&C yellow #6 lake, FD&C blue #1 lake, titanium dioxide
Vitamin K1	100 µg	Cellulose, $CaHPO_4$, stearic acid, Mg stearate, croscarmellose sodium
Vitamin K2	50 µg	Cellulose, Mg stearate, silica

Table 1. Fat-soluble vitamin formulations.

Experimental

System:	ACQUITY UPC^2 consisting of ACQUITY UPC^2 Binary Solvent Manager, Sample Manager, Convergence Manager, Column Manager, and PDA Detector
Columns:	ACQUITY UPC^2 BEH, 3.0 x 100 mm, 1.7 µm ACQUITY UPC^2 HSS C18 SB, 3.0 x 100 mm, 1.8 µm ACQUITY UPC^2 HSS C18 SB, 2.1 x 150 mm, 1.8 µm
Data system:	Empower 3 Software
Separation methods:	Details of each separation method are included in the individual results and discussion sections of this application note

Sample preparation

Oil-filled capsules (vitamins A, A + D3, D3) – contents of four individual capsules were removed and dissolved in 10 mL of iso-octane. No further pre-treatment was used. The contents of one individual vitamin E capsule was removed and dissolved in 10 mL of iso-octane. No further pre-treatment was used.

Eight crushed tablets of vitamin K1 were sonicated with iso-octane for 30 minutes. Following settling, an aliquot of the extract was filtered directly into a sample vial through a 1.0-µm glass fiber filter.

Contents of eight powder-filled vitamin K2 capsules were removed and sonicated for 30 minutes with iso-octane. Following settling, an aliquot of the extract was filtered directly into a sample vial

through a 1.0-µm glass fiber filter.

Results and Discussion

Vitamin A

This formulation of vitamin A was labeled as derived from fish liver oil and contained soy oil, gelatin, glycerin, and water as inactive ingredients. Two primary forms of vitamin A palmitate (*cis* and *trans* isomers, 1.325 and 1.394 minutes, respectively) were noted and resolved well from the small excipient peaks, as shown in Figure 1, which elute in the range of 2.0 to 2.5 minutes. This separation was accomplished using a gradient of carbon dioxide and methanol (containing 0.2% formic acid) 97:3 to 90:10 over 3 minutes with an Active Back Pressure Regulator (ABPR) setting of 2176 psi. Further details are contained in Table 2. Using this separation method, vitamin A acetate, palmitate, and retinol were easily resolved, as seen in Figure 2.

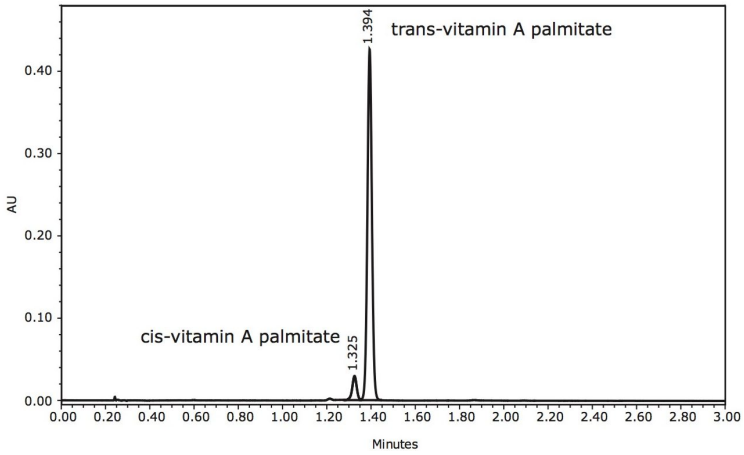

Figure 1. UPC^2 separation of the components of a vitamin A capsule.

Column:	ACQUITY UPC2 HSS C_{18} SB, 3.0 x 100 mm, 1.8 µm
Flow rate:	2.0 mL/min
Gradient:	97:3 to 90:10 over 3 minutes
Mobile phase A/B:	CO_2 and methanol containing 0.2% formic acid
Detection:	UV at 320 nm, compensated (500 to 600 nm)
Injection volume:	1 µL
ABPR pressure:	2176 psi
Column temp.:	50 °C

Table 2. Separation method details of vitamin A.

