

# Analysis of Choline and its Metabolites on the Finnigan LTQ

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# Goal:

What we set out to demonstrate ...

- The applicability of the Finnigan LTQ to a quantitative method previously established on the LCQ Advantage
- The sensitivity and dynamic range of choline and its metabolites on the Finnigan LTQ
- The precision and quantitative characterization of the LTQ
- The limits of detection (LOD) and quantitation (LOQ) for this method on the LTQ

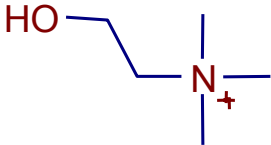
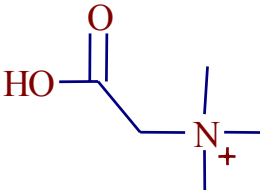
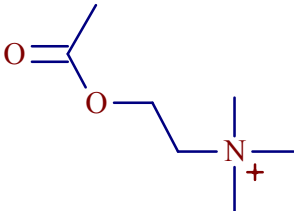
## Background:

# What's the interest in cholines and its metabolites?

## Choline

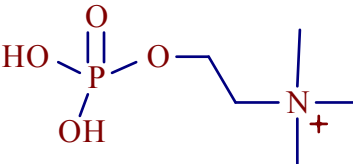
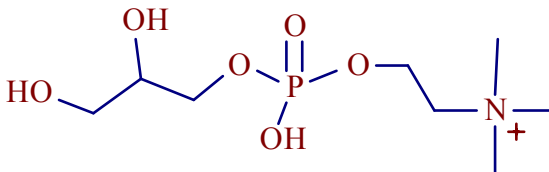
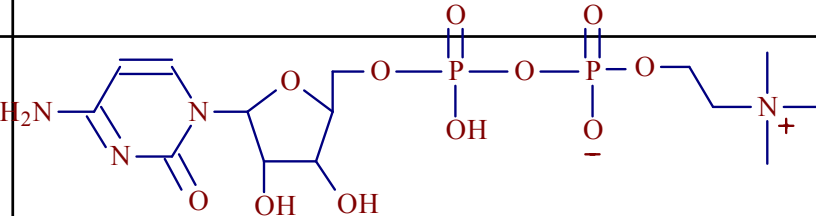
- Part of several major phospholipids that are critical for normal cell membrane structure and lipoprotein function (1).
- A precursor of betaine, which is used by kidney to maintain water balance (2), and by liver as a source of methyl groups for methionine formation (3).
- Also used to produce acetylcholine, an important neurotransmitter (4).
- If deprived of choline, cells die of apoptosis (5-7) and humans develop liver dysfunctions (8).
- While the essentiality of cholines in several animals has long been recognized, a dietary recommendation for humans was only recently recommended by the US National Academy of Sciences (9).

# Choline Compounds

Analyte	Structure	MH+	Normalized Collision energy	Product ions
<i>Betaine</i>		118	40	58 and 59
<i>Choline</i>		104	40	60
<i>Acetylcholine</i>		146	32	87

Generated with Mass Frontier software

# Choline Compounds (2)

Analyte	Structure	MH+	Normalized Collision energy	Product ions
<b>Phosphocholine</b>		184	32	86
<b>Glycerophosphocholine</b>		258	27	104
<b>CDP choline</b>		489	27	401

Generated with Mass Frontier software

# LC Conditions

## Surveyor MS Pump and Surveyor Autosampler

### – *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)

### – *Column*

20 x 2.1 mm id packed with 5  $\mu$ m Silica particles

### – *Injection volume*: 10 $\mu$ L

– *Gradient*: At 100% B for 0.5 min and 400  $\mu$ L/min; to 60% B in 5.0 min at 400  $\mu$ L/min; to 100% B in 2 min at 300  $\mu$ L/min at 100% B for 3 min at 400  $\mu$ L/min to 100% A in 1 min at 500  $\mu$ L/min at 100% A for 6 min at 500  $\mu$ L/min

