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應用手冊

Analysis of Fat-Soluble Vitamins Using UPLC with PDA and the SQ Detector

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Abstract

This application note describes a 5-minute reversed phase analysis of fat-soluble vitamins is described for the

simultaneous detection of vitamins, A, D, E, and K using the Waters ACQUITY UPLC System with the PDA and SQ Detector. The ACQUITY UPLC System is an advanced separation system that utilizes a 1.7 µm stationary phase particle size to improve resolution and peak shape in a shorter runtime. The SQ Detector is compatible with both Waters Empower and MassLynx MS Software. The MS setup parameters are made easy with the functionality of IntelliStart Technology, which has been incorporated into both software packages.

Introduction

Vitamins are essential nutrients that perform various roles to maintain good health. Eating a normal, well-balanced diet should provide sufficient levels of vitamins; however, modern-day life-styles can lead to deficiencies. As a result, the practice of enriching foods with vitamins in order to provide the recommended daily allowance (RDA) has become commonplace.

Vitamin A (retinol) plays an important role in bone growth, tooth development, reproduction, cell division, and gene expression.Sources of vitamin A are dairy products, carrots, pumpkin, dark green leafy vegetables, and apricots.

Vitamin D helps increase the amounts of calcium absorbed from the small intestine and helps form and maintain bones. Children need adequate amounts of vitamin D to help develop strong bones and healthy teeth. Common sources of vitamin D include a variety enriched dairy products, most notably milk. Vitamin D can also be obtained through sunlight exposure of the skin.

Vitamin E is a fat-soluble anti-oxidant, and protects vitamins A and C, red blood cells and essential fatty acids from destruction. Sources of vitamin E are margarines and vegetable oils.

Vitamin K plays an essential role in normal blood clotting and helps promote bone health. Sources of vitamin K are green vegetables (spinach, broccoli), cauliflower, cabbage, and certain vegetable oils (soybean oil, cottonseed oil, canola oil, and olive oil).

ACQUITY UPLC with PDA and SQ Detector.

Experimental

UPLC Conditions

LC system:	ACQUITY UPLC
Solvent A:	Water:acetonitrile (90:10)
Solvent B:	Acetonitrile:methanol (50:50)
Flow rate:	0.7 mL/min

Column temp:	35 °C
Column:	ACQUITY BEH C_{18} 2.1 mm x 100 mm, 1.7 µm ACQUITY UPLC with PDA and SQ Detector

Time (min)	%age A	%age B	Curve
Initial	10	90	Initial
0.10	10	90	9
2.00	0	100	6
3.00	0	100	6
3.50	10	90	6
5.00	10	90	6

Table 1. UPLC solvent method.

ACQUITY UPLC PDA

Wavelength range:	205 to 450 nm
Resolution:	1.2 nm
Sampling rate:	20 spectra/s

Waters 996 PDA

Wavelength range:	205–450 nm
Resolution:	1.2 nm
Sampling rate:	20 spectra/s

SQ Detector Settings

The single quadrupole MS was run in APCI mode.

Corona current:	15.0 uA
Cone voltage:	35.0 V
Source temp:	145 °C
Probe temp:	575 °C
Cone gas:	200 L/Hr
Desolvation gas:	600 L/Hr

(See Table 2 for the *m/z* values used for the SIR method)

Data Acquisition and Processing

The total system was controlled with MassLynx MS Software (creation of methods and data-processing). The SQ Detector parameters were set up via the IntelliStart Software and using the on-line fluidics. IntelliStart monitors the performance of the mass spectrometer and will initiate corrective action if any problems are diagnosed. Processes such as tuning and calibration can be performed by the click of a button using the on-line fluidics.

Results and Discussion

Published methods for the detection of fat-soluble vitamins can require 15 to 60 minutes. A standard solution of vitamins is shown in Figure 1 with an analysis time of 5 minutes by UPLC. Table 2 shows the values used for the each vitamins analyzed.