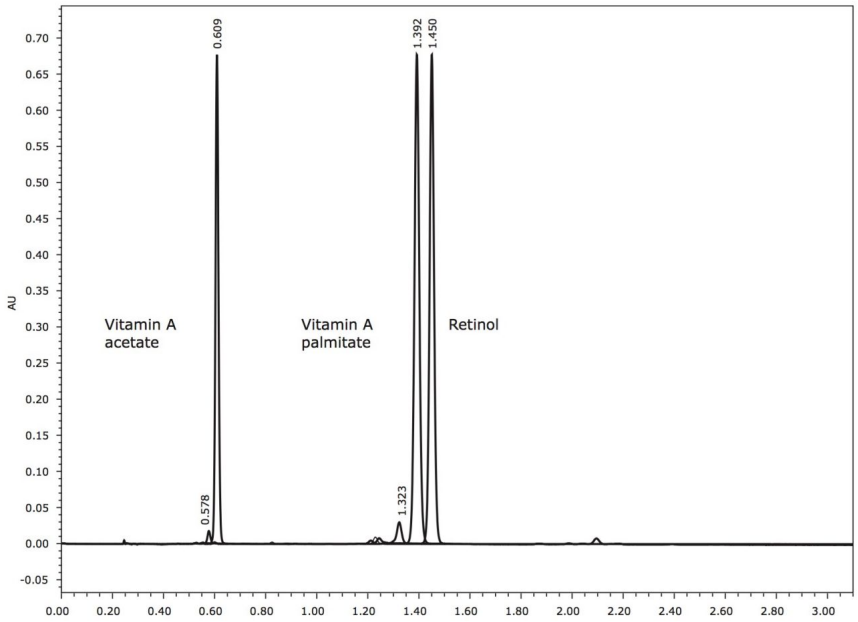

Figure 2. Separation of vitamin A acetate, vitamin A palmitate, and retinol.

Vitamin A + D3

Similar to the previous example, this formulation of vitamins A + D was also labeled as derived from fish liver oil and contained soy oil, gelatin, glycerin, and water as inactive ingredients. Again, two forms of vitamin A palmitate (***cis*** and ***trans*** isomers, 2.626 and 2.851 minutes, respectively) were noted before the bulk of excipient peaks. To fully resolve vitamin D3 (cholecalciferol, 6.862 minutes) from the major excipient materials and a number of other compounds contained in the formulation, shown in Figure 3, it was necessary to use a longer column that provided enough separation efficiency to accomplish this goal. The system provided enough sensitivity to easily detect the vitamin D3 peak, as shown in Figure 3 inset.

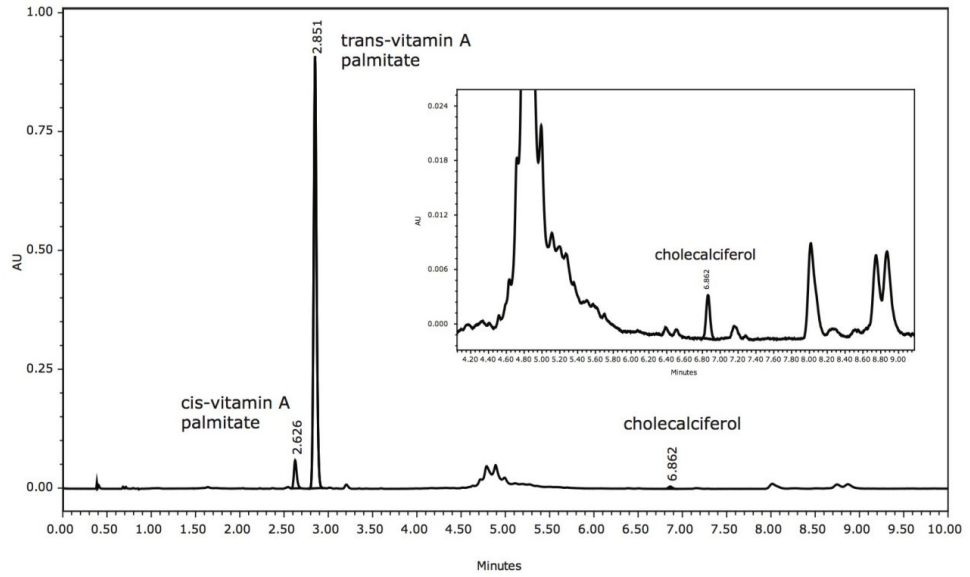

Figure 3. UPC^2 separation of the components of a vitamin A + D3 capsule.

