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Food Analysis / LCMS-8045

Quantitative Analysis of Vitamin D3 in Milk Powders by LC/MS/MS Following AOAC Method 2011.11

Yin Ling Chew, Zhe Sun, Zhaoqi Zhan and Jie Xing

1. Introduction

Vitamin D3 (cholecalciferol) is an essential micronutrient for the well-being of human body. Deficiency of vitamin D3 will lead to illnesses such as osteoporosis and cardiovascular diseases. It is synthesized in skin upon exposure to UV sunlight or from diet[1]. Due to its importance, vitamin D3 is fortified in food and dairy products such as infant and adult formulas. Vitamin D3 is monitored in foods by authorities around the world. The AOAC standard method 2011.11 describes the analysis method of vitamin D3 in infant and adult nutritional formula[2]. In this study, a LC/MS/MS method with ESI ionization for sensitive detection of vitamin D3 in infant and adult formula was developed. The extraction method followed the AOAC standard method 2011.11 with LC modification. The reliability of the LC/MS/MS method was verified and confirmed with the analysis of vitamin D3 content in a standard reference material (SRM), NIST 1849a.

2. Experimental

The vitamin D3 (VD3) standard was obtained from AccuStandard while the isotope-labelled VD3 [$^2H_3$] internal standard dissolved in ethanol was obtained from IsoSciences. Stock solution of vitamin D3 was prepared in mixed solvent of ethanol and methanol (1:1). The stock solutions were diluted into different vitamin D3 concentrations as standard calibrants with acetonitrile. The sample preparation method followed the AOAC standard analysis method 2011.11 with slight modification as shown in Figure 1. The sample was analyzed using a Shimadzu LCMS-8045 with a heated ESI and a Shim-pack GIS C18 column. The LC-MS/MS conditions are given in Table 1.

Table 1. Analytical conditions of vitamin D3 on LCMS-8045

Column	Shim-Pack GIS C18 2um, (2.1 mm I.D. x 100 mm L)
Flow	0.3 mL/min
Mobile phase	A : Milli-Q water with 0.1% formic acid B : Methanol with 0.1% formic acid
Oven Temp	40°C
Injection vol	10 µL
Elution gradient	B% : 70% (0.0 min) ➡90% (0.5 min) ➡100% (0.8 to 6.0 min) ➡70% (7.0 to 10.0 min)

Interface	**ESI (heated)**
MS Mode	Positive mode
CID gas	Argon, 230 kPa
Block Temperature	400 °C
DL Temperature	250 °C
Interface Temp.	400 °C
Nebulizing Gas Flow	Nitrogen, 3.0 L/min
Drying Gas Flow	Nitrogen, 10.0 L/min
Heating Gas Flow	Zero Air, 10.0 L/min

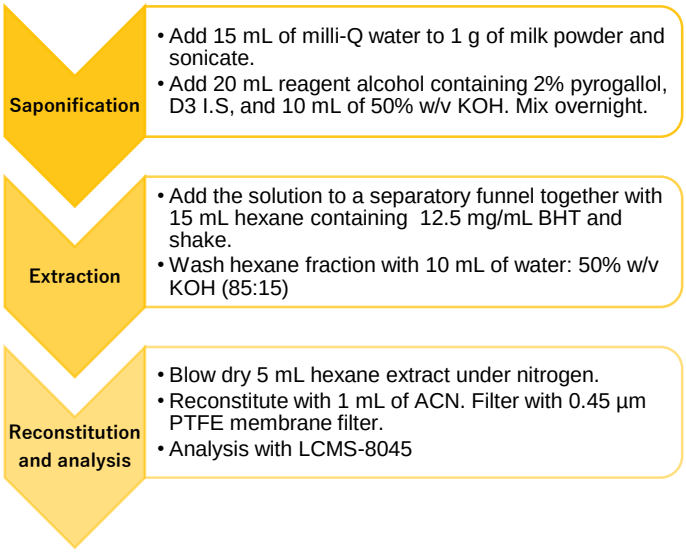

Figure 1. Sample preparation flowchart for Vitamin D3 in milk powders following AOAC 2011.11 method with slight modification

3. Results and Discussion

3.1 MRM optimisation

MRM optimisation of both vitamin D3 and the VD3 [$^2H_3$] I.S are performed using automated MRM program of the LabSolutions. Table 2 shows the MRM optimisation results of vitamin D3 and VD3 [$^2H_3$] I.S. Instead of APCI interface as described in AOAC method 2011.11, heated ESI ionisation is used. Good sensitivity was achieved using ESI ionisation.

