EXTRACTION AND ANALYSIS OF FAT-SOLUBLE VITAMINS IN FORTIFIED FOOD PRODUCTS AND INFANT FORMULA

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OVERVIEW

AIM

- To develop a simple, reliable and sensitive method to extract and analyze fat-soluble vitamin compounds in fortified food products and infant formula.

METHODS

- Simultaneous extraction of fat-soluble vitamins using solid phase extraction (SPE).
- Analysis performed using UPLC coupled to a tandem quadrupole MS in APCI positive mode.

RESULTS

- The use of a two-step elution method with Oasis® HLB cartridges yielded extraction recoveries of between 77 and 112%, with RSDs of less than 5%.
- A rapid six minute UPLC-MS/MS method was successfully developed for the simultaneous analysis of 7 fat-soluble vitamin compounds.
- Untargeted contaminants in the food sample matrix were successfully identified with the use of RADAR functionality.

INTRODUCTION

The presence of fat-soluble vitamins (A, D, E and K) in humans is of vital importance because of their catalytic functions in anabolic and catabolic pathways. Reliable and sensitive determination of fat-soluble vitamins in fortified food and beverage products are essential for both nutritional and economic reasons.

Official analytical methods for the determination of fat-soluble vitamins are mainly HPLC and UV based, which typically take 15 to 60 minutes per run, and generally require separate analyses for each vitamin. More recently the trend within industry has been the adoption of multi-analyte methods to improve lab efficiency.

Another issue is sample preparation: this is often the rate limiting step of the analysis and the most challenging for labs that routinely test for the presence of vitamins in dairy products and infant formula.

In this study, we developed a solid phase extraction (SPE) method for the simultaneous extraction of fat-soluble vitamins from fortified food products and infant formula. A rapid six-minute UPLC-MS/MS method using positive atmospheric pressure chemical ionization (APCI) ionization was utilized for the analysis of the fat-soluble vitamin compounds.

METHODS

Sample Preparation & Extraction

- Sample matrix subjected to ethanol extraction followed by solid phase extraction (SPE) using Waters Oasis HLB Cartridge (60 mg, 3 cc) as described in Figure 1.
- Combine both eluted fractions, evaporate to dryness, and reconstitute with ethanol.
- Analyze by LC-MS/MS.

Oasis Sample Extraction

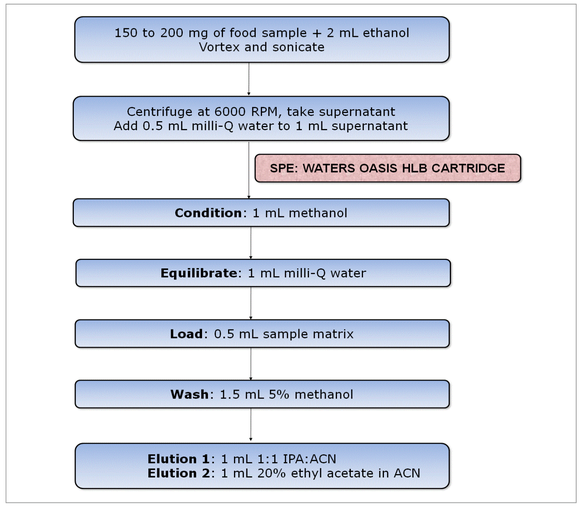

Figure 1. Illustration of FSV extraction protocol

LC Conditions

LC system: ACQUITY UPLC system
Column: ACQUITY UPLC BEH C18, 2.1 x 100 mm, 1.7 µm
Column temp: 40 oC
Flow rate: 0.6 mL/min
Mobile phase A: 90:10 Acetonitrile:Water
Mobile phase B: Methanol
Gradient:

Time (min)	%A	%B
0.00	99.9	0.1
0.50	99.9	0.1
2.50	0.1	99.9
4.50	0.1	99.9
4.51	99.9	0.1
6.00	99.9	0.1