Electrospray, positive ionization

Capillary temperature 310 C

Spray voltage 4.5 kV

Sheath gas 50

Aux gas 10



# LC/MS/MS Analysis of 3 Cholines

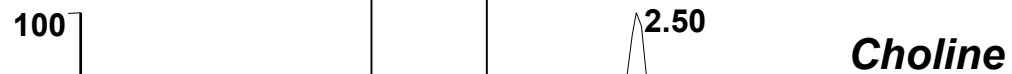
RT: 0.00 - 6.00 SM: 7G



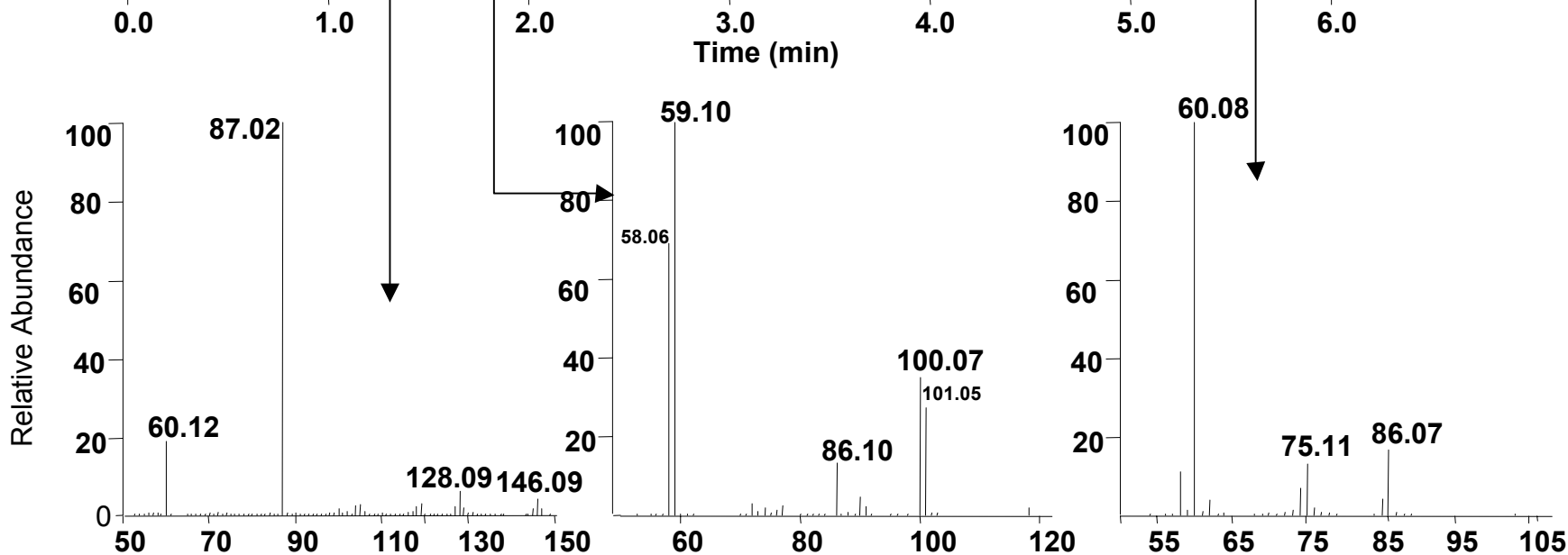
Base Peak m/z= 86.50-87.50  
F: ITMS + c ESI Full ms2  
146.00@32.00 [50.00-150.00]  
MS Genesis new\_sample\_16



Base Peak m/z=57.50-58.50+58.50-59.50  
F: ITMS + c ESI Full ms2  
118.10@40.00 [50.00-122.00] MS  
Genesis new\_sample\_16

