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Fat-soluble vitamins / LCMS-8045

Application News

Quantitative Determination of Fat-Soluble Vitamins in Infant Formula by LC-MS/MS Method with Supported Liquid Extraction (SLE)

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User Benefits

- A LC-MS/MS method with sample preparation using supported liquid extraction (SLE) column is established for quantitative determination of fat-soluble vitamins (A, 1, D3, E and K1) in infant formula on LCMS-8045.
- The reliability of the method was verified and confirmed with analysis of fat-soluble vitamins in FAPAS QC quality control material. The analysis results indicate the acceptable accuracy and precision of the vitamin analysis.

■ Introduction

Fat-soluble vitamins are classified into four categories: Vitamin A, D, E and K. They have important roles in several functions of the human body, such as vision (vitamin A), calcium absorption (Vitamin D), antioxidative protection in cell membranes (Vitamin E), and blood coagulation (Vitamin K). Infant formula, as a main source of feeding for infants, must be able to provide sufficient vitamins. Due to complexity and instability of Fat-soluble vitamins during extraction, their quantitation remains challenging. Thus, extraction is the critical step in determination of fat-soluble vitamins in milk samples. [1] In this application notes, a sensitive MRM method is described for the quantitative determination of fat-soluble vitamins in infant formula with supported liquid extraction (SLE) on LCMS-8045, a tandem LC-MS/MS system with an APCI interface.
*FAPAS: "Food analysis performance Assessment Scheme" is the proficiency scheme for food and water testing, organized by the Food and Environment Research Agency, UK.

■ Experimental

Reagents and standards

Retinol, pyrogallol, potassium hydroxide, and butylated hydroxytoluene were purchased from Sigma-Aldrich. A fat-soluble Vitamin Kit (containing Vitamin D3, Vitamin E and Vitamin K1) was purchased from AccuStandard. Hexane and reagent alcohol of HPLC grade were used. Formic acid of LCMS grade were used as additives in the mobile phase prepared from LC/MS grade Methanol and Milli-Q water.

LC-MS/MS conditions

The analytical conditions on LCMS-8045 are compiled in Table 1.

The MRM transitions (quantifier ion and reference ion) and their optimized collision energy (CE) of the four compounds are compiled into Table 2.

Table 1 Analytical conditions on LCMS-8045

LC Conditions (Nexera)	
Column	Shim-pack™ GIST C18 column (2.1 x 100 mm, 3 µm) P/N: 227-30008-05
Flow Rate	0.4 mL/min
Mobile Phase	A: 0.1% Formic acid in Mili-Q water B: 0.1% Formic acid in Methanol
LC program	Gradient elution, 15 minutes
Oven Temp.	40°C
Injection Vol.	10 µL
Elution gradient	B%: 80% (0.5min)→ 100% (5min to 12 min)→ 80% (12.01min to 15min)
MS Conditions (LCMS-8045)	
Interface	APCI
Interface Temp.	350°C
DL Temp.	250°C
Heat Block Temp.	200°C
Nebulizing Gas	3 L/min
Drying Gas Flow	5 L/min
Mode	MRM, Positive mode

Table 2 MRM transitions and optimized CE

Name	Quantifier	CE (V)	Ref. Ion	CE (V)
Vitamin A1	269.3> 92.9	-24	269.3>107.2	-18
Vitamin D3	385.4>259.1	-14	385.4>367.2	-11
Vitamin E	431.5>165.2	-21	431.5>137.3	-43
Vitamin K1	451.5>187.1	-25	451.5>57.2	-35

Sample preparation

A schematic procedure of sample preparation is shown in Fig. 1. Quality control material were prepared according to the procedure.

pH adjustment before loading of sample onto the SLE column is important as it will affect the retention of fat-soluble vitamins in the column. Experiment result shows that sample pH of around 6-7 would be the best condition for good recovery of fat-soluble vitamins.

