

# Determination of Choline and Its Metabolites Using an LTQ Linear Ion Trap Mass Spectrometer

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## Key Words

- LTQ™
- Lipids
- Metabolites
- Sensitivity
- Quantitation

## Introduction

Development of the linear ion trap with increased ion storage capacity enables quantitative analysis to be performed with greater accuracy and precision than possible with 3D ion traps. This is demonstrated with the Thermo Scientific LTQ and its major *in vivo* metabolites in mouse liver. Choline is a component of many foods and serves as a precursor for a variety of compounds including acetylcholine, betaine and phospholipids. Phospholipids are critical for normal cell membrane structure and lipoprotein function.<sup>(1)</sup> Acetylcholine is an important neurotransmitter,<sup>(2)</sup> whereas betaine is used by the kidney to maintain water balance,<sup>(3)</sup> and by the liver as a source of methyl groups for methionine formation.<sup>(4)</sup> If deprived of choline, cells die of apoptosis<sup>(5-7)</sup> and liver dysfunctions develop in humans.<sup>(8)</sup> While cholines have long been recognized as essential in several animals, a dietary recommendation for humans was only recently made by the U.S. National Academy of Sciences.<sup>(9)</sup>

## Goal

- 1) Demonstrate the accuracy and precision for quantitation using the LTQ.
- 2) Determine the limits of detection (LOD) and quantitation (LOQ) for this method on the LTQ.
- 3) Determine the sensitivity and dynamic range for full-scan LC-MS/MS analysis of choline and its metabolites using the LTQ.

## Experimental Conditions

### HPLC

HPLC System: Thermo Scientific Surveyor™ MS pump with Surveyor autosampler  
Column: 20×2.1 mm ID packed with 5 µm silica particles  
Injection volume: 10 µL  
Mobile phase: A: Acetonitrile/water/ethanol/1M ammonium acetate/glacial acetic acid (800/127/68/3/2 v/v)  
B: Acetonitrile/water/ethanol/1M ammonium acetate/glacial acetic acid (500/500/85/27/18 v/v)

## Gradient

Time	% A	% B	Flow Rate (mL/min)
0	100	0	0.4
5.5	40	60	0.3
7.5	0	100	0.3
10.5	0	100	0.4
11–17	100	0	0.5

## Mass Spectrometer

Mass spectrometer: LTQ linear ion trap mass spectrometer  
Ionization mode: Positive electrospray ionization (ESI)  
Capillary temperature: 310 °C  
Spray voltage: 4.5 kV  
Sheath gas: On  
Sweep gas: On

## Standards

Choline chloride, betaine, acetylcholine chloride, phosphocholine, glycerophosphocholine, and cytidine diphosphocholine (CDP choline) were obtained from Sigma Chemicals (St. Louis, MO). Choline-[N,N,N-trimethyl-d9] chloride was used as an internal standard for generation of calibration curves and was obtained from Cambridge Isotope Laboratories (Andover, MA).

## Liver extracts

Choline and its metabolites were extracted from mouse liver and partitioned into aqueous and organic phases using the method described by Koc et al.<sup>(10)</sup> The aqueous phase fraction contains choline, betaine, acetylcholine, phosphocholine, glycerophosphocholine and cytidine diphosphocholine. The individual fractions were spiked with respective deuterated internal standards and analyzed by normal phase chromatography using a 4.1 mm×150 mm column packed with 5 µm silica particles.

## Results and Discussions

### Full-Scan LC-MS/MS Analysis

Figure 1 shows extracted ion chromatograms obtained from normal phase chromatography with full-scan MS/MS analysis of acetylcholine, betaine and choline, whereas Figure 2 shows chromatograms for the analysis of phosphocholine, glycerophosphocholine and CDP-choline. The MS/MS spectra of these closely eluting and co-eluting choline compounds are also shown in the figures. Table 1 lists the product ions used for obtaining the extracted ion chromatograms.