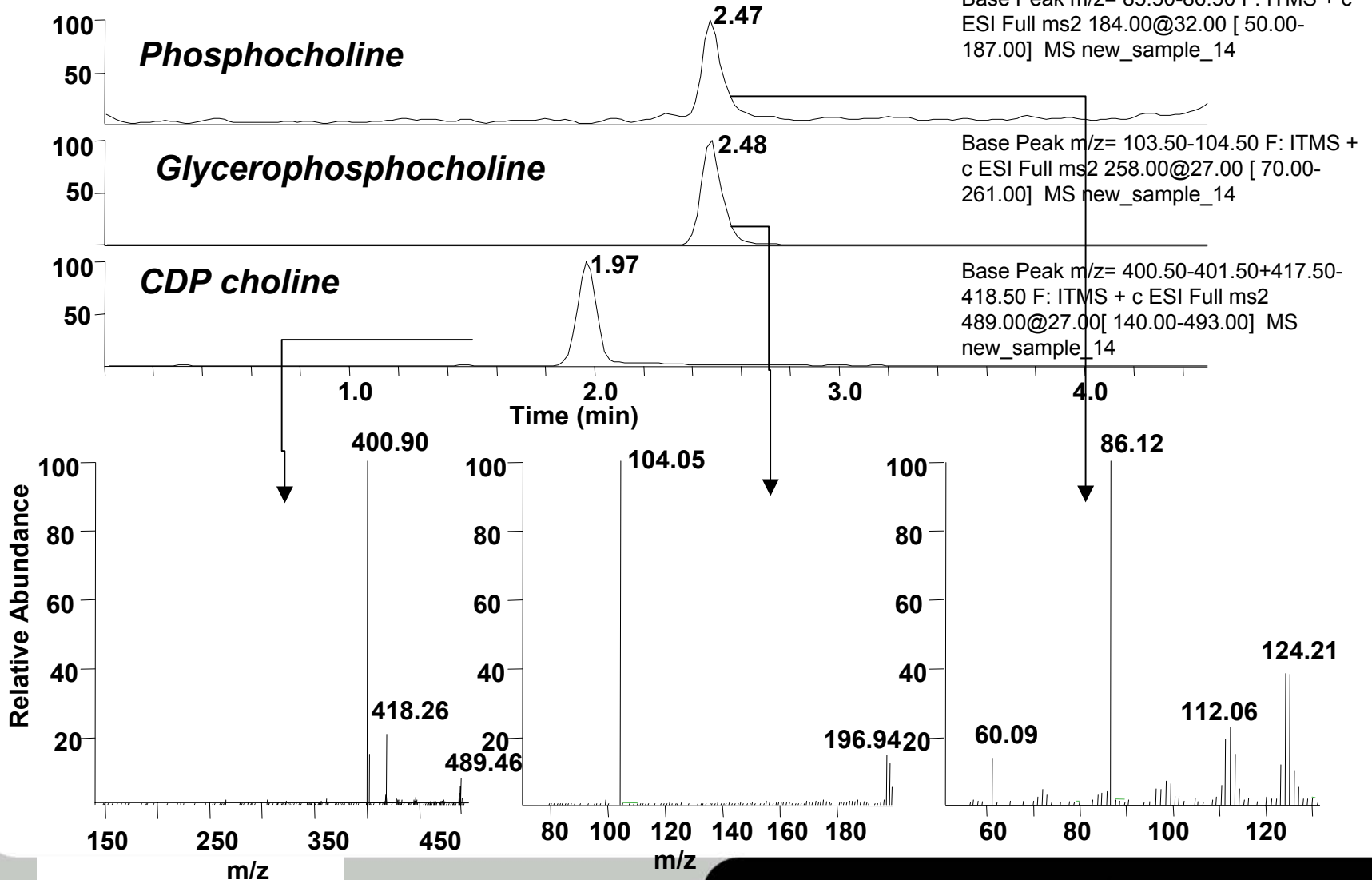


Base Peak m/z= 59.50-60.50 F:  
ITMS + c ESI Full ms2  
104.10@42.00 [50.00-108.00]  
MS new\_sample\_16