This separation was accomplished using a gradient of carbon dioxide and methanol (containing 0.2% formic acid), 99:1 to 90:10 over 10 minutes. Further details are outlined in Table 3.

Column:	ACQUITY UPC2 HSS C_{18} SB, 2.1 x 150 mm, 1.8 µm
Flow rate:	1.0 mL/min
Gradient:	99:1 to 90:10 over 10 minutes
Mobile phase A/B:	CO_2 and methanol containing 0.2% formic acid
Detection:	UV at 263 nm, compensated (500 to 600 nm)
Injection volume:	1 µL
ABPR pressure:	2176 psi
Column temp.:	50 °C

Table 3. Separation method details of vitamin A + D3 and D3 only.

Vitamin D3

Using identical separation conditions as those used for vitamins A + D3, as shown in Table 3, vitamin D3 (cholecalciferol, 6.867 minutes) was easily resolved from the capsule excipient material, which was labeled as primarily sunflower oil, shown in Figure 4 and Table 3.

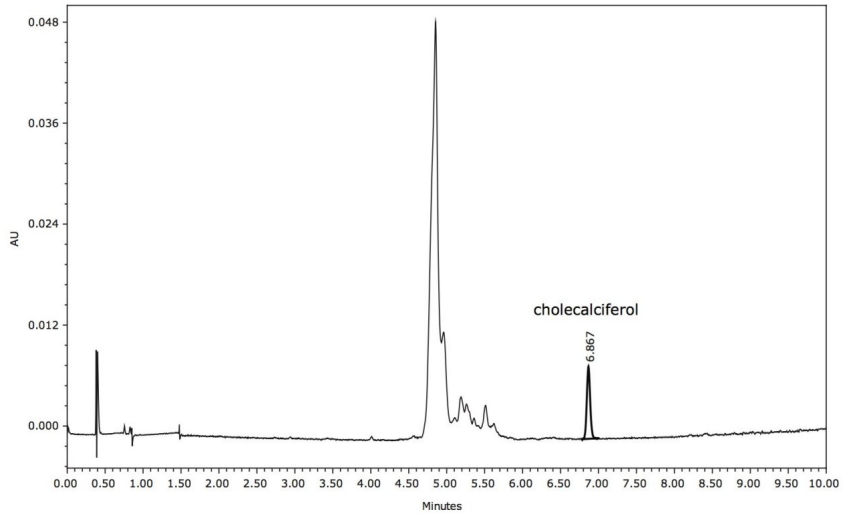

Figure 4. UPC^2 separation of the components of a vitamin D3 capsule..

Vitamin E

A very rapid gradient analysis (~ 90 second run time) that easily provided baseline resolution of the four tocopherol isomers (d-alpha, d-beta, d-gamma, d-delta) was developed for the vitamin E capsule, shown in Figure 5. This separation was accomplished using a gradient of carbon dioxide and methanol, 98:2 to 95:5 over 1.5 minutes. Further details are shown in Table 4.

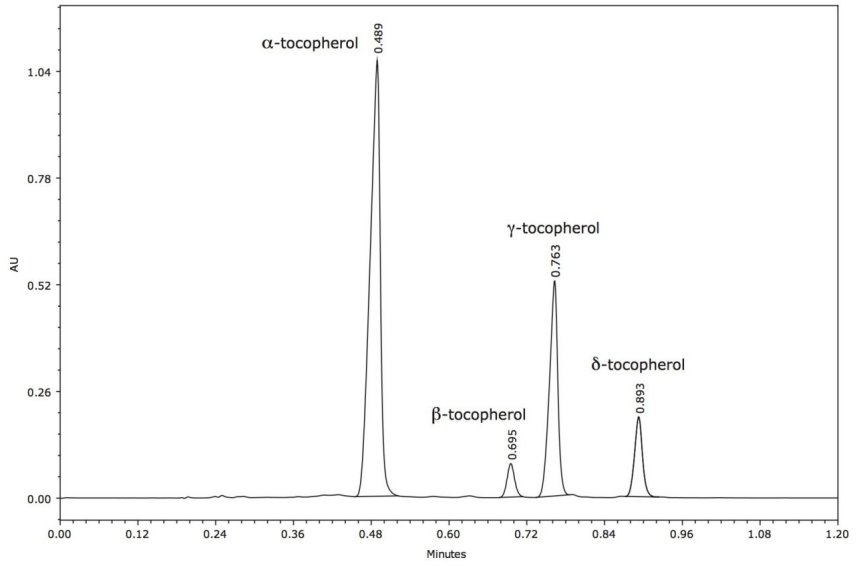

Figure 5. UPC^2 separation of the components of a vitamin E capsule.

