MS/MS APPROACHES TO CLINICAL TESTING FOR INBORN ERRORS OF METABOLISM

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Presented at the 2011 Stanford Mass Spectrometry Users' Meeting

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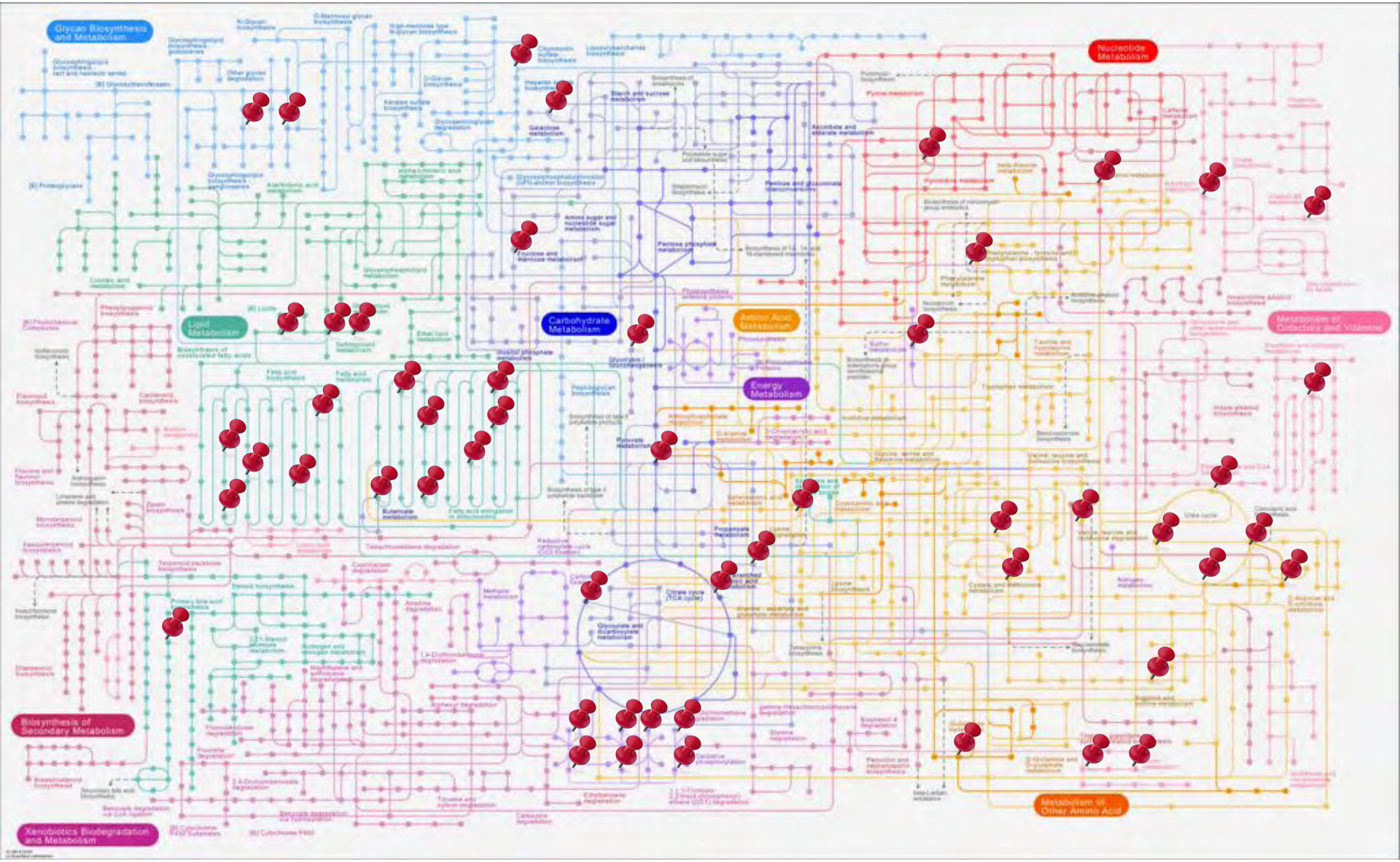
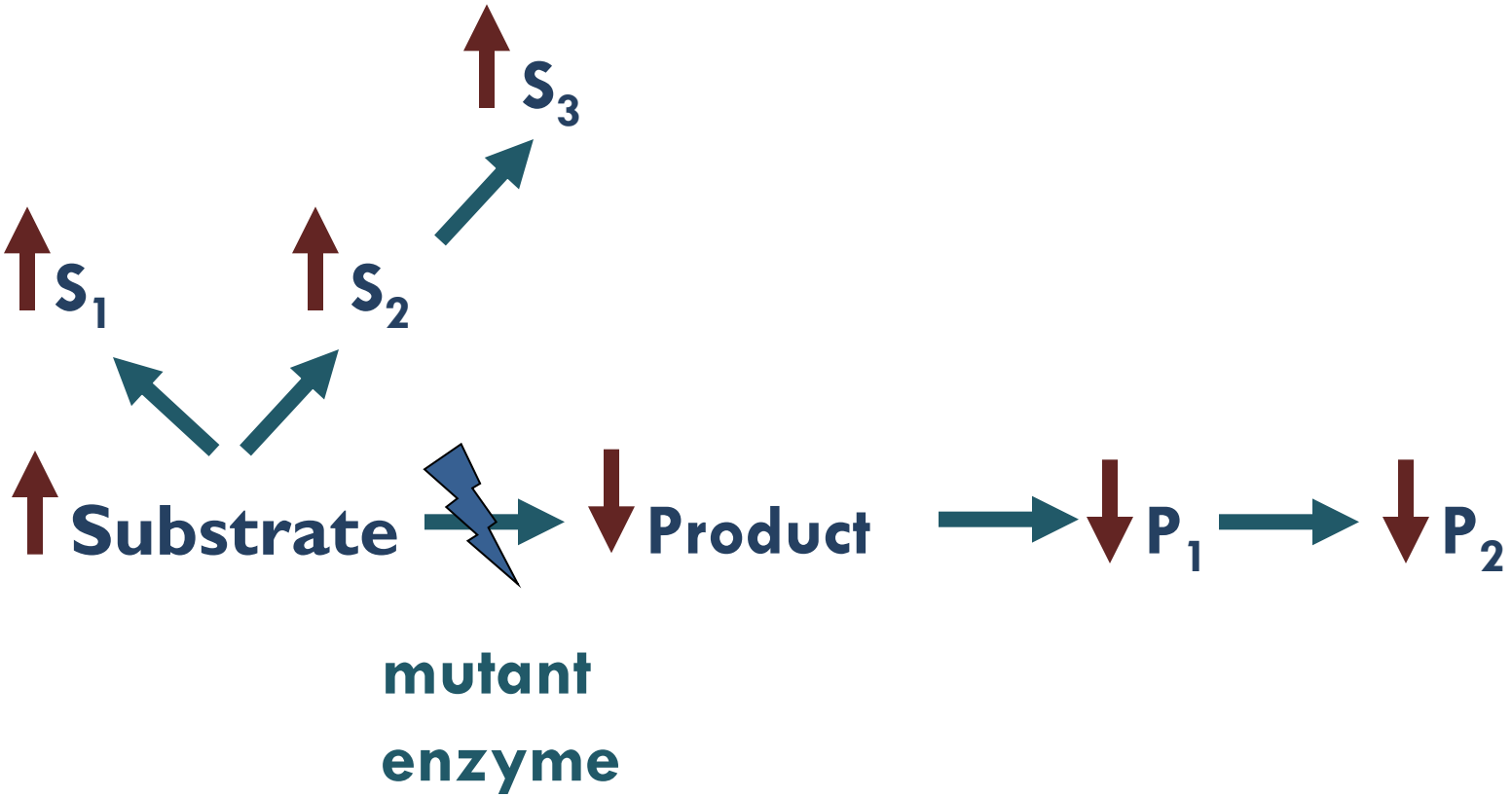
Metabolic Pathways