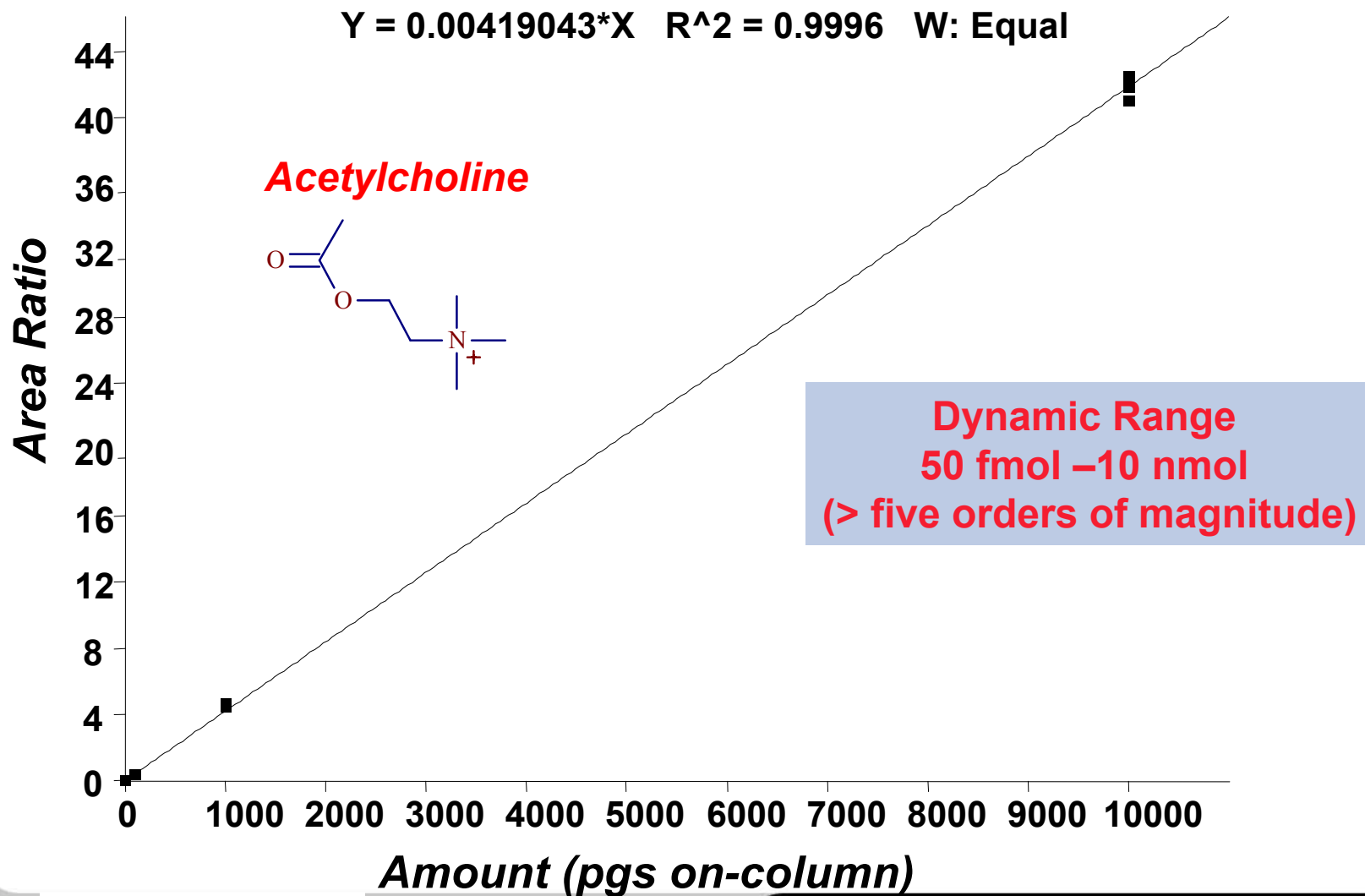


# LC/MS/MS of 3 Cholines

RT: 0.00 - 4.50 SM: 7G



# Calibration Curve for Acetylcholine

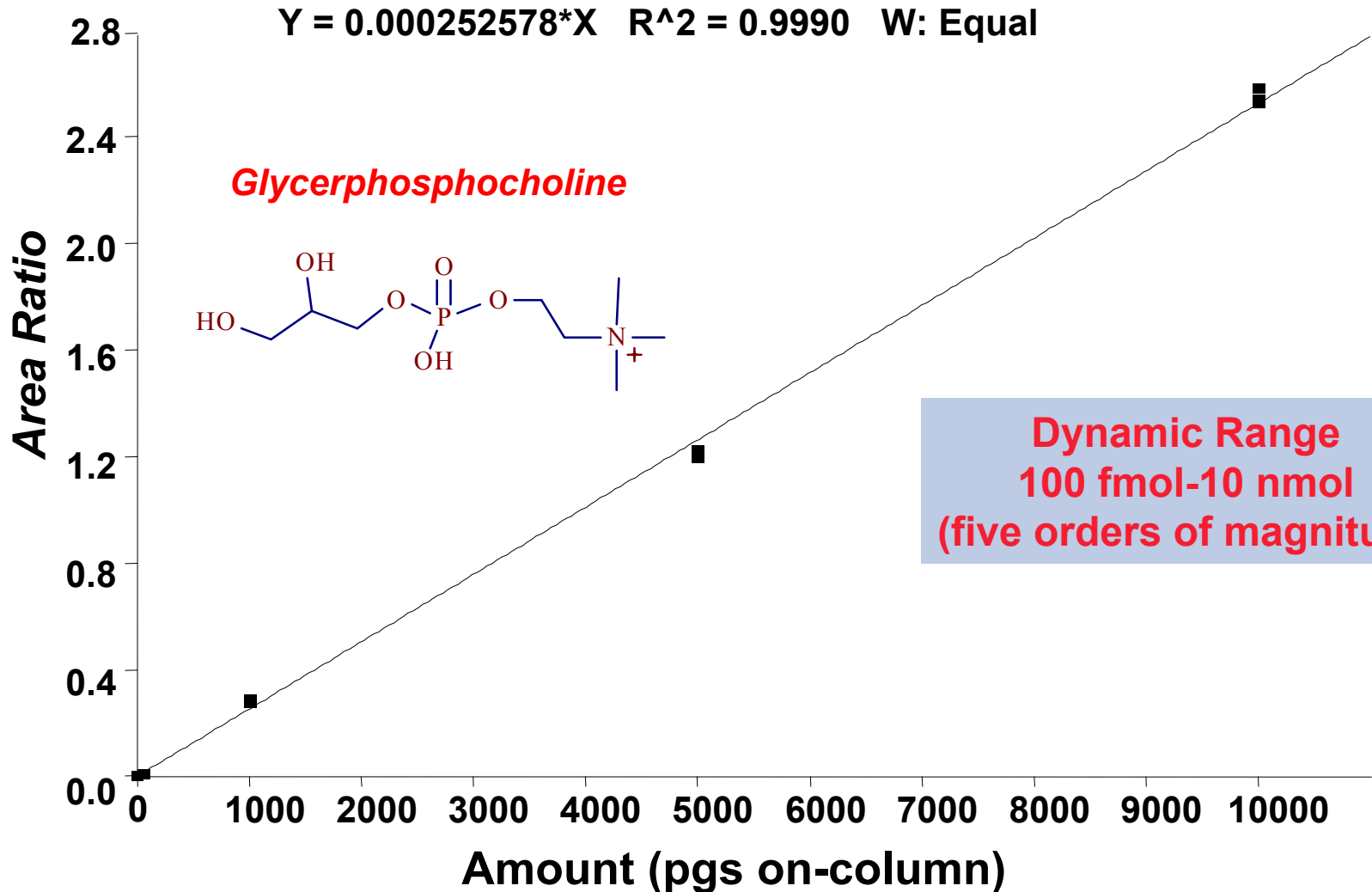


# Same-Day Assay Precision for Acetylcholine

<b>Amount on column</b>	<b>% CV</b>
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

