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Application Data Sheet

No. AD-0055

LCMS-2020

Quantitative Analysis of Choline in Milk Powder and Dietary Supplements by LC/MS Method

As an essential nutrient for the normal function of human cells, choline must be consumed in the diet. Many foods like egg, milk as well as some dietary supplements are the main sources of choline. A HPLC method with ELSD detection and HILIC separation for quantitative determination of choline in multivitamin tablet was developed recently [1]. Here, we report a LC/MS method for quantitation of choline in milk powders and dietary supplements. The advantages of the LC/MS method include not only superior sensitivity and mass selectivity, but also faster and simpler in sample preparation.

❑ LC/MS Method & Conditions

The analytical conditions are shown in Table 1. The HILIC separation was carried out in isocratic elution mode using acetonitrile and water-5mM ammonium acetate as the mobile phase. The retention time of choline under this condition was at 6.25 min. The SIM chromatograms of choline standards on LCMS-2020 are shown in Figure 1. With ESI positive ionization mode, choline was detected as molecular ion M^+ (m/z 104.2). In SIM mode only choline was detected selectively, which made the analysis of choline more selective and reliable than the HPLC-ELSD method. A linear calibration curve was established using choline

Table 1: LC and MS analytical conditions for choline analysis

Column	ZIC®-pHILIC column (PEEK 150 x 4.6 mm, 5µm)
Flow Rate	0.80 mL/min
Elution Mode	Isocratic elution
Mobile Phase	5 mM NH_4Ac/ACN (20/80, v/v)
Oven Temp.	40 ºC
Injection Volume	10 µL

Interface	ESI
MS Mode	Positive mode (SIM)
Block Temp.	200 ºC
DL Temperature	250 ºC
Nebulizing Gas Flow	Nitrogen, 1.5 L/min
Drying Gas Flow	Nitrogen, 15.0 L/min

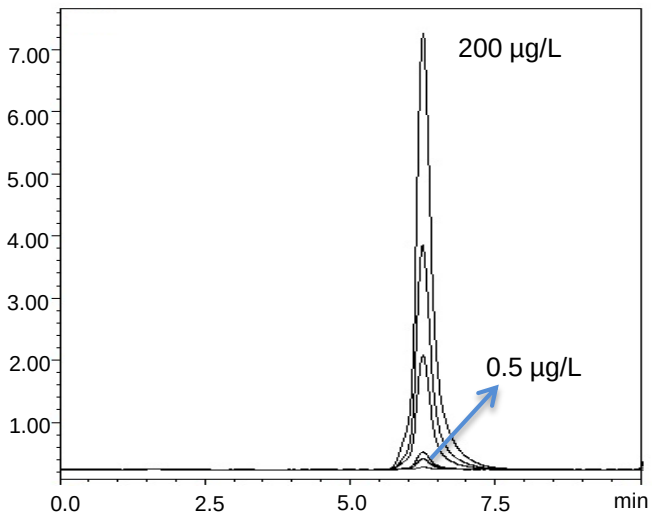

Figure 1: LCMS Chromatogram of choline standards in SIM mode (m/z 104.2)

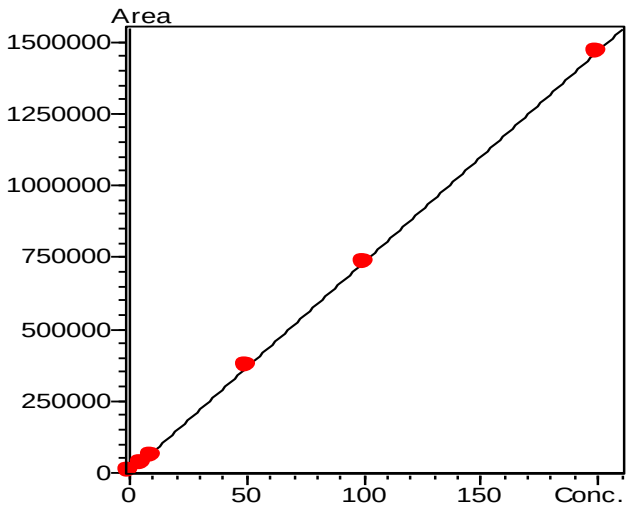

Figure 2: Calibration curve of choline (µg/L), 0.5 ug/L ~ 200 ug/L (ppb)

standard solutions of concentrations of 0.5, 5, 10, 50, 100 and 200 µg/L (ppb). The linearity of the calibration curve was $r^2 > 0.999$ (Figure 2). The limit of detection (LOD) of choline of the method was estimated to be at 0.2 µg/L. The repeatability of the system was evaluated and the RSD (%, n=6) of peak area and retention time are shown in Table 2.