http://www.genome.jp/kegg/pathway/map/map01100.html

Inborn Errors of Metabolism



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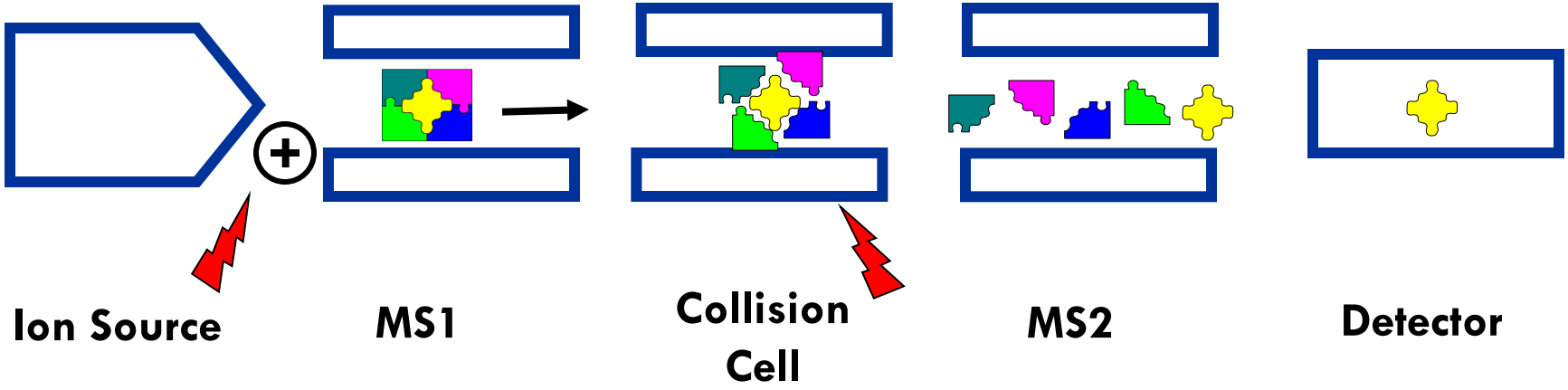
The Triple Quadrupole Tandem Mass Spectrometer



Inborn Errors Of Metabolism

HPLC/ spectrophotometry

- **Amino acids**
  - **PKU, MSUD, Urea cycle defects**

GC/MS

- **Organic acids**
  - **Methylmalonic acidemia, propionic acidemia**

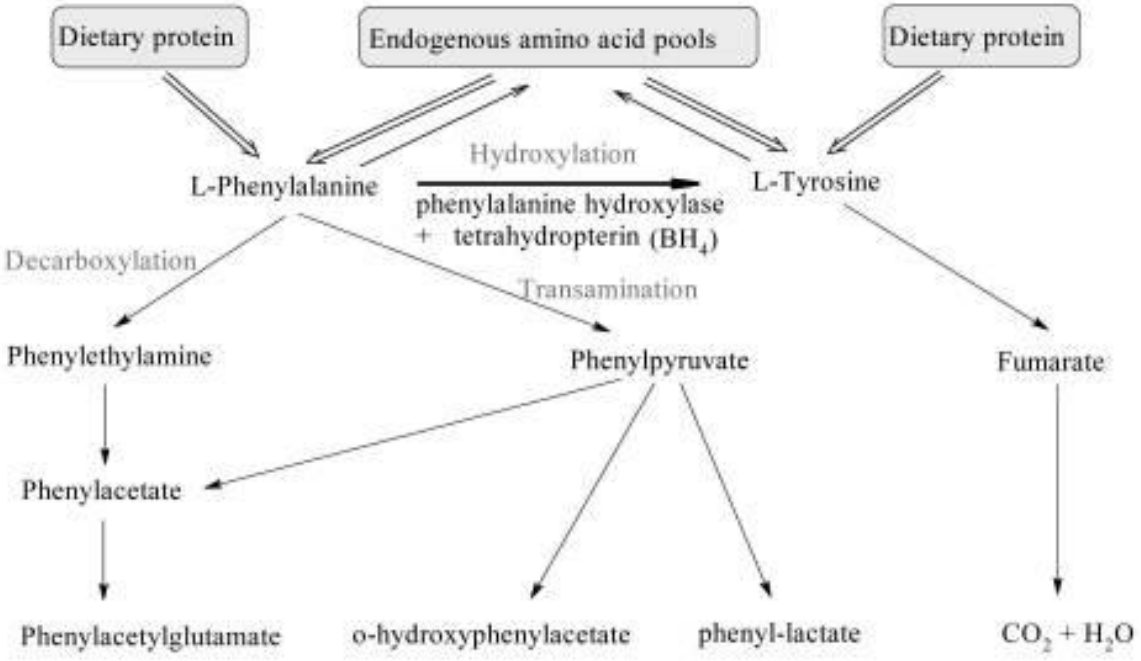
MS/MS

- **Fatty acids**
  - **MCAD, VLCAD**

Enzyme assays

- **Organelles**
  - **Lysosomal storage diseases**
  - **Mitochondrial myopathies**
  - **Perixosomal disorders**