■ Results and Discussion

Optimization of LC-MS/MS

MRM optimization of 4 non-polar fat-soluble vitamins were performed in APCI positive mode. To separate the fat-soluble vitamins, a C18 column was used. Initial percentage of mobile phase B was set at 80% to shorten the total analysis time and to achieve sharper peaks. Fig. 2 shows the MRM chromatograms of a 50 ng/mL mixed standards in pure solvent.

Saponification

- Add 1.5mL of Mili-Q water to 0.1g of milk powder and sonicate.
- Add 2mL 2% pyrogallol in reagent alcohol and 1mL of 50% KOH.
- Mix overnight.

pH Adjustment (target pH around 6-7)

- Add 250µL Formic acid and mix.
- Adjust pH of the sample solution to around 6-7.

Extraction

- Load all sample solution into SLE column, wait for 5mins
- Add 8mL of 0.1% BHT in hexane and allow to flow under gravity.
- Add 2 x 8mL of hexane
- Collect the eluent in a glass tube.
- Apply vacuum for 10-30 seconds to complete elution.
- Blow dry eluent under gentle stream of Nitrogen.

Reconstitution and Filtration

- Reconstitute with 0.5mL of Methanol.
- Filter with 0.22µm Nylon membrane filter into a 1.5mL amber vial.
- Analyse with LCMSMS.

Fig. 1 Sample preparation procedure of infant formula sample by supported liquid extraction (SLE).

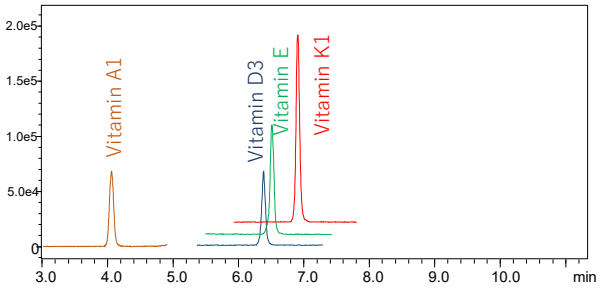

Fig. 2 MRM peaks of 50 ng/mL mixed standards

Calibration curve and sensitivity

The calibration curves were established with mixed standards in pure solvent from 10 ng/mL to 400 ng/mL (Fig. 3). Good linearity was obtained for all vitamins with R^2 value greater than 0.999. The repeatability was evaluated by 6 consecutive injections of mixed standards solution at 50ng/mL concentration. The linearity, LOD, LOQ and %RSD results are tabulated in Table 3.

Table 3 Linearity, LOD, LOQ and %RSD of fat-soluble vitamins

Vitamin	RT (min)	R^2	LOD (ng/mL)	LOQ (ng/mL)	%RSD Area (n=6)	%RSD RT (n=6)
A	4.090	0.9998	2.8	8.5	0.44	0.22
D3	6.499	0.9997	2.1	6.3	3.41	0.06
E	7.025	0.9998	1.6	4.8	0.73	0.03
K1	8.561	0.9998	2.5	7.4	2.05	0.02

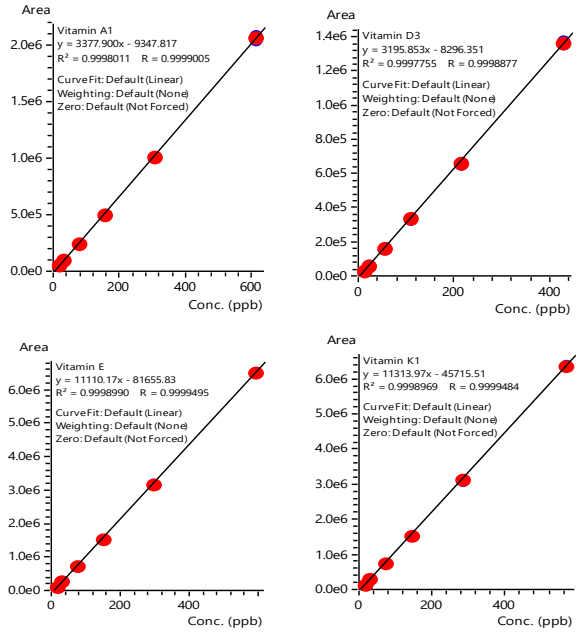

Fig. 3 MRM calibration curves of vitamin A1, D3, E, and K1 in the range of 10~600 ng/mL .