### Quantitation and Linear Dynamic Range

Full-scan MS/MS analysis using the LTQ yielded a linear dynamic range of more than five orders of magnitude for standards prepared in aqueous solution. The standard calibration curve for acetylcholine (Figure 3) shows linearity over the range from 50 fmol–10 nmol, whereas glycerophosphocholine (Figure 4) demonstrates linearity from 100 fmol -10 nmol. The correlation coefficients for acetylcholine and glycerophosphocholine are 0.9996 and 0.9990, respectively. Tables 2 and 3 show the within-day assay precision from three replicate injections for these analytes. The coefficient of variation (%CV) for glycerophosphocholine is less than 6% over the entire linear dynamic range, and for acetylcholine is less than 8%, except near LOQ where the (%CV) is 11.6%.

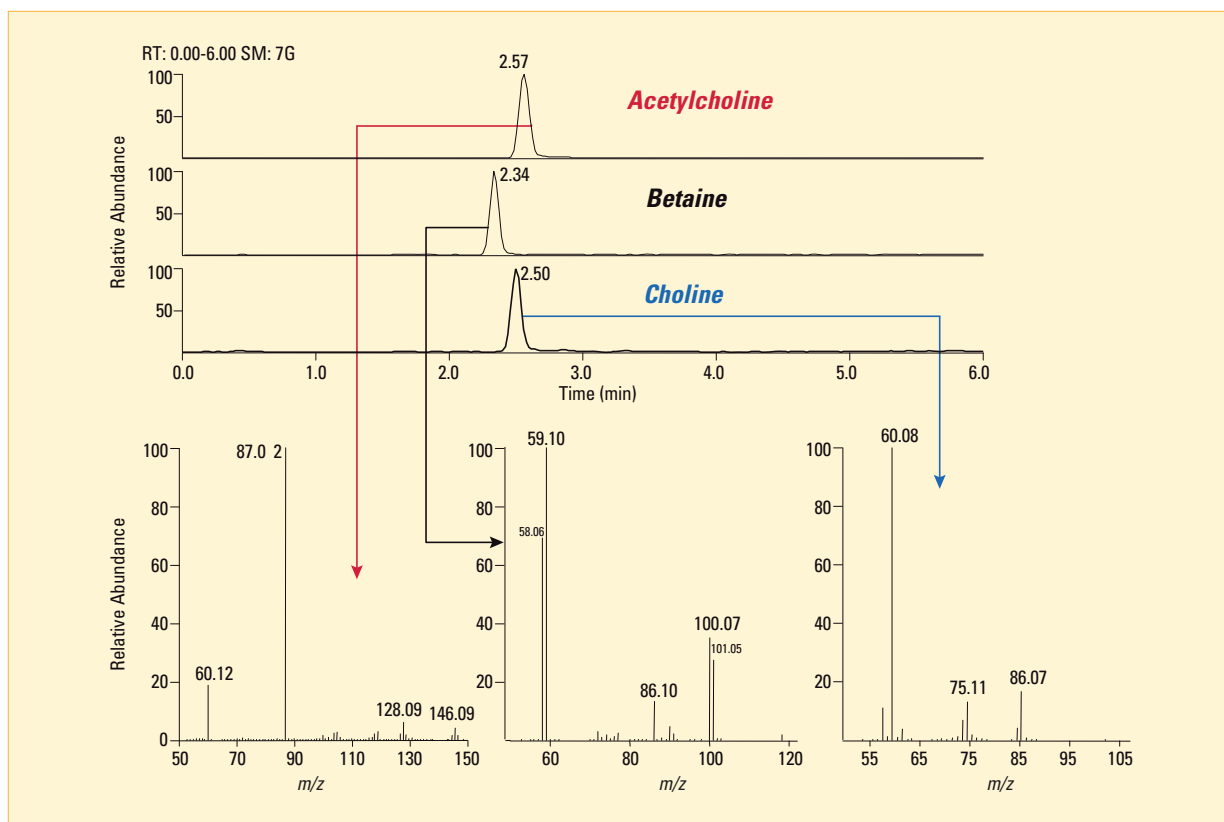


Figure 1: Extracted ion chromatograms and MS/MS spectra for acetylcholine, betaine, and choline

Analyte	MH+	Normalized Collision Energy™ %	Product ions
Betaine	118	40	58 and 59
Choline	104	40	60
Acetylcholine	146	32	87
Phosphocholine	184	32	86
Glycerophosphocholine	258	27	104
CDP choline	489	27	401

Table 1: Normalized collision energy and product ions used for analysis of choline and its metabolites