PKU: An Inherited Disorder of Phenylalanine Metabolism



CLIN. CHEM. 39/1, 66–71 (1993)

Rapid Diagnosis of Phenylketonuria by Quantitative Analysis for Phenylalanine and Tyrosine in Neonatal Blood Spots by Tandem Mass Spectrometry

Donald H. Chace,[1] David S. Millington,[1] Naoto Terada,[2] Stephen G. Kahler,[1] Charles R. Roe,[1] and Lindsay F. Hofman[3]

Because the mass-spectrometric method also recognizes other aminoacidemias simultaneously and is capable of automation, it represents a useful development toward a broad-spectrum neonatal screening method.

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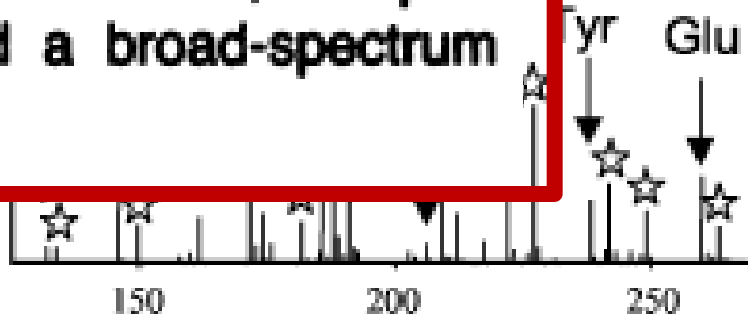
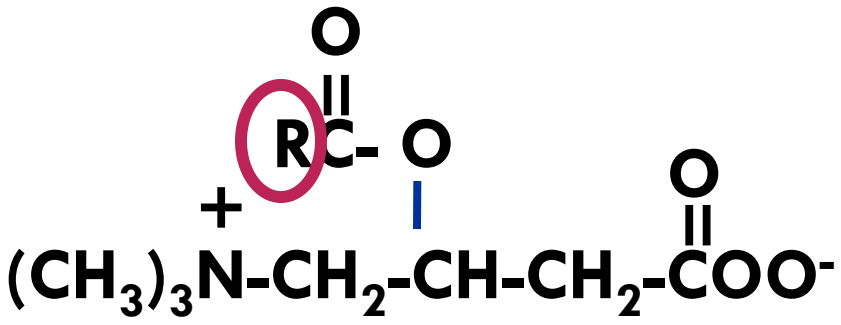