\* For three injections

# Calibration Curve for Glycerophosphocholine

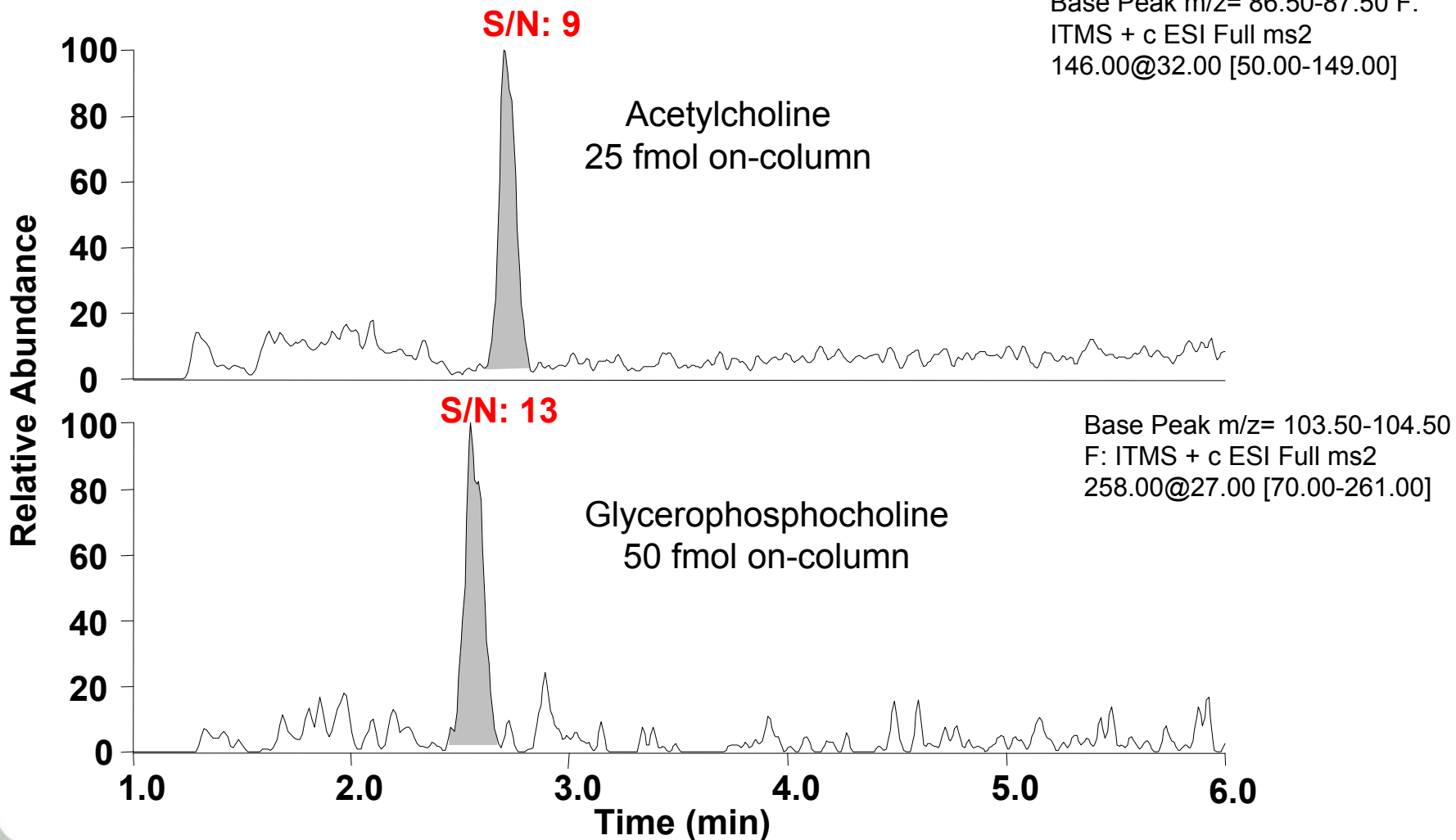


# Same-Day Assay Precision for Glycerophosphocholine

<b>Amount on column</b>	<b>% CV</b>
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

\* For three injections

# LOD for Acetylcholine and Glycerphosphocholine



# LOD\* and LOQ\*

<b>Analyte</b>	<b>LOD (on-column)</b>	<b>LOQ (on-column)</b>
Acetylcholine	25 fmol	50 fmol
Glycerophosphocholine	50 fmol	100 fmol

