Analysis of Fat-Soluble Vitamins Using UltraPerformance Convergence Chromatography (UPC^2)

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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 analysed using the ACQUITY UPC^2™ System. The examined formulations contained vitamin A only, vitamins A + D_3, vitamin E, vitamin D_3 only, vitamin K_1 only, and vitamin K_2 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.

Table 1. Fat-soluble vitamin formulations.

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

Results And Discussion

Vitamin A

This formulation of vitamin A was labelled 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*.

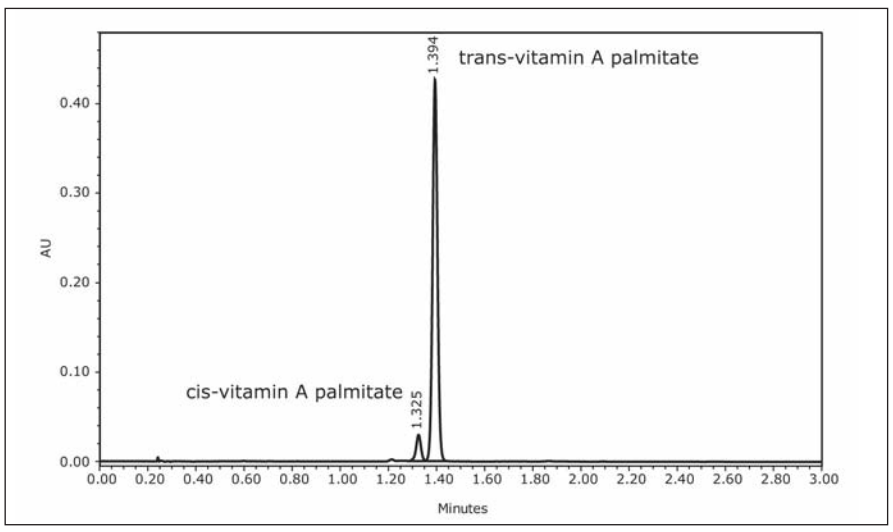

Figure 1. UPC^2 separation of the components of a vitamin A capsule.

Table 2. Separation method details of vitamin A.

Column	ACQUITY UPC^2 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

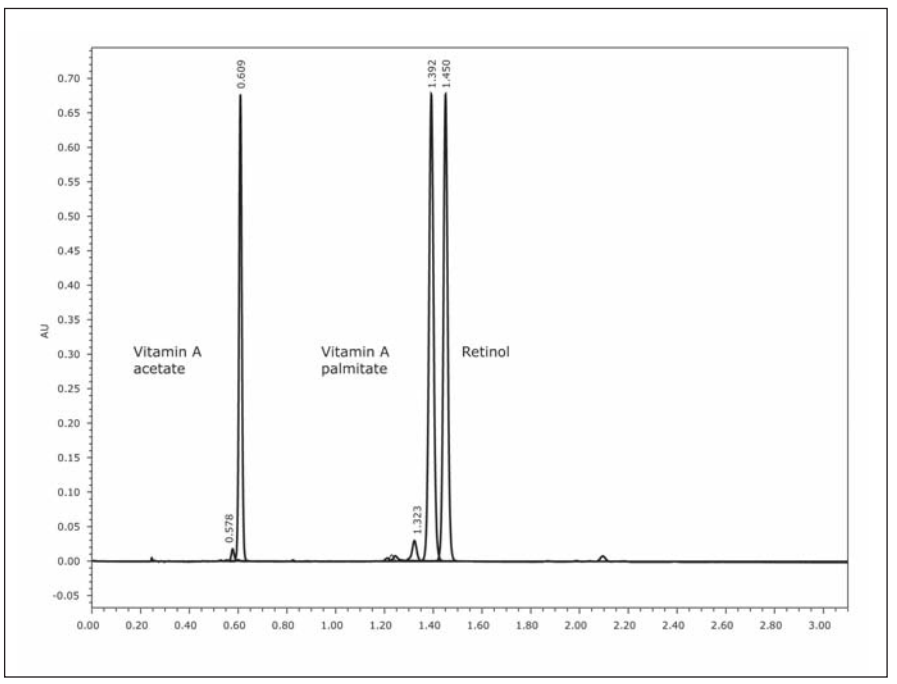

Figure 2. Separation of vitamin A acetate, vitamin A palmitate, and retinol.