Table 2. MRM transitions of vitamin D3 and VD3 [$^2H_3$] I.S

Name	MRM 1	MRM 2
Vitamin D3	385.40>259.20	385.40>159.10
VD3 [$^2H_3$] I.S	388.15>259.10	388.15>110.10

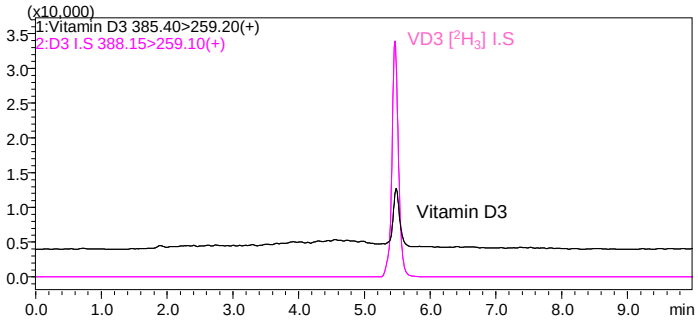

Figure 2. MRM chromatogram of vitamin D3 at 1 ppb and VD3 [$^2H_3$] I.S at 50 ppb (scale factor of 0.10)

3.2 Establishment of LC/MS/MS method

A calibration curve was established from the range of 1 ~ 100 ppb. A good linearity with r2 value of 0.9990 was obtained. The detection limit (LOD) and quantitation limit (LOQ) were 0.06 ppb and 0.185 ppb, respectively. The repeatability was evaluated by injecting 6 consecutive injections of vitamin D3 standard at 10 ppb concentration. The results are shown in Table 3.

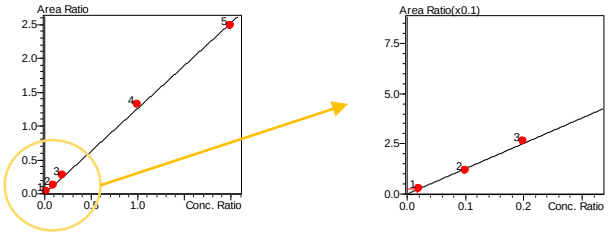

Figure 3. Internal standard calibration curve of vitamin D3

Table 3. Linearity, LOD, LOQ and % RSD results of vitamin D3

RT (min)	Range (ppb)	Linearity	LOQ (ppb)	LOD (ppb)	% RSD (n=6)
5.455	1 - 100	0.9990	0.185	0.06	0.711

3.3 Quantitation of Vitamin D3 in SRM NIST 1849a and sample

The established method was applied to analyse a milk powder sample A obtained from a supplier. To ensure reliability and the analysis, standard reference material (SRM) NIST 1849a was analysed before analysis if the unknown sample A. The vitamin D3 concentration (0.107 mg/kg) determined falls precisely in the range of vitamin D3 content in NIST 1849a (0.111 ± 0.017 mg/kg). The results of SRM and Sample A are shown in Table 4.

Table 4. Quantitation results of vitamin D3 in NIST 1849a and unknown Sample A.

No.	Sample name	Vitamin D3 content (mg/kg)
1	Sample A	0.176
2	NIST 1849a	0.107

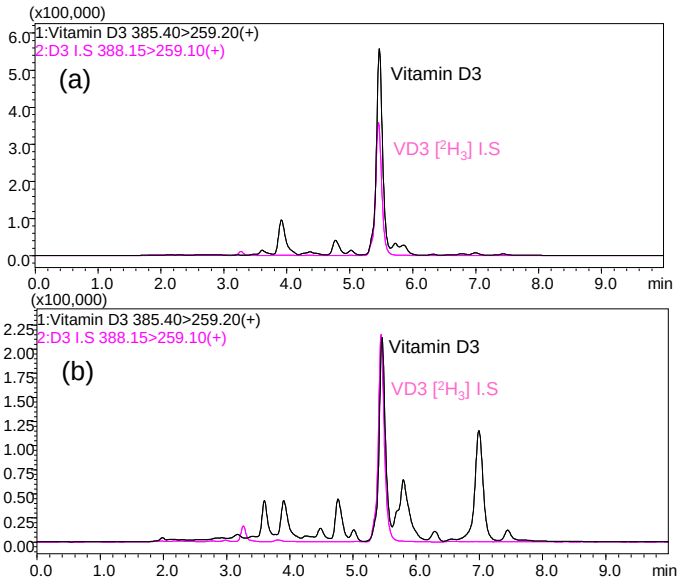

Figure 4. (a) MRM chromatogram of sample A. (b) MRM chromatogram of NIST 1849a

4. Conclusions

Following AOAC 2011.11, a LC-MS/MS method and sample preparation procedure were established for accurate quantitation of vitamin D3 in milk powders. The LOD and LOQ are 0.06 ppb and 0.185 ppb, respectively. Good linearity was achieved with r2>0.999 in a range of 1~100 ppb. To ensure the reliability and accuracy, the method was used to determine SRM NIST 1849a and the vitamin D3 content falls precisely in the range of official value.

References

1. Mostafa,W.; Hegazy, R. Vitamin D And The Skin: Focus On A Complex Relationship: A Review. Journal of Advanced Research 2015, 6(6), 793-804.
2. Official Methods of Analysis of AOAC INTERNATIONAL (2012) 19th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method 2011.11

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SHIMADZU (Asia Pacific) Pte. Ltd
79 Science Park Drive, #02-01/08 Cintech IV, Singapore 118264,
www.shimadzu.com.sg; Tel: +65-6778 6280 Fax: +65-6778 2050