Determination of Quality Control Material (QCM)

The established method was applied to analyze FAPAS quality control material (T21125QC) [3] to determine the reliability of the method. Six separate analyses were performed on the QC material. The analysis results of the QC material were summarized in Table 4. %RSD of the 6 results are less than 10%, indicating acceptable precision of the method.

Table 4 Results of 6 analysis on QC material T21125QC.

Sample	A (µg/100g)	D3 (µg/100g)	E (µg/100g)	K1 (µg/100g)
QC1	436.8	10.2	22.1	31.4
QC2	450.0	9.7	22.1	33.0
QC3	444.1	9.1	21.3	39.4
QC4	436.7	8.9	21.6	36.0
QC5	468.3	9.3	22.6	37.4
QC6	455.7	8.7	21.9	36.0
%RSD	**2.3**	**4.8**	**1.7**	**6.9**

Quantitation results of all six analyses are within the acceptable range of the QC material. The average concentration of the six analysis, assigned value and their acceptable range are shown in Table 5.

Table 5 Quantitation results, assigned value and acceptable range of vitamin A, D3, E and K1 in QC material T21125QC (Unit: ug/100 g)

Vitamin	Ave. conc. Meas. (n=6)	Assigned value	%Recovery	Acceptable range
A	448.6	443	101.3	355-532
D3	9.3	10.6	87.8	7.7-13.5
E	22.0	21.6	101.8	17.3-25.9
K1	35.5	40.6	87.4	25.7-55.5

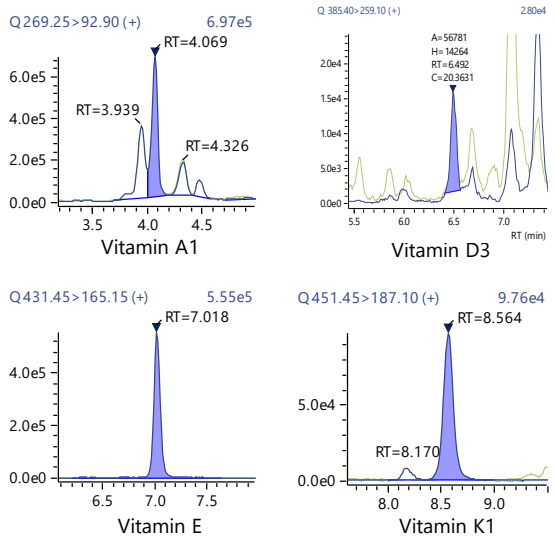

Fig. 4 MRM peaks of vitamin A, D3, E and K in QC material.

Inter-day repeatability of the method was evaluated. %RSD of retention time and concentration are tabulated in Table 6. Inter-day repeatability were found to be less than 10% for vitamin A1 and vitamin E, and less than 15% for vitamin D3 and vitamin K1.

Table 6 Inter-day %RSD of RT and concentration of vitamin A, D3, E and K1 detected in QC material T21125QC (n=6 x 2 days).

Vitamin	Ave. Conc., µg/100g	%RSD, RT	%RSD, Conc.
A	437.9	0.33	3.5
D3	10.0	0.09	10.8
E	21.8	0.09	3.6
K1	38.0	0.11	14.9

■ Conclusion

An LC-MS/MS method was established for the quantitative determination of fat-soluble vitamins including vitamin A1, D3, E and K1 in infant formula. Sample preparation using a supported liquid extraction (SLE) column was adopted. Good linearity was achieved with R^2> 0.999 in a range of 10-400 ng/mL. Analysis of a QC material (FAPAS) verified that the method is well acceptable in accuracy and precision without use of internal standards.

■ References

1. E. Karrar, Isam A.Mohamed Ahmed, M. F. Manzoor,W.W., F.Sarpong, X.Wang, Food Chemistry 373 (2022) 131436
2. Y.L Chew, Shimadzu Appl. News AD-0208
3. FAPAS QC Material Data Sheet (T21125QC)

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