Vitamin A + D_3

Similar to the previous example, this formulation of vitamins A + D was also labelled 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 D_3 (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 D_3 peak, as shown in *Figure 3* inset.

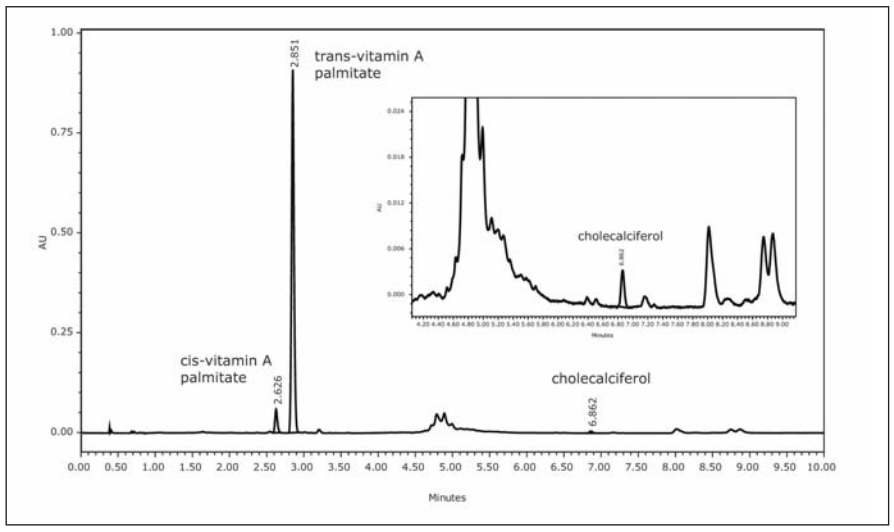

Figure 3. UPC² separation of the compnents of a vitamin A + D_3 capsule.

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*.

Table 3. Separation method details of vitamin A + D_3 and D_3 only.

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

Vitamin D_3

Using identical separation conditions as those used for vitamins A + D_3, as shown in *Table 3*, vitamin D_3 (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*.

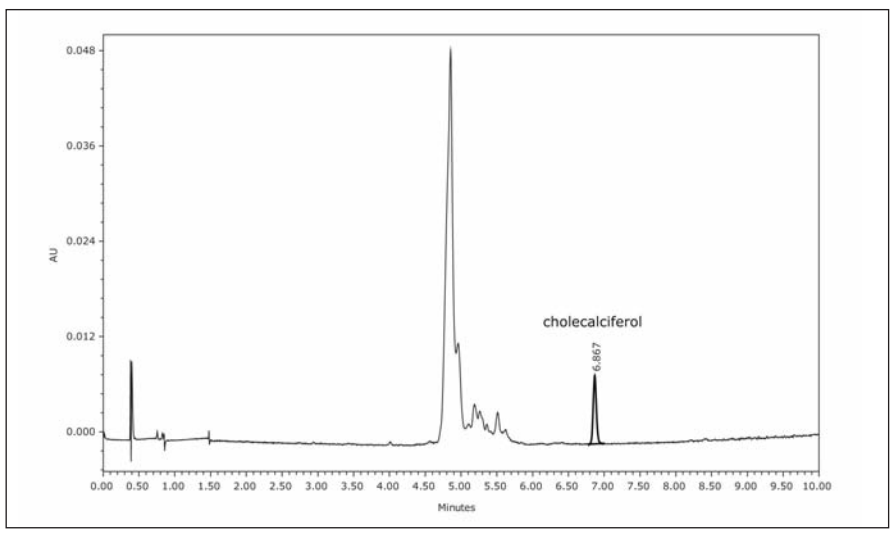

Figure 4. UPC² separation of the components of a vitamin D_3 capsule.