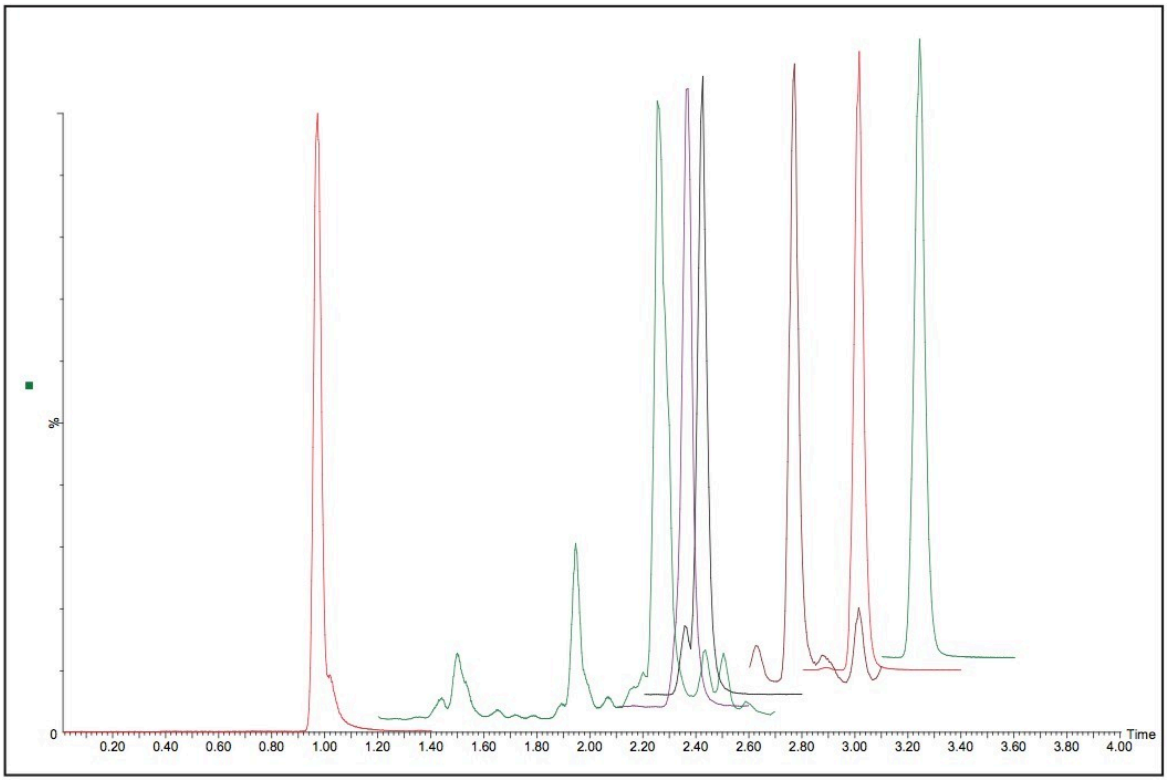

Figure 1. Standard mixture of fat-soluble vitamins.

RT	Vitamin	UVmax	SIR - RT windows	SIR -m/z
0.98	Vitamin A_1	325	0.00-1.40	269
2.25	Vitamin K_2	265 245	1.20-2.70	445
2.37	Vitamin D_2	265 [2]	2.10-2.60	397
2.43	Vitamin D_3	265 [2]	2.20-2.80	385
2.77	Vitamin E	291	2.60-3.10	431
3.02	Vitamin E acetate	285	2.80-3.40	473
3.24	Vitamin K_1	265 240	3.10-3.60	451

Table 2. Retention times, UV maxima and m/z values (used for SIR) for the fat-soluble vitamins.

Vitamin A is the first to elute and Vitamin K is the last to elute on the ACQUITY BEH C_{18} Column.

Figure 2 shows the presence of vitamin E (*m/z* 431) and vitamin E acetate (*m/z* 473) from the standard mixture (left) and from a butter sample (right) that has undergone liquid-liquid extraction.1 Selected ion recording (SIR) was used to increase the selectivity of the vitamins in the butter.