Column:	ACQUITY UPC^2 BEH, 3.0 x 100 mm, 1.7 µm
Flow rate:	2.5 mL/min
Detection:	UV at 293 nm, compensated (500 to 600 nm)
Gradient:	98:2 to 95:5 over 1.5 minutes
Mobile phase A/B:	CO_2 and methanol
Injection volume:	1 µL
ABPR pressure:	1885 psi
Column temp.:	50 °C

Table 4. Separation method details of vitamin E.

Vitamin K1

The vitamin K1 tablets generated two fully resolved (Rs > 2.0), distinct peaks with a simple isocratic method consisting of 99% CO_2 and 1% methanol/acetonitrile 1:1, shown in Figure 6. UV spectra (collected simultaneously as the UV at 246 nm channel) of both peaks were similar, indicating that the peaks were related, as displayed in Figure 7. Although not confirmed (individual standards of each of the isomers were not available at time of analysis), it is likely that the two peaks are stereoisomers of phylloquinone (vitamin K1). Further details are shown in Table 5.

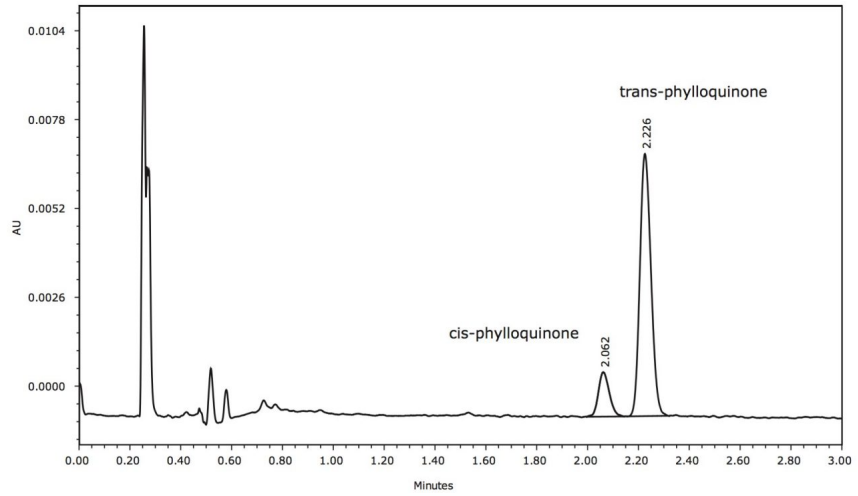

Figure 6. UPC[2] separation of the components of a vitamin K1 tablet.