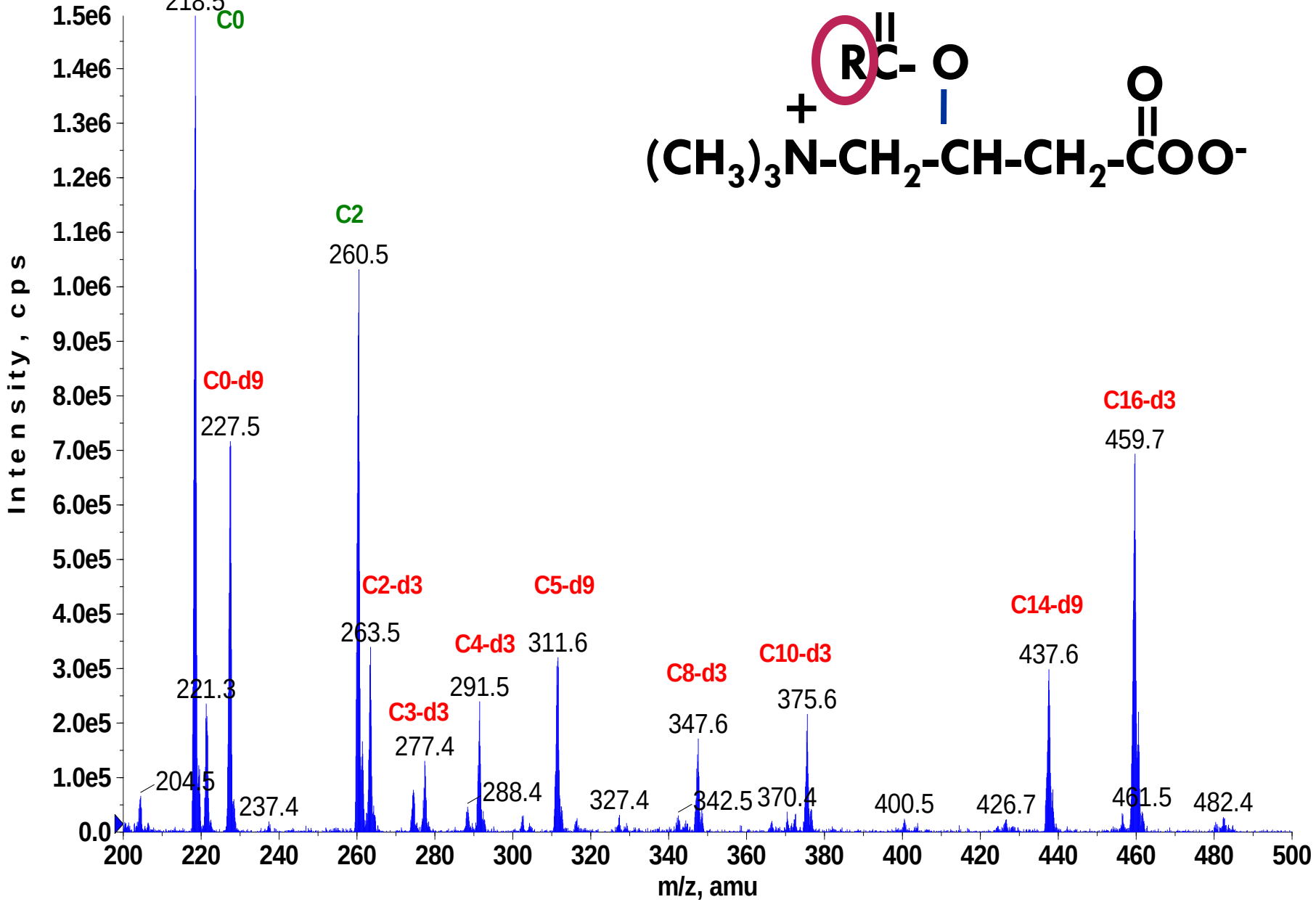
Neutral loss (*m*/*z* 102)

Acylcarnitine Profile: precursors of *m/z* 85

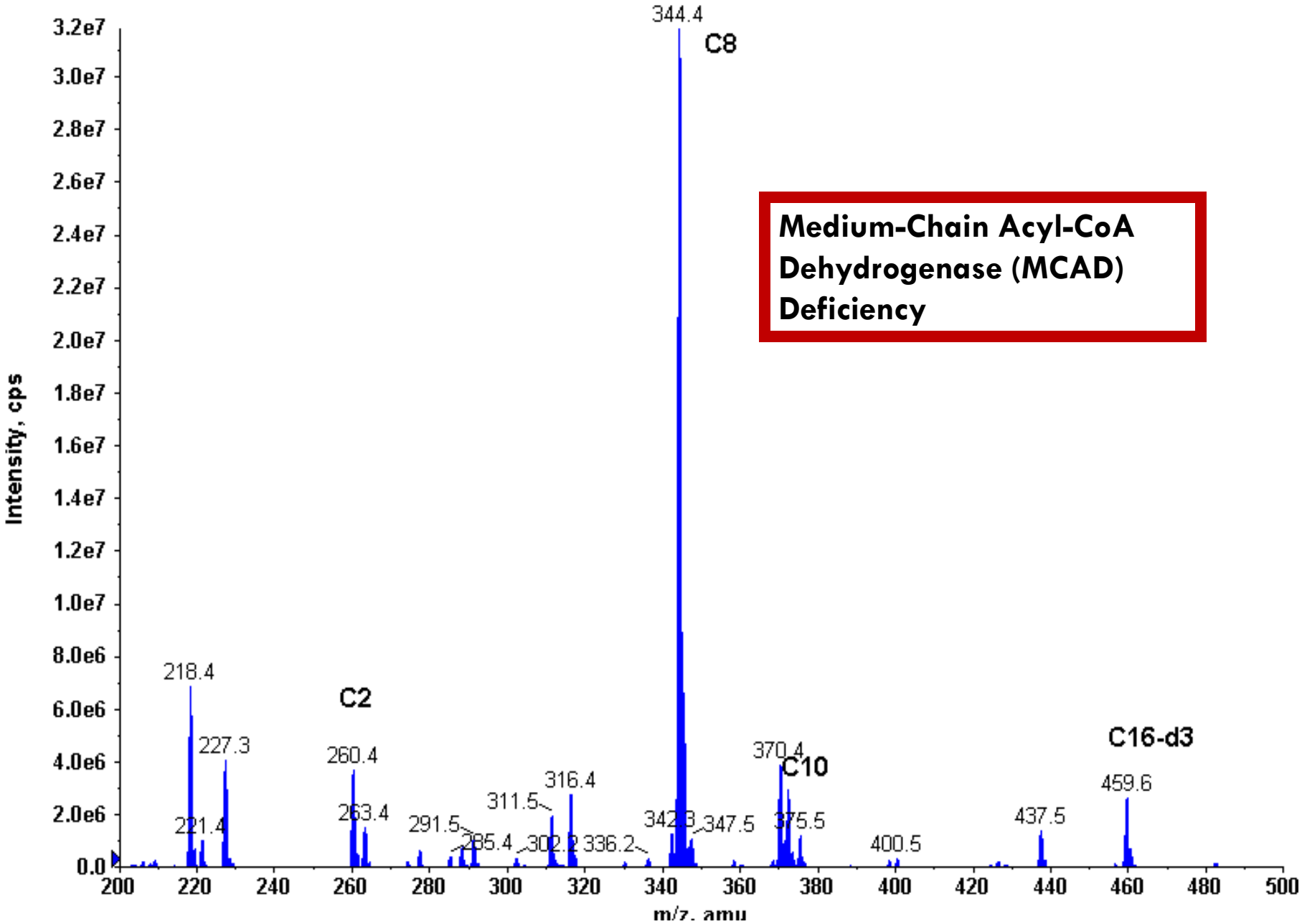
+Precursor (85.0): 28 MCA scans from Sample 6 (H38870 Asa-Nunies, W) of Data081004.wiff

