A novel fast and simple quantification method for vitamins, complements and contaminants in milk infant formulas by LC-MS/MS

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1. Introduction

Milk infant formulas are generally enriched with vitamins and complements which are essential for normal health and growth. The manufacturers must insure that their content is controlled properly. Also they must certify the absence of certain contaminants. Traditional methods to measure complements and contaminants in this matrices are based on HPLC thanks to the possibility of rapid separation and quantification. Mass spectrometry detection is the gold standard due to its specificity, precision and sensitivity. However, because of the high variability of structures and chemical properties of this analytes, several methods are needed for both their extraction and their separation by chromatography. We here report a unified solution for the quantification of all of this compounds in milk infant formulas.

2. Methods and Materials

The quantitative analysis of vitamins, complements and contaminants was performed using commercially available milk infant formulas from several manufacturers. Method is simplified by using only two extraction procedures followed by a unique separation by HPLC coupled to mass spectrometry (Figure 1.). The analytes were monitored using UHPLC-MS/MS system (Nexera X2 and LCMS-8050, Shimadzu, Kyoto). Main MRM transitions are detailed in Table 1. Two sample preparations were performed: one for water-soluble vitamins and polar complements and contaminants, based on a simplified extraction with acidified methanol, and a second one, for fat-soluble vitamins, based on Biotage ABN SPE extraction. Analytical performance of the method was monitored using calibrators and QC prepared in milk infant formulas, using standard addition strategy.

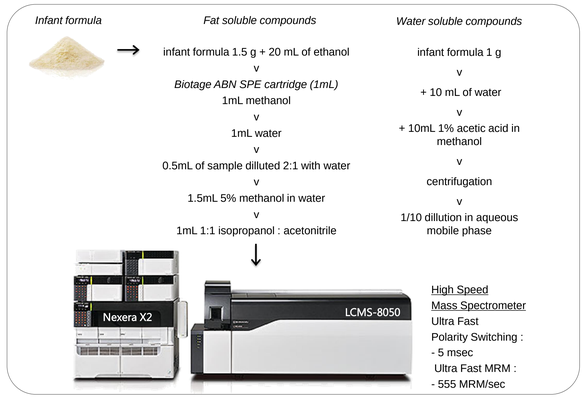

Figure 1. Sample workflow overview.

UHPLC conditions : Nexera X2

Column : reverse phase 150 x 2.1, 3µm,
Oven temperature : 60°C,
Mobile Phases: water and acetonitrile
Additives : ammonium formate and formic acid
Flow rate : 400 µL/min,

MS conditions : LCMS-8050

Heating Gas : 10 L/min (Air),
Nebulizing Gas : 2.5 L/min (N2),
Drying Gas : 10 L/min (N2),
HESI : 400°C
DL : 100°C
HB : 300°C
Pause time : 1 msec
Polarity switching : 5 msec
Points per peak : > 30

Transitions :

Compound Name	MRM
Inositol (+)	202.85 > 22.95
Aflatoxin M1 (+)	329 > 273
Taurine (-)	124.2 > 80
Vitamin C Ascorbic Acid (-)	175 > 115.05
Melamine (+)	127.1 > 85
Choline (+)	104.1 > 60.1
L-carnitine (+)	162.1 > 103.15
Vitamin B3 Nicotinic Acid (+)	124 > 80
Vitamin B6 Pyridoxyne (+)	170 > 134.15
Vitamin B5 Pantothenic Acid (+)	220.05 > 90.15
Vitamin B9 Folic Acid (+)	442.1 > 295.15
Vitamin B9 Folic Acid (-)	439.9 > 311.1
Vitamin B12 Cyanocobalamin (+)	678.5 > 147.1
Vitamin B2 Riboflavin (+)	377.15 > 243.15
Vitamin B8 Biotin (+)	245.1 > 227.15
Vitamin B1 Thiamine (+)	265 > 122
Chlorate (-)	83.1 > 66.9
Perchlorate (-)	99.1 > 82.9
Vitamin A Retinol (+)	269.2 > 93.15
Vitamin D3 Cholecalciferol (+)	385.3 > 91.2
Vitamin E Tocopherol (+)	431.1 > 165.05
Vitamin K1 Phylloquinone (+)	451.3 > 187.05

Table 1. Main MRM transition for each compound.