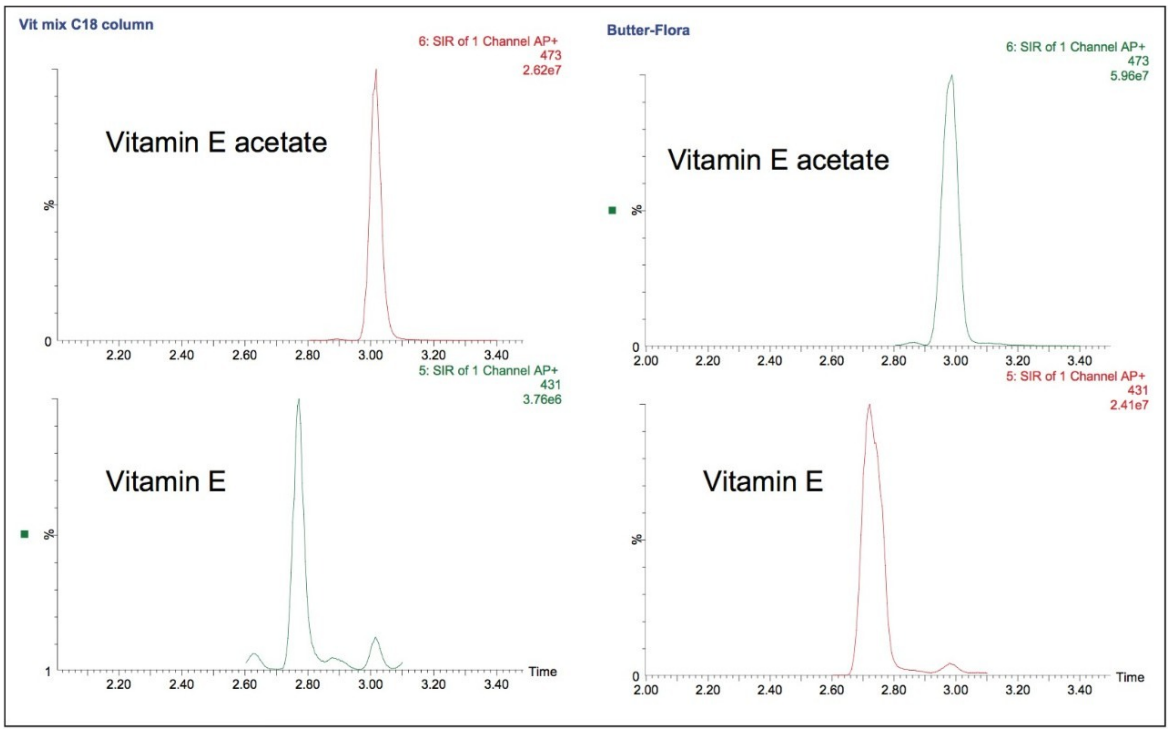

Figure 2. Vitamin E and Vitamin E acetate in standard solution (left), and in butter (right).

Figure 3 shows the resulting data from PDA detection. While the chromatogram can be simplified for this particular butter sample by selecting the specific maximas for each vitamin, the complexity of certain systems can cause interferences that prevent comprehensive analysis via a single detector. By employing complementary techniques such as those described in this note it may be possible to discern additional components and obtain required data.

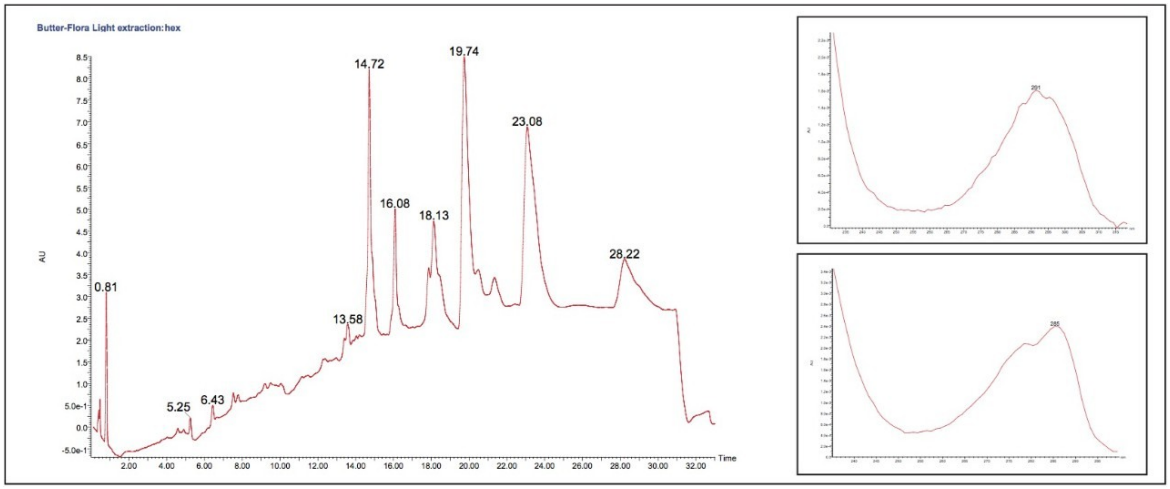

Figure 3. PDA chromatogram of butter using the extended method where the presence of vitamin E (maxima 291) and vitamin E acetate (maxima 285) have been confirmed by their UV spectra (shown at the right hand side of the figure).

Conclusion

A 5-minute method utilizing UPLC has been developed to detect fat-soluble vitamins. Current published methods typically require at least 15 minutes to perform the same analysis. While identification of these vitamins is possible using a PDA, samples are often complex, requiring additional techniques to detect all components of interest. The PDA and SQ Detectors are complementary techniques that allow not only for the identification of vitamins but also for components that may not be amenable to PDA detection alone.

References

1. D Blanco, M P Fernández, M D Gutiérrez. *Analyst*, 2000: 125, 427–431.
2. F Zonta, B Stancher, J Bielawny. *Chrom,* 1982: 246, 105–112.

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- ACQUITY UPLC System <https://www.waters.com/514207>
- SQ Detector 2 <https://www.waters.com/134631584>
- MassLynx MS Software <https://www.waters.com/513662>

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