Max. 1.5e6 cps.





Acylcarnitine Profile: precursors of *m/z* 85



Evolution of Clinical Testing by MS/MS

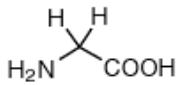
- Amino Acids (limited panel)
- Acylcarnitine Profile
- Single analyte determinations
  - Methylmalonic acid
  - Orotic acid
  - S-Sulfocysteine
  - Guanadinoacetate/creatinine
  - Succinylacetone
  - Carnitine
  - Many more
- Immunosuppressive drugs (e.g., mycophenolic acid)
- Steroid hormones
- Vitamin D

Testing in the Clinical Laboratory
Elements of Method Validation

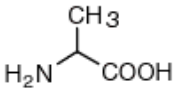
- Accuracy
- Precision
- Linearity
- Analytic measurement range
- LLOD, LLOQ
- Recovery or correlation with existing method

Amino Acid Analysis

Small

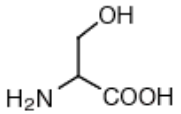


Glycine (Gly, G)
MW: 57.0

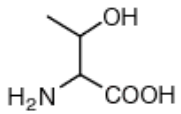


Alanine (Ala, A)

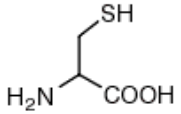
Nucleophilic



Serine (Ser, S)

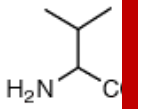


Threonine (Thr, T)



Cysteine (Cys, C)
, pK_a = 8.35

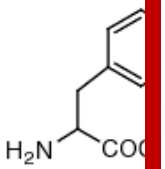
Hydrophob



Valine (Va
MW: 99.

COOH
ro, P)
.12

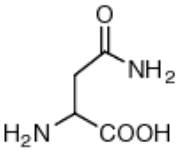
Aromatic



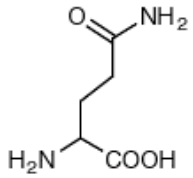
Phenylalanine (
MW: 147.