Table 2: Repeatability of the method for choline (n=6)

Conc. (µg/L)	% RSD, peak area	% RSD, RT
0.5	4.23	0.15
50	3.52	0.03
200	0.22	0.02

❑ Analysis Results of Samples

The established LC/MS method was applied to quantitation of different dietary supplement samples including infant milk powder formula, multivitamin tablet and weight loss support supplement. Take 1g of milk powder added with 15mL of 1% TFA solution and 5mL of acetonitrile, the sample was sonicated for 30min followed by 10min of centrifugation. The supernatant was separated and further centrifuged for 30min. The supernatants were filtered and diluted 1000 times before LC/MS analysis. The tablet sample (multivitamin / weight loss support) was ground to fine powders. A certain amount of the powders that is supposed to contain 1mg of choline according to the labelled content were added with 100mL pure water and sonicated for 30min. The sample solution was filtered with 0.2µm syringe filter and diluted 100 times before injection onto LC/MS. The SIM chromatograms of the sample tested are shown in Figure 3. The quantitation results of choline in these dietary supplements are shown in Table 3.

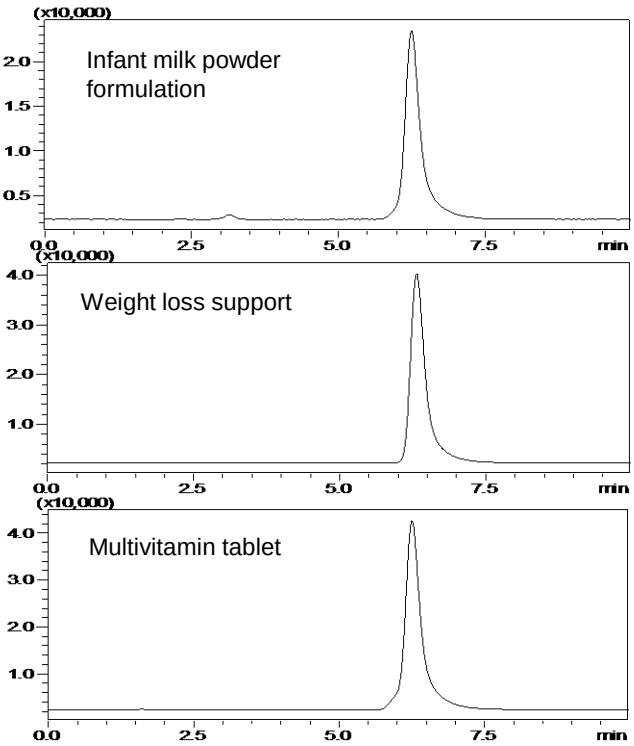

Figure 3: Chromatograms of choline in SIM mode (m/z 104.2) in three samples

Table 3: Quantitation results of choline in milk powder and dietary supplements samples

Sample	Content / tablet	Choline conc. (µg/L)	Result (µg/L)	% of labeled
Infant milk powder formulation	105mg/100g	52.5	57.1	108
Multivitamin tablet	25mg	100	111.8	111
Weight loss support	7.5mg	100	108.6	108

❑ Conclusions

A LC/MS SIM mode method was established for quantitative determination of choline in milk powder and dietary supplement samples. The method demonstrated high sensitivity (ppb level), excellent repeatability and selectivity, allowing its uses in analysis of choline in various food and dietary supplements.

❑ Reference:

1. Application Data Sheet, AD-0054 (http://www.shimadzu.com/an/literature/hplc/apl213035.html)

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