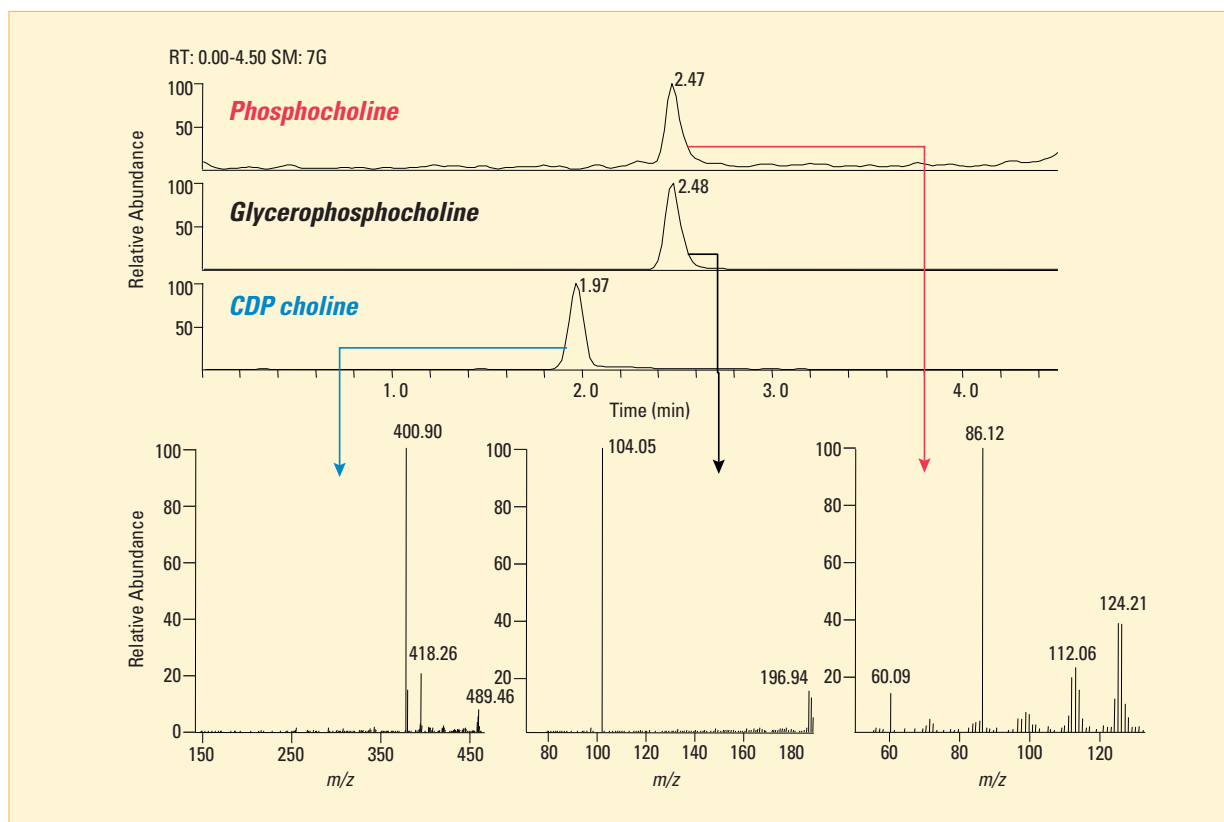


Figure 2: Extracted ion chromatogram and MS/MS spectra for phosphocholine, glycerophosphocholine and CDP choline