3. Results

3-1. Method conditions

The method enables the quantification, in infant formulas, of the all the compounds of interest (see bellow). Linearity was confirmed for all compounds in the generally expected target range (concentrations in µg/100g) : 10 to 300 for Vitamin A (Retinol), 500 to 15000 for Vitamin E (Tocopherol), 0.5 to 50 for Vitamin D3 (Cholecalciferol), 1 to 30 for Vitamin K1 (Phylloquinone), 50 to 600 for Vitamins B1 (Thiamine), B2 (Riboflavin) and B6 (Pyridoxine), 100 to 5000 for Vitamin B3 (Nicotinic acid), 100 to 20000 for Vitamin B5 (Pantothenic acid), 1 to 60 for Vitamin B8 (Biotin), 20 to 400 for Vitamin B9 (Folic acid), 0.1 to 2 for Vitamin B12 (Cyanocobalamin), 1000 to 200000 for Vitamin C (Ascorbic acid), 5000 to 100000 for Choline, 10000 to 200000 for Inositol, 10000 to 200000 for Taurine, 2000 to 40000 for L-carnitine, 0.05 to 1 for Aflatoxin M1, 0.002 to 0.04 for Chlorate, 0.001 to 0.02 for Perchlorate, and 100 to 2000 for Melamine.

The r^2 of linearity models were above 0.98, with S/N > 10 for all LLOQ levels.

3-2. Typical chromatograms

Figure 2. presents the chromatograms for all analyzed compounds. Total run time is 14 min.

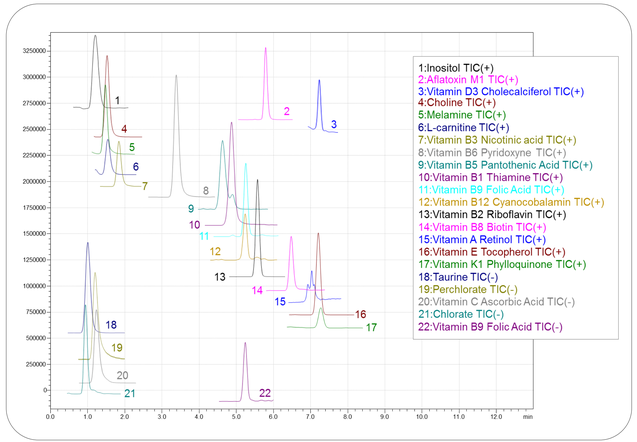

Figure 2. Typical chromatograms for all analyzed compounds.

3-3. Fat soluble vitamins extraction recovery

The fat soluble vitamins were successfully retained and eluted. Table 2. shows the percent recoveries for vitamins A, D3, E and K1, extracted from commercial infant formula.

Compound	Conc. µg/100g	SPE recovery
Vitamin A	10	107% ± 8%
Vitamin D3	50	83% ± 3%
Vitamin E	5	97% ± 5%
Vitamin K1	2500	91% ± 7%

Table 2. Fat soluble vitamins extraction recovery.

3-4. Water soluble compounds recovery

Table 3. presents the recoveries obtained for water soluble compounds.

Compound	Conc. µg/100g	Recovery
Vitamin B1	1000	102% ± 1%
Vitamin B12	5	80% ± 4%
Vitamin B2	5000	88% ± 4%
Vitamin B5	10000	102% ± 1%
Vitamin B6	1000	100% ± 6%
Vitamin B8	50	94% ± 3%
Vitamin B9	500	79% ± 1%
Vitamin B3	10000	112% ± 3%
Taurine	100000	98% ± 9%
Vitamin C	500000	97% ± 2%
Aflatoxine M1	0.5	108% ± 8%
Choline	50000	100% ± 2%
Inositol	100000	91% ± 8%
L-carnitine	20000	100% ± 1%
Melamine	1000	102% ± 5%

Table 3. Water soluble compounds extraction recovery.

4. Novel Aspect

Fast quantification of vitamins, complements and contaminants in infant formulas by LCMSMS, using two extractions and a unique HPLC separation.