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*.

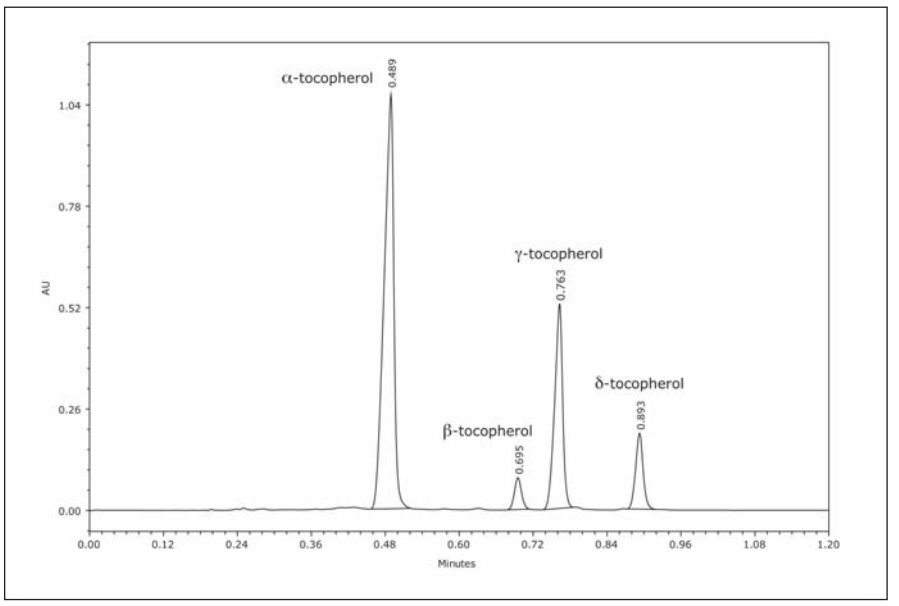

Figure 5. UPC² separation of the components of a vitamin E capsule.

Table 4. Separation method details of vitamin E.

Column	ACQUITY UPC² 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

Vitamin K_1

The vitamin K_1 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 K_1). Further details are shown in *Table 5*.

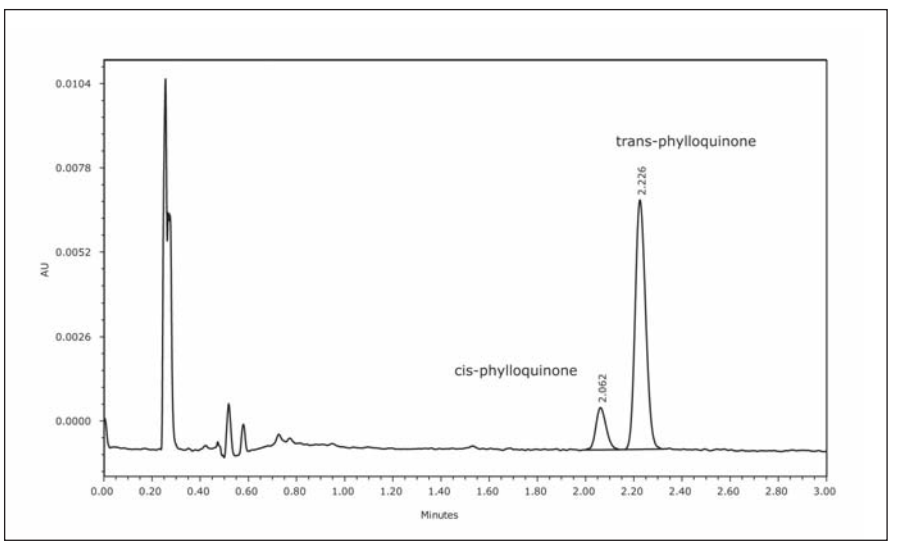

Figure 6. UPC² separation of the components of a vitamin K_1 tablet.

Table 5. Separation method details of vitamin K_1.