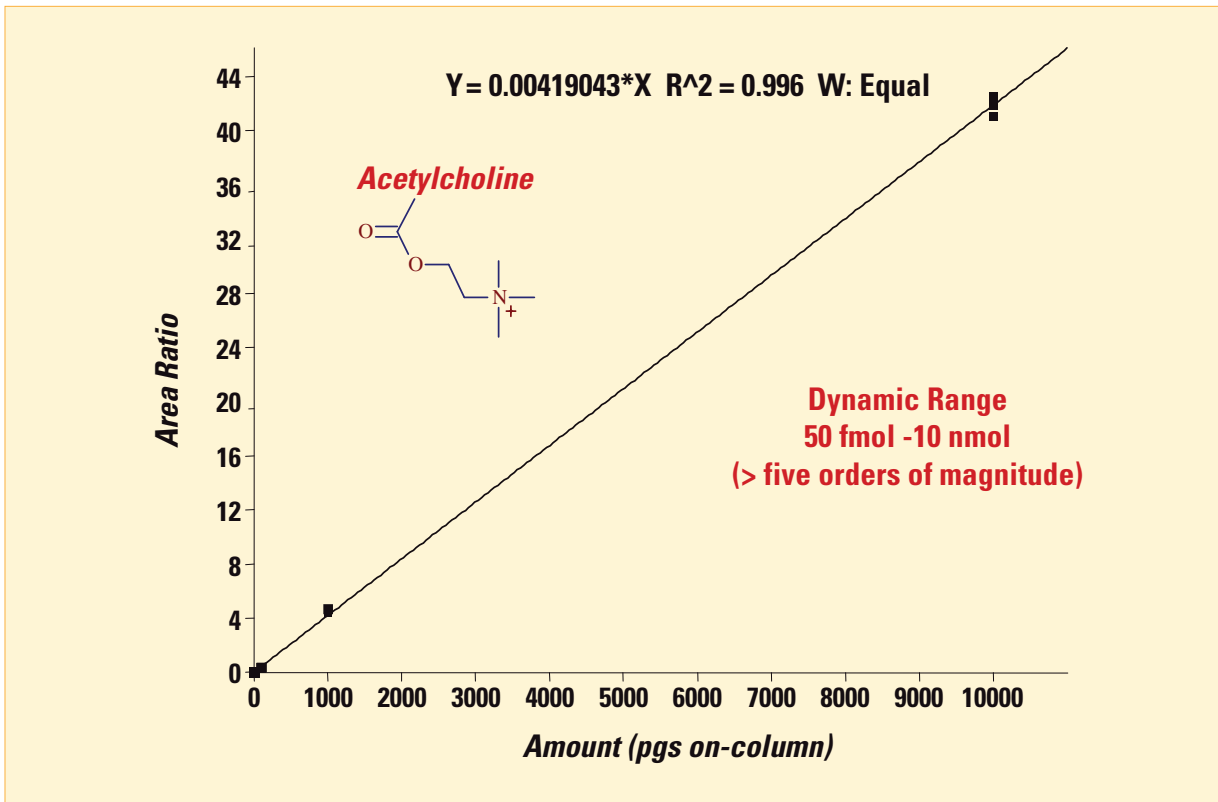


Figure 3: Calibration curve for acetylcholine in aqueous solution

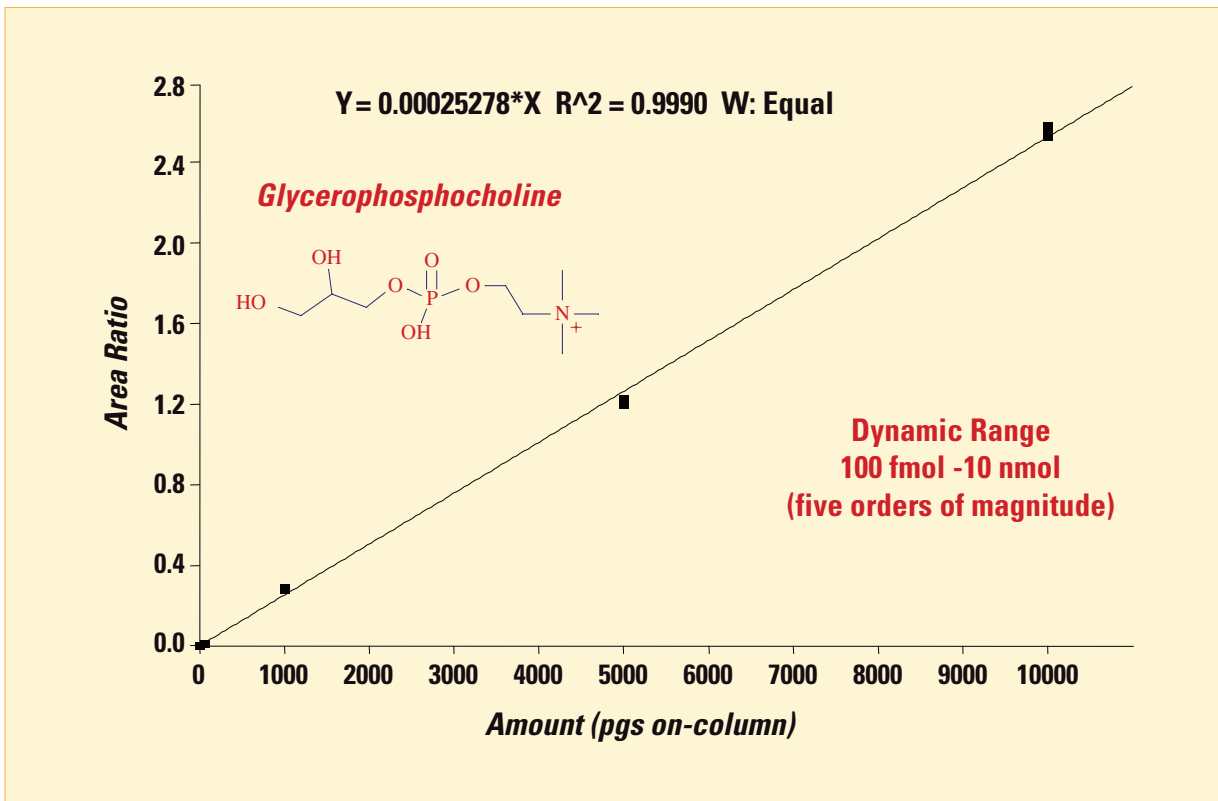


Figure 4: Calibration curve for glycerophosphocholine in aqueous solution

### Limit of Detection and Quantitation

Figure 5 shows extracted ion chromatograms at the limit of detection (LOD) for assay of acetylcholine and glycerophosphocholine in full-scan MS/MS mode. The LOD for acetylcholine was determined to be 25 fmol (on-column), with a signal to noise ratio of 9:1, whereas that for glycerophosphocholine was determined to be 50 fmol (on-column) with a signal to noise of 13:1. Table 4 summarizes the LOD and LOQ values (on-column) for acetylcholine and glycerophosphocholine, and demonstrates these analytes can be detected at low levels using the LTQ.

Amount on column	% CV
100 fmol	3.95
500 fmol	5.63
5 pmol	2.14
50 pmol	3.88
100 pmol	3.09
1 nmol	2.74
5 nmol	1.24
10 nmol	1.12

Table 2: Within day precision (from three replicate injections) for assay of glycerophosphocholine in aqueous solution

### Determination of choline compounds in mouse liver extracts

Figure 6 shows MS/MS quantitation of betaine, choline and glycerophosphocholine in pooled mouse liver extract. The sample was spiked with 20 nmoles of the respective deuterated compounds. The concentration of betaine, choline and glycerophosphocholine were determined to be 47.9 nmol, 6.2 nmol and 13.8 nmol, respectively.

Amount on column	% CV
50 fmol	11.6
100 fmol	7.56
1 pmol	5.02
10 pmol	1.32
100 pmol	1.85
1 nmol	2.03
10 nmol	1.82

Table 3: Within day precision (from three replicate injections) for assay of acetylcholine in aqueous solution

