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Application Data Sheet

No. AD-0054

Prominence HPLC- ELSD

Quantitative Analysis of Choline in Multivitamin Tablet by HPLC with ELSD Detection

Choline is a dietary component essential for the normal function of human cells. The main functions of choline in biological process include fat and cholesterol transportation, energy metabolism, cell and nerve signaling, etc. Although choline is biosynthesized in human body, additional dietary intake is still required for maintaining human health. In 1998, the US Institute of Medicine's Food and Nutrition Board established dietary reference intake levels of 550 mg/day for male and 425 mg/day for female. It recommends higher intake for women during pregnancy (450 mg/day) and lactation (550 mg/day). Choline are present in a number of foods such as egg yolk, chicken, soybeans, peanuts etc. In addition, choline can be obtained from supplements like multivitamin tablet and milk powder formulation. Analytical methods of choline have been reported in open literature including HPLC methods with chemiluminescence detection and electrochemical detection [1-2], as well as LC/MS methods [3-4]. Here, we report a new method using HPLC with ELSD detector for quantitative analysis of choline in multivitamin tablet. A ZIC®-pHILIC column which is suitable for separation of polar and hydrophilic compounds was employed in this method.

❑ Analysis Conditions

Choline bitartrate standard was obtained from Sigma-Aldrich. A stock solution of choline bitartrate was prepared with the mobile phase (Table 1). Standard solutions of concentration of 20, 100, 200, 500 and 1000 µg/mL were prepared for the establishment of the standard calibration curve.

Sample solutions were prepared by grinding two multivitamin tablets into fine powders, transferred into a clean glassware and added with 50 mL of pure water. The samples were sonicated for 8 minutes at room temperature, followed by filtered with 0.45 µm syringe filters. The clear sample solutions were further diluted before HPLC analysis.

Table 1: HPLC conditions for analysis of choline

System	Prominence HPLC (Shimadzu)
Mobile Phase	A: 5 mM ammonium acetate B: Acetonitrile A:B = 40:60, v/v
Flow Rate	1.0 mL/min
Column	Merck SeQuant®ZIC®-pHILIC, 150 x 4.6 mm
Column Temp	35 ºC
Detector	ELSD-LT II
ELSD Condition	Temp 30 ºC, pressure 350 KPa, gain 6
Injection Vol	20 µL

❑ Calibration Curve of Choline

Choline bitartrate standards of different concentrations were analyzed by HPLC with ELSD-LT II detector. The retention time of choline peak was found at 12.5 min (see Figure 1). This peak was further confirmed by injecting choline chloride and sodium chloride into HPLC under the same running condition. Comparing the LC chromatograms of these standards, the peak at 12.5 min was confirmed to be choline component.

A calibration curve of choline was established with the calibrants prepared. A linear calibration curve of Ln (area) and Ln (conc) was obtained with the R^2 of 0.9972 as shown in Figure 2.

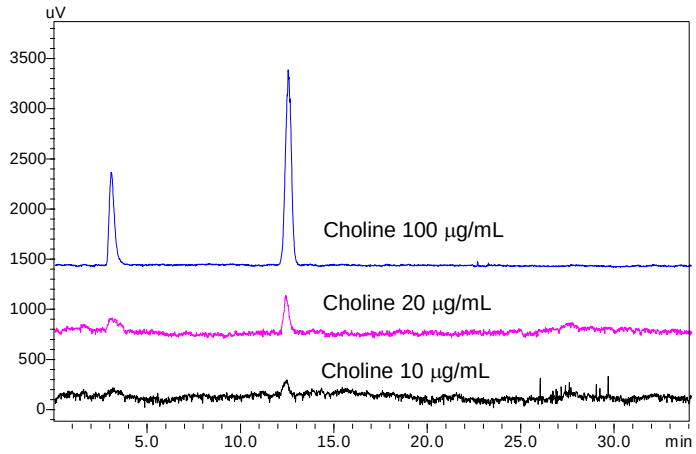

Fig 1: Chromatographic peaks of choline detected by ELSD.

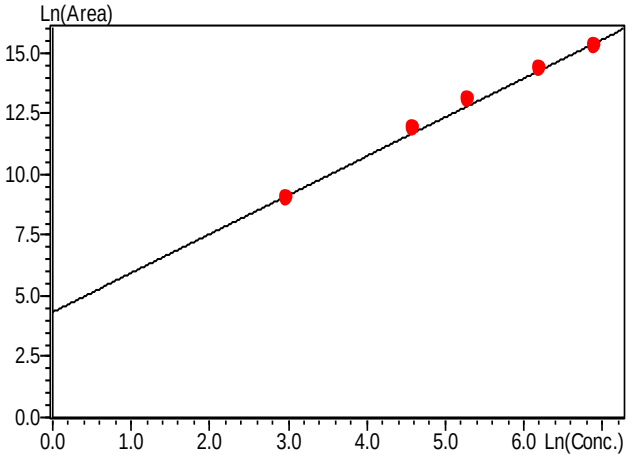

Fig 2: Exponential calibration curve of choline standard for concentrations of 20, 100, 200, 500, 1000 µg/mL.

❑ Results of Multivitamin Tablets

The established HPLC-ELSD method was applied to analysis of choline in the multivitamin tablet samples. The quantitation results of four preparations are shown in table 2. The accuracy of choline content of the four preparations was at 102-105%. The limit of detection (LOD) of the method is 3.6 µg/mL.

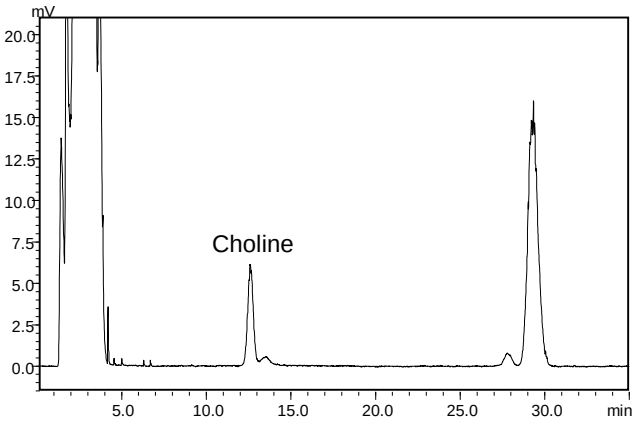

Fig 3: Chromatographic peaks of multivitamin tablet sample

Table 2: Quantitation result of choline in the multivitamin tablet sample by HPLC-ELSD

Tablet sample	Spec of choline bitartrate in tablet (mg)	Quantitation result (mg)	Accuracy (%)
1	25.0	25.56	102.2
2	25.0	26.34	105.3
3	25.0	25.71	102.8
4	25.0	26.21	104.9

❑ Summary

A new HPLC-ELSD method for quantitative determination of choline in multivitamin tablet was established on Prominence HPLC system using ELSD-LT II detector.

❑ Reference

1. P. Van Zoonen, C. Gooijer, N.H. Velthorst, R.W. Frei, Journal of Pharmaceutical and Biomedical Analysis, vol 5 (5), 485-492 (1987)
2. A. Shen, A.G.W. Murray, and F. Mitchelson, Journal of Pharmacological and Toxicological Methods, 34, 215-218 (1995)
3. Bruce SJ, Guy PA, Rezzi S, Ross AB, J Agric Food Chem.,58(4), 2055-61, Feb 24 (2010)
4. Yuan-Yuan Zhao, Yeping Xiong, Jonathan M. Curtis, Journal of Chromatography A, 5470-5479, 1218 (2011)

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