Total run time: 6.0 min
Injection volume: 5 µL

MS Conditions

MS system: Waters XevoTM TQ MS system
Ionization: APCI positive
Corona current: 15 µA
Source temp: 150 oC
APCI probe temp: 550 oC
Desolvation gas: 1000 L/hr
Acquisition: Multiple Reaction Monitoring (MRM) with RADAR full scan
Collision gas: Argon at 3.5 x 10-3 mbar

Analyte	Parent (m/z)	Dau 1/ Dau 2 (m/z)	CV (V)	CE 1/ CE 2 (eV)	RT (min)
Vitamin A	268.9	93	20	22	0.98
(palmitate form in IF)		81		24	(4.48)
Vitamin K2	445.5	187.1	24	22	2.45
		81		46	
Vitamin D2	397.5	107	20	20	2.53
		379.4		12	
Vitamin D3	385.5	367.4	20	14	2.59
		107		24	
Vitamin E	431.5	165	18	26	2.91
		137		40	
Vitamin E acetate	473.6	207.1	28	18	3.12
		165.1		40	
Vitamin K1	451.5	187.1	34	24	3.34
		128		74	

Table 1. MRM parameters for analysis of fat-soluble vitamin compounds

RESULTS

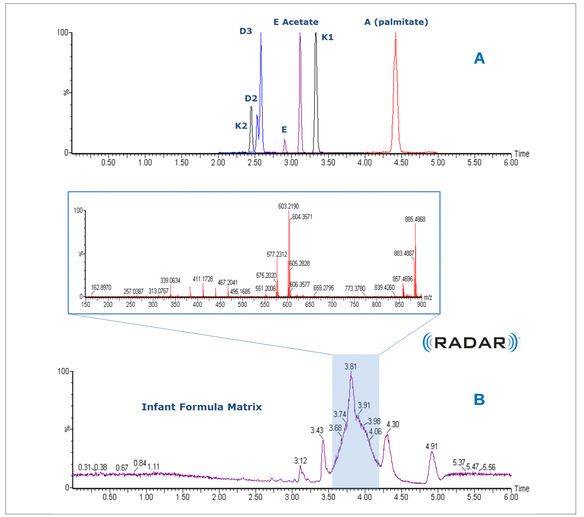

Figure 2. RADAR technology allows for simultaneous acquisition of MRM (A) and full scan data (B) in a single analysis run. Spectrum identifying untargeted contaminants in sample matrix (infant formula) are shown in the insert of the full scan data.

Recovery and Repeatability

Recovery was determined by comparing the pre-extracted spiked samples with the post-extracted spiked samples. Experiments were performed on 2 different days with 6 replicates being performed on each day. Average extraction recoveries range from 77.7 to 112.9% in the food matrices tested, with RSDs less than 5% for 6 replicates.

Breakfast Cereals

Vitamin Compound	Recovery (%)	%RSD (n=6)
A	90.6	1.6
D2	100.1	4.1
D3	99.0	2.3
E	80.6	1.7
E acetate	96.5	2.8
K1	100.6	1.6
K2	98.5	2.4

Chocolate

Vitamin Compound	Recovery (%)	%RSD (n=6)
A	83.7	2.7
D2	82.9	2.7
D3	94.8	3.3
E	112.9	4.1
E acetate	107.6	3.9
K1	84.6	4.4
K2	84.0	3.3

Infant Formula

Vitamin Compound	Recovery (%)	%RSD (n=6)
A	91.0	2.0
D2	89.0	3.8
D3	77.7	2.3
E	93.2	2.5
E acetate	96.5	2.0
K1	95.7	5.0
K2	92.1	4.4

CONCLUSION

- A muliti-analyte method replaces separate, lengthy methods for the extraction and analysis of fat-soluble vitamin compounds in fortified food products, leading to greater lab efficiency.
- Results showed good recoveries and %RSDs even in the presence of oils and other interferences in food products.
- RADAR technology allows for monitoring of matrix interferences, impurities and degradants in samples, allowing analysts to make informed decisions when assessing matrix effects.

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