Column	ACQUITY UPC² 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

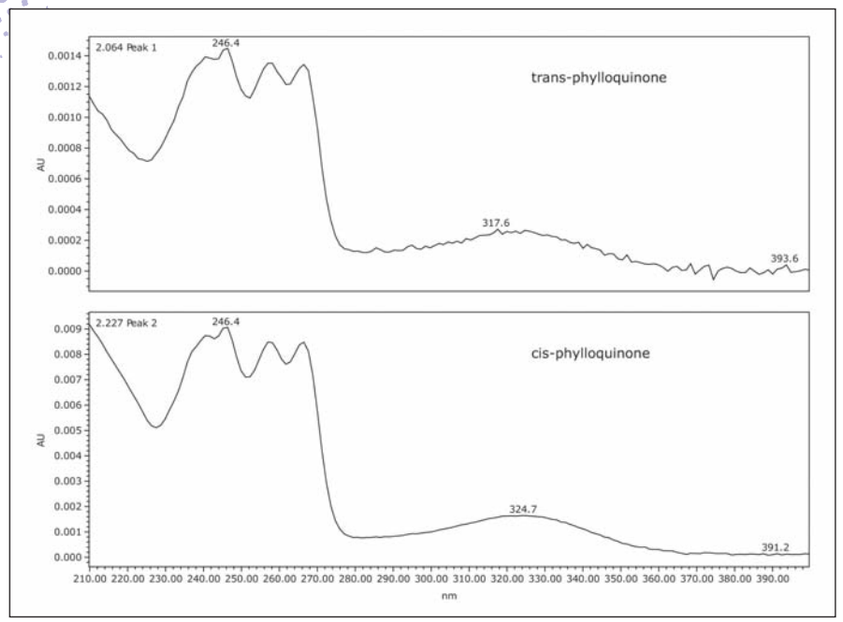

Figure 7. UV Spectra of vitamin K peaks observed at 2.064 and 2.227 minutes.

Vitamin K_2

Vitamin K_2 consists of menaquinone (MK) forms MK-3 through MK-14. The various forms of vitamin K_2 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*.

Table 6. Separation method details of vitamin K_2.

Column	ACQUITY UPC² 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

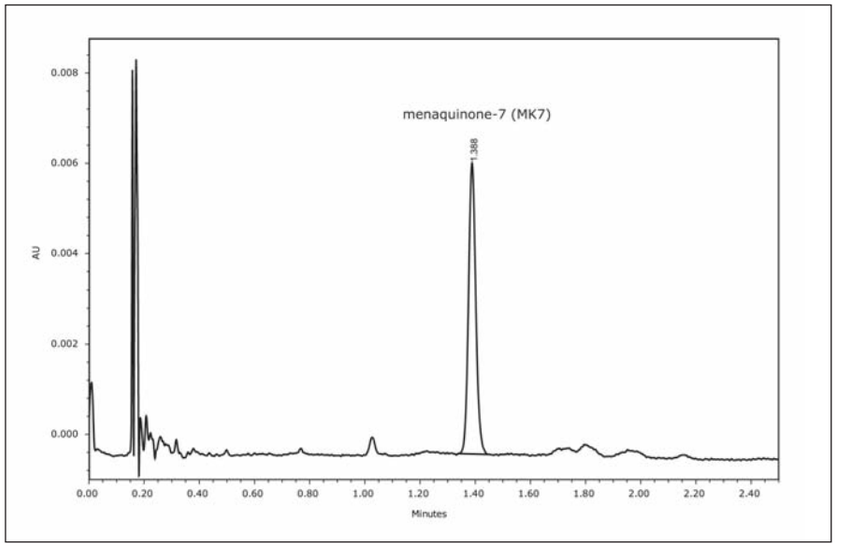

Figure 8. UPC² separation of the components of a vitamin K_2 capsule.

Conclusions

- Waters' ACQUITY UPC² System was able to successfully analyse six different formulations of fat-soluble vitamins.
- Each of the FSV formulations were analysed rapidly with components of interest resolved from excipient materials.
- Isomers of vitamins A, E, and K_1 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 analyse a wide range of FSV formulations.

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