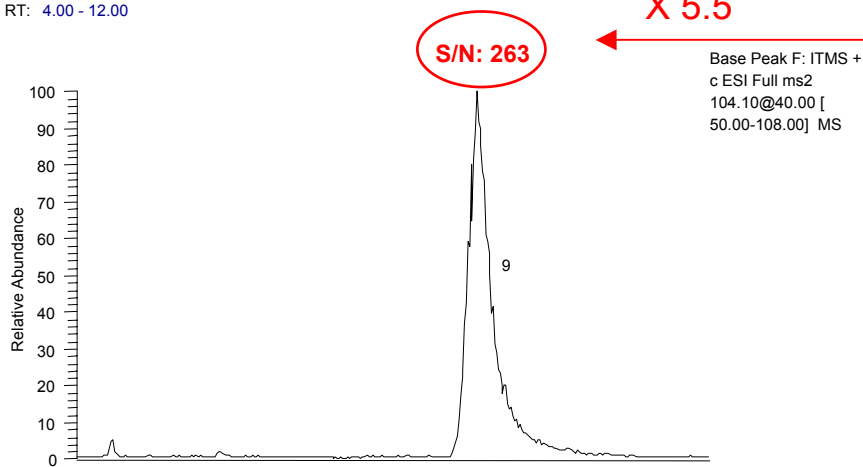
\* In water

# Choline and Betaine from Pooled Liver Extract S:N Comparison

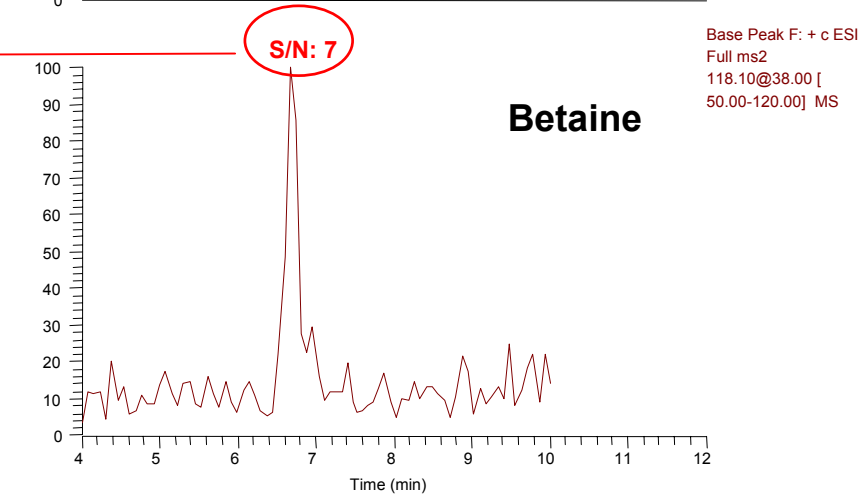
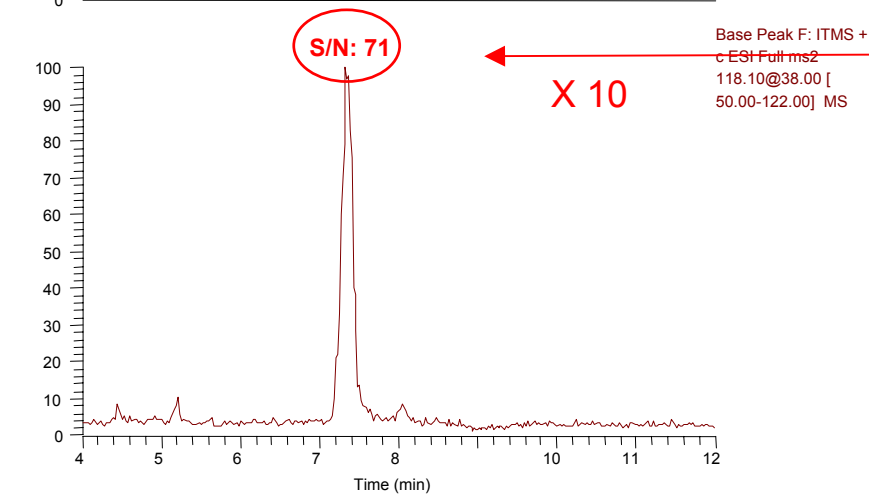
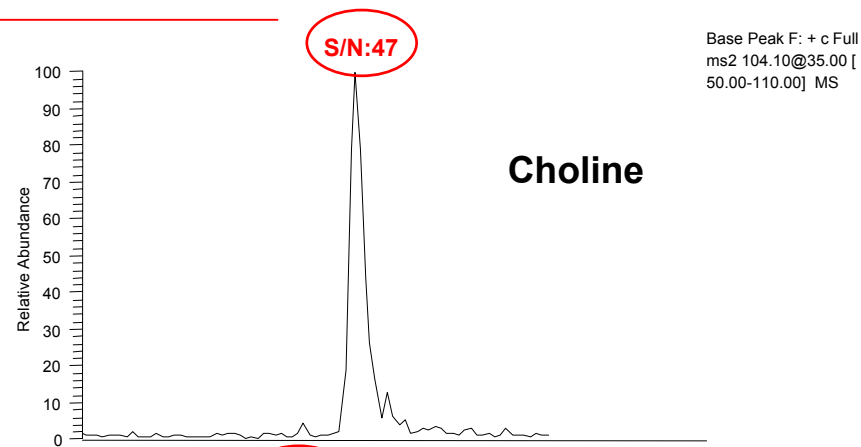
## Finnigan LTQ