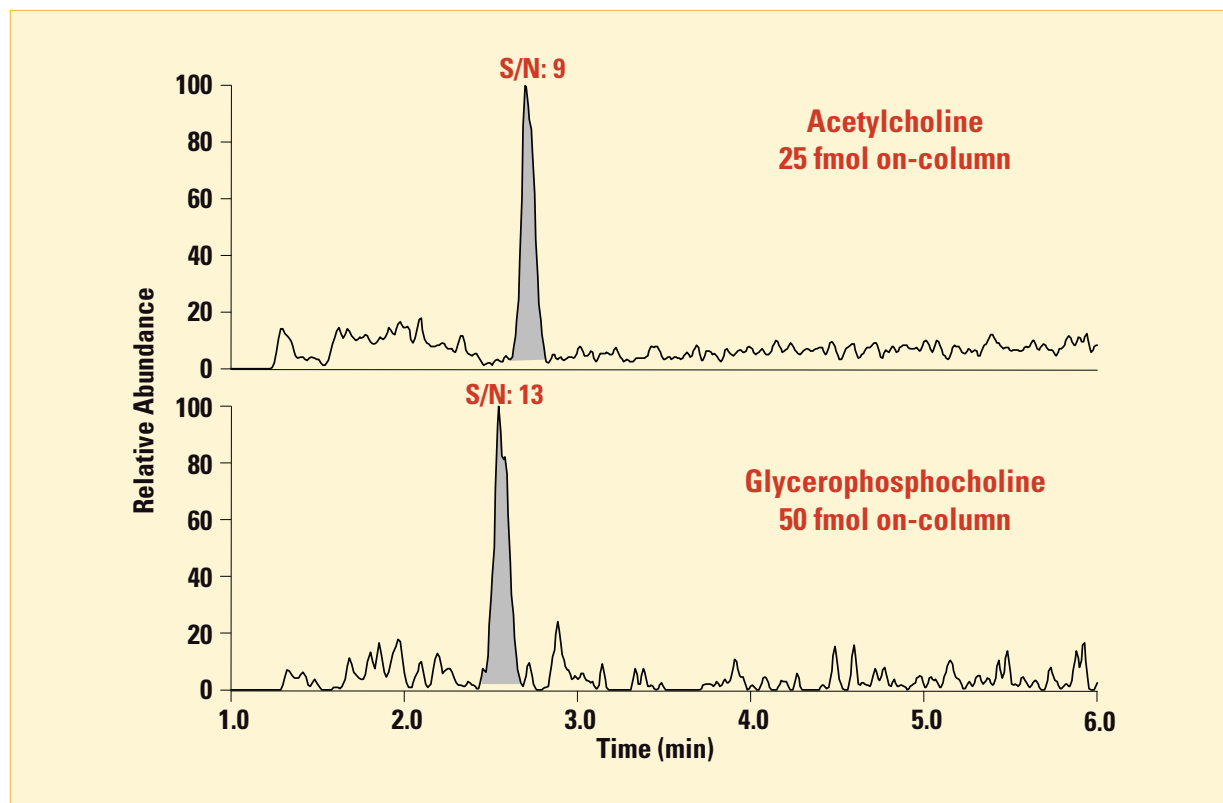


Figure 5. Extracted ion chromatograms for acetylcholine and glycerophosphocholine at the LOD in water

Analyte	LOD (on-column)	LOQ (on-column)
Acetylcholine	25 fmol	50 fmol
Glycerophosphocholine	50 fmol	100 fmol

Table 4: Limit of Detection (LOD) and Quantitation (LOQ) for acetylcholine and glycerophosphocholine in aqueous solution