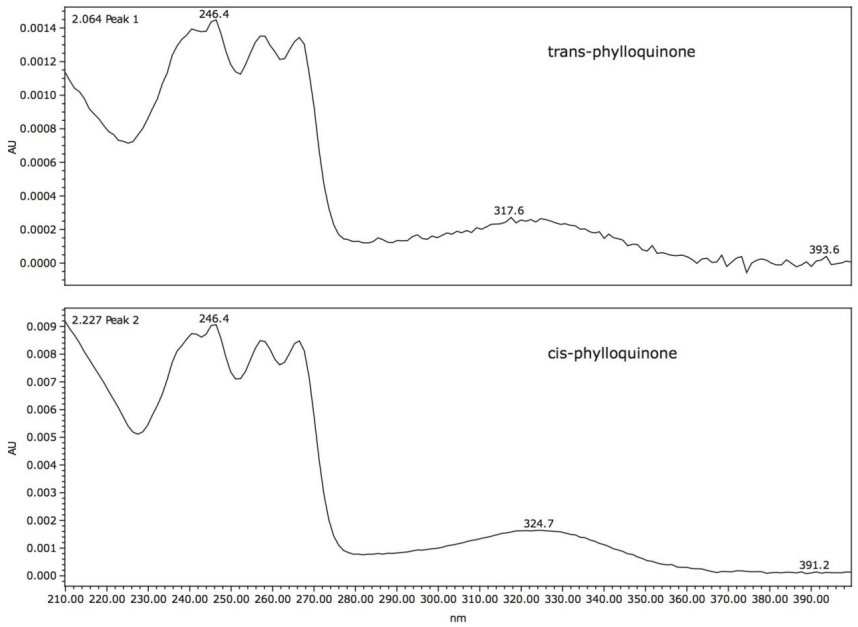

Figure 7. UV spectra of vitamin K peaks observed at 2.064 and 2.227 minutes.

Column:	ACQUITY UPC^2 HSS C_{18} SB, 2.1 x 150 mm, 1.8 µm
Flow rate:	1.5 mL/min
Isocratic:	99% A and 1% B
Mobile phase A/B:	CO_2 and methanol/acetonitrile 1:1
Detection:	UV at 248 nm, compensated (300 to 400 nm)
Injection volume:	2 µL
ABPR pressure:	1885 psi
Column temp.:	50 °C

Table 5. Separation method details of vitamin K1.

Vitamin K2

Vitamin K2 consists of menaquinone (MK) forms MK-3 through MK-14. The various forms of vitamin K2 have side chain lengths comprised of a variable number of unsaturated isoprenoid units. This tablet formulation showed one predominant peak and several smaller ones, as seen in Figure 8, using an isocratic separation of 95:5 CO_2 /methanol, and was identified as MK-7 (data not shown). This result is consistent with the capsule label claim, which indicated that this formulation should have contained predominantly MK-7. Further method details are shown in Table 6.

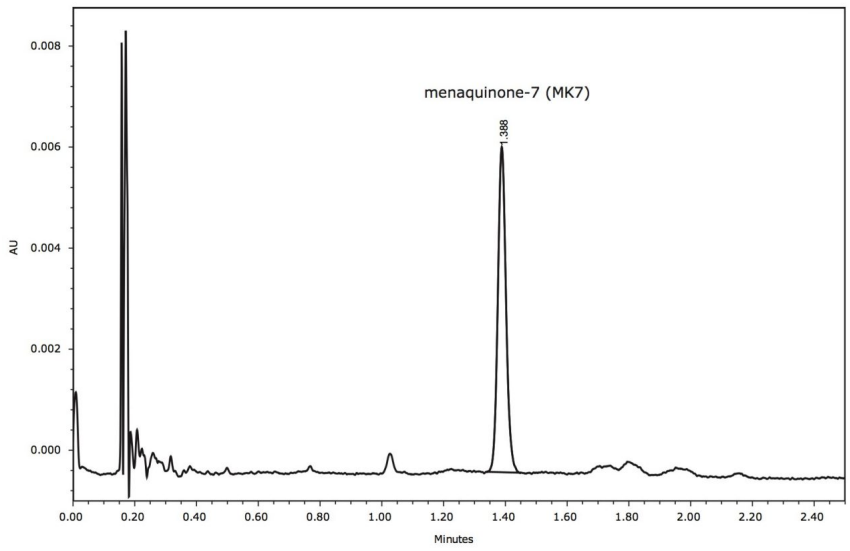

Figure 8. UPC^2 separation of the components of a vitamin K2 capsule.

Column:	ACQUITY UPC^2 HSS C_{18} SB, 3.0 x 100 mm, 1.8 µm
Flow rate:	3.0 mL/min
Isocratic:	95% A and 5% B
Mobile phase A/B:	CO_2 and methanol
Detection:	UV at 248 nm, compensated (500 to 600 nm)
Injection volume:	1 µL
ABPR pressure:	1885 psi
Column temp.:	50 °C

Table 6. Separation method details of vitamin K2.

Conclusion

- Waters ACQUITY UPC^2 System was able to successfully analyze six different formulations of fat-soluble vitamins.
- Each of the FSV formulations were analyzed rapidly with components of interest resolved from excipient materials.
- Isomers of vitamins A, E, and K1 were successfully resolved from each other.
- This system can greatly streamline FSV analysis by enabling laboratories to use a single technique on a single system to analyze a wide range of FSV formulations.

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