## Finnigan LCQ Advantage

RT: 4.00 - 12.00



RT: 4.00 - 12.00

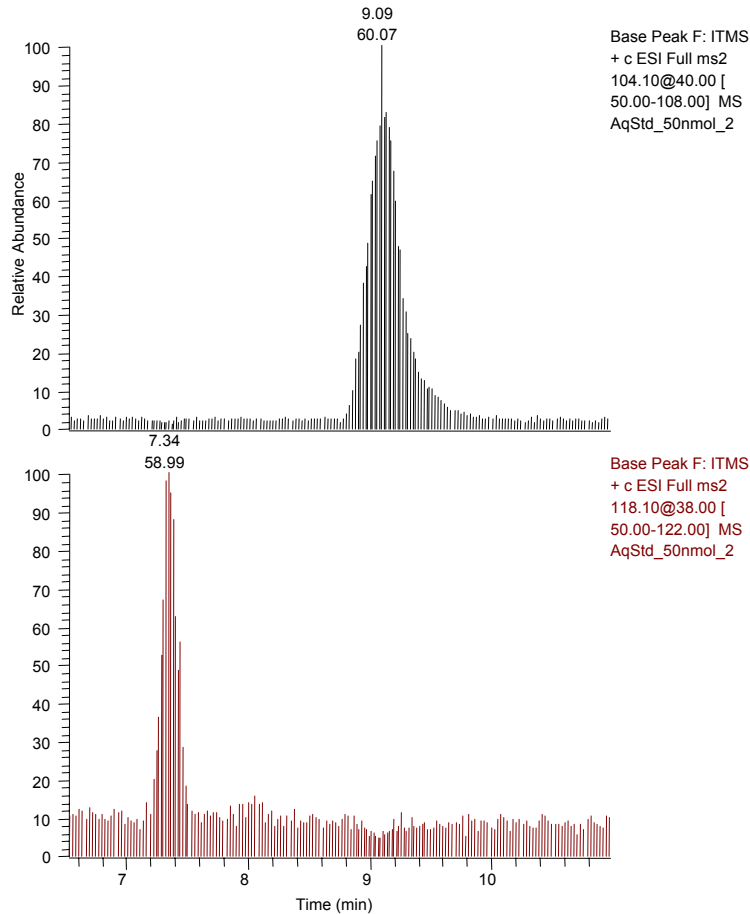


X 5.5

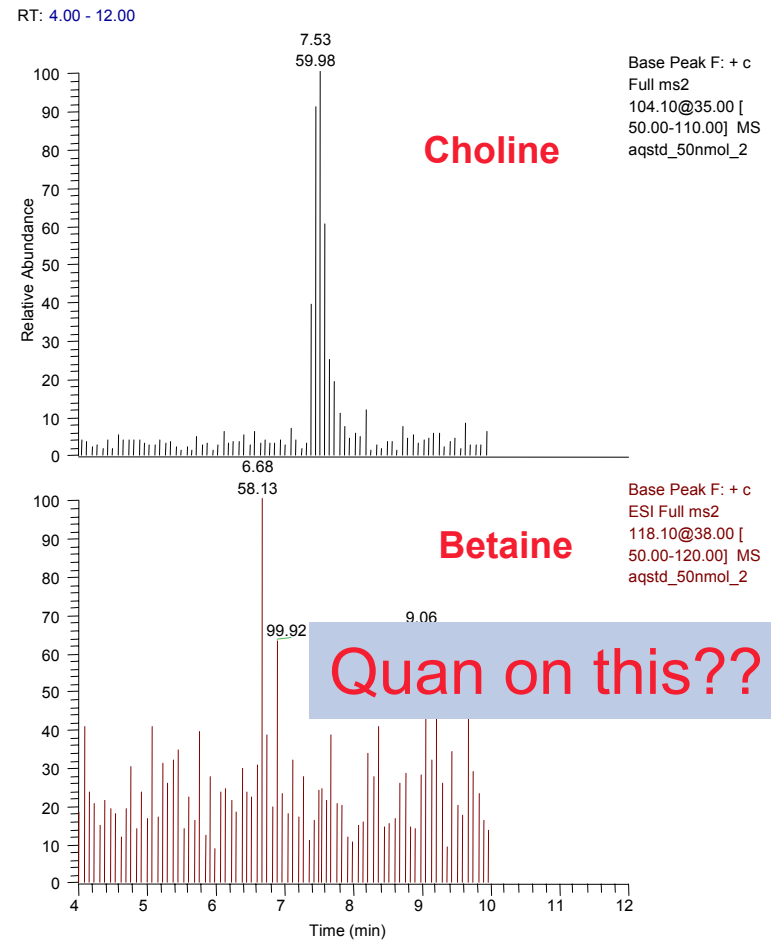
X 10

# Choline and Betaine from Pooled Liver Extract # Scans Across the LC Peak

## Finnigan LTQ



## Finnigan LCQ Advantage



# Conclusions

- Full scan MS/MS analysis with the Finnigan LTQ linear ion trap mass spectrometer provides the selectivity and sensitivity for the analysis of choline compounds
- Accurate quantitation of choline compounds with a linear dynamic range over five orders of magnitude is achieved on the Finnigan LTQ.
- The Finnigan LTQ provides excellent within-day assay precision for the analysis of choline compounds. The CV for glycerophosphocholine is less than 6% for the entire linear dynamic range. The CV for acetylcholine is less than 8% except near LOQ where the CV is 11.6%.
- The limit of quantitation is determined from the calibration curves as 50 fmol for acetylcholine and 100 fmol for glycerophosphocholine.
- The LOD for acetylcholine is determined to be 25 fmol (on-column) with a signal to noise ratio of 9:1, whereas that for glycerophosphocholine is determined to be 50 fmol (on-column) with a signal to noise of 13:1

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- Rohan Thakur

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