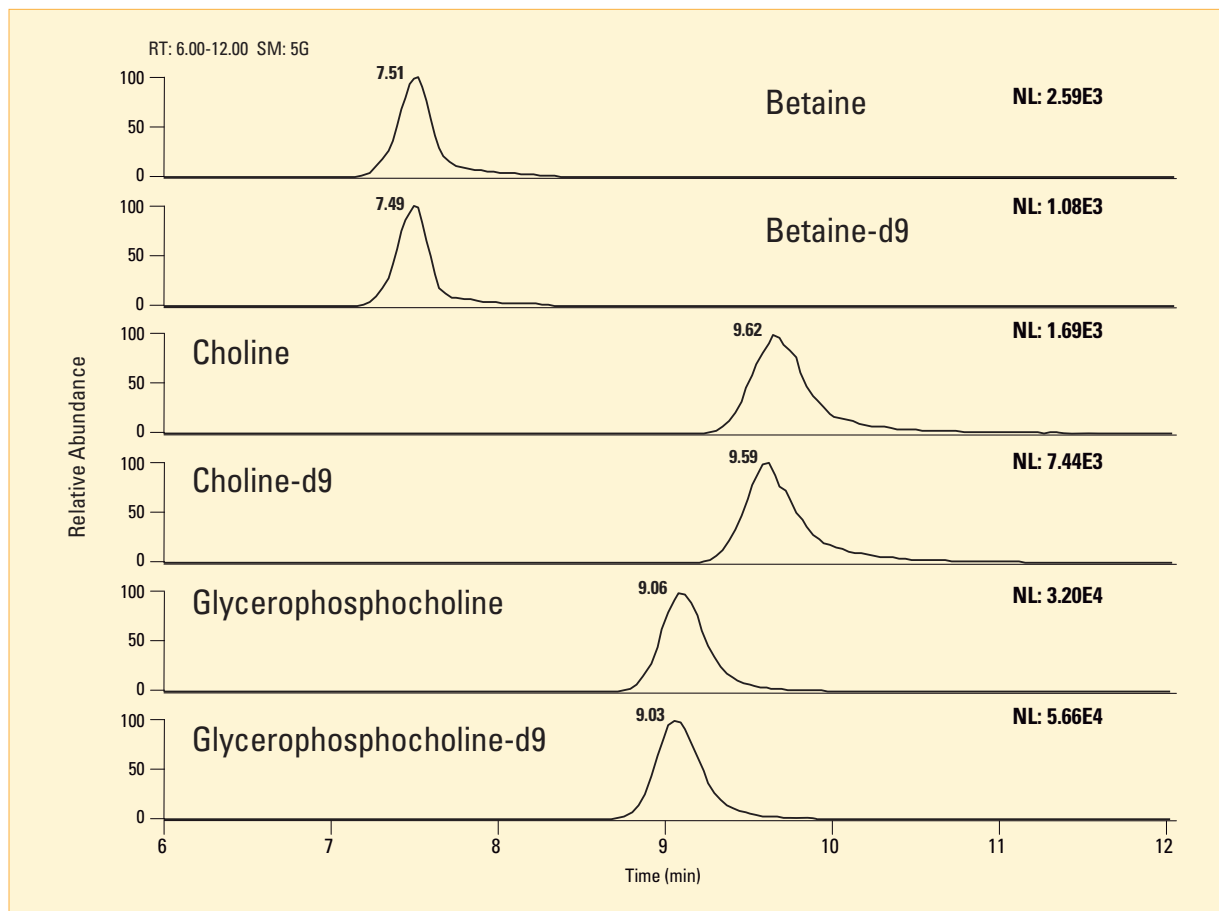


Figure 6: Extracted ion chromatograms for betaine, choline and glycerophosphocholine obtained from pooled mouse liver extract. (Internal standard spiked at 20 nmol)

## Conclusions

Full-scan MS/MS quantitative analysis using the LTQ linear ion trap mass spectrometer provides excellent selectivity and sensitivity for the determination of choline and its *in vivo* metabolites in mouse liver extracts. Accurate quantitation of choline compounds with a linear dynamic range of over five orders of magnitude was achieved using the LTQ. Quantitative assays performed in full-scan MS/MS mode provide the added benefit of structural information resulting in more reliable compound identification and quantitation.

## References

- 1) Zeisel, S.H. *Nutrition* 2000, 16, 669-671.
- 2) Burg, M. *Am. J. Physiol.* 1995, 268, F983-F996
- 3) Dudman, N.; Guo, X.; Gordon E.; Dawson, P.; Wilcken D. *J. Nutr.* 1996, 126, 1295S-1300S
- 4) Haubrich, D.R.; Wang, P. F.; Herman, R.L.; Clody, D.E., *Life Sci.* 1975, 17, 739-747
- 5) Holmes-McNary, M.Q.; Loy, R.; Mar, M.H.; Albright, C.D.; Zeisel, S.H. *Dev. Brain Res.* 1997, 101, 9-16.
- 6) Yen, C.L.; Mar, M.H.; Zeisel, S.H. *FASEB J.* 1999, 13, 135-142
- 7) Albright, C.D.; Salganik, R.I.; Kaufmann, W.K.; Vrablic, A.S.; Zeisel, S.H. *J. Nutr. Biochem.* 1998, 9, 476-481
- 8) Zeisel, S.H.; DaCosta, K.A.; Franklin, P.D.; Alexander, E.A.; Lamont, J.T.; Sheard, N.F.; Beiser, A. *FASEB J.* 1991, 5, 2093-2098
- 9) Institute of Medicine: National Academy of Sciences U.S.A. Dietary reference intakes of folate thiamin riboflavin, niacin, vitamin B12, pantothenic acid, biotin, and choline; National Academy press: Washington, DC 1998
- 10) Koc, H.; Mar, M.H.; Ranasinghe, A.; Swenberg, J.A.; Zeisel, S.H.; *Anal. Chem.* 2002, 74, 4734-4740.

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