OH
COOH
id (Glu, E)
, pK_a = 4.07

Amide

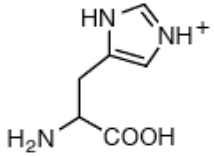


Asparagine (Asn, N)
MW: 114.11

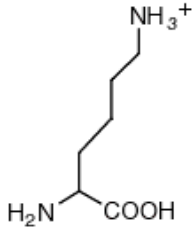


Glutamine (Gln, Q)
MW: 128.14

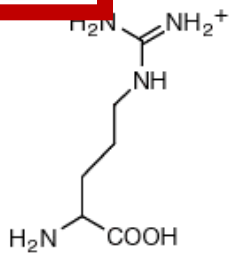
Basic



Histidine (His, H)
MW: 137.14, pK_a = 6.04

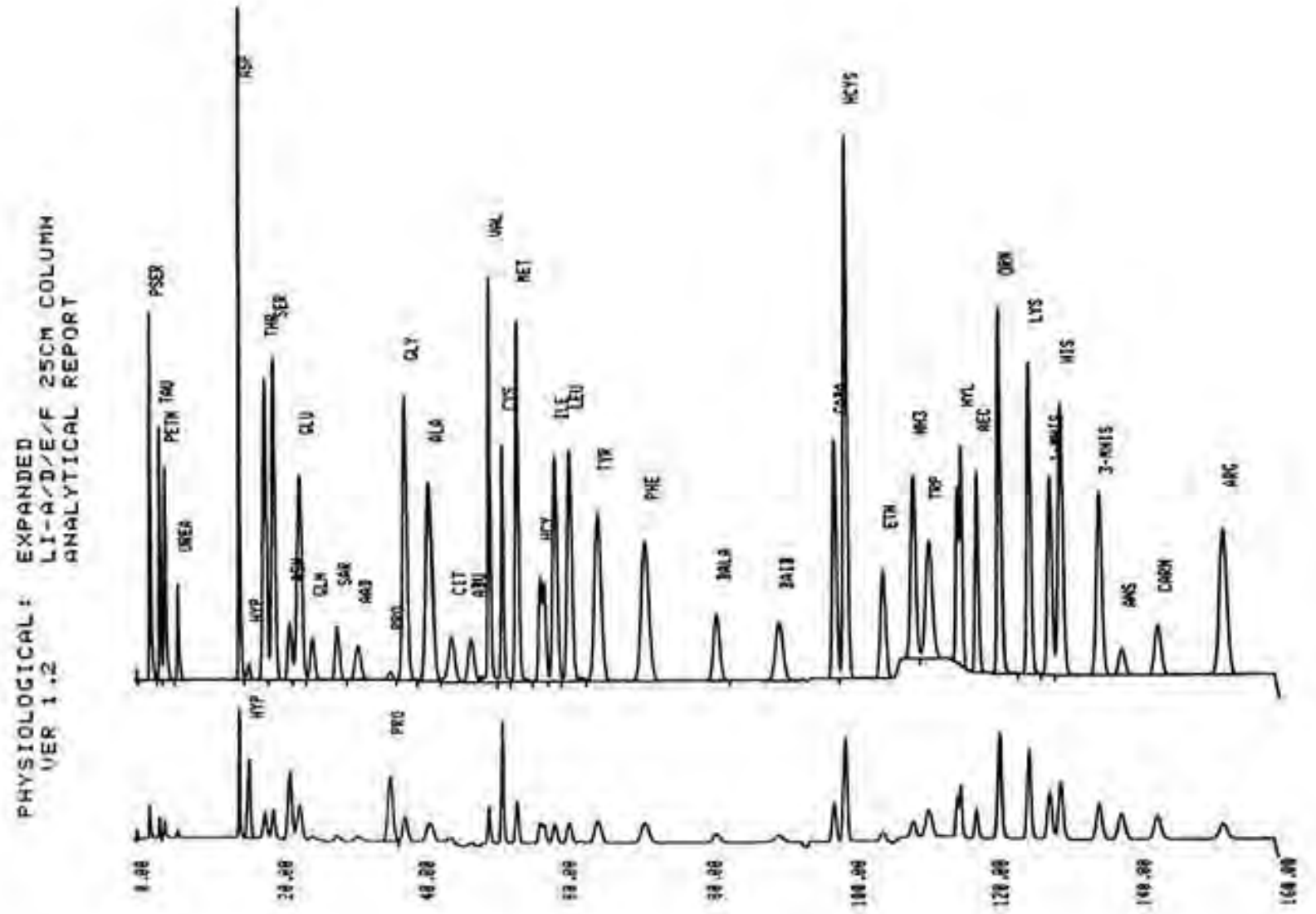


Lysine (Lys, K)
MW: 128.17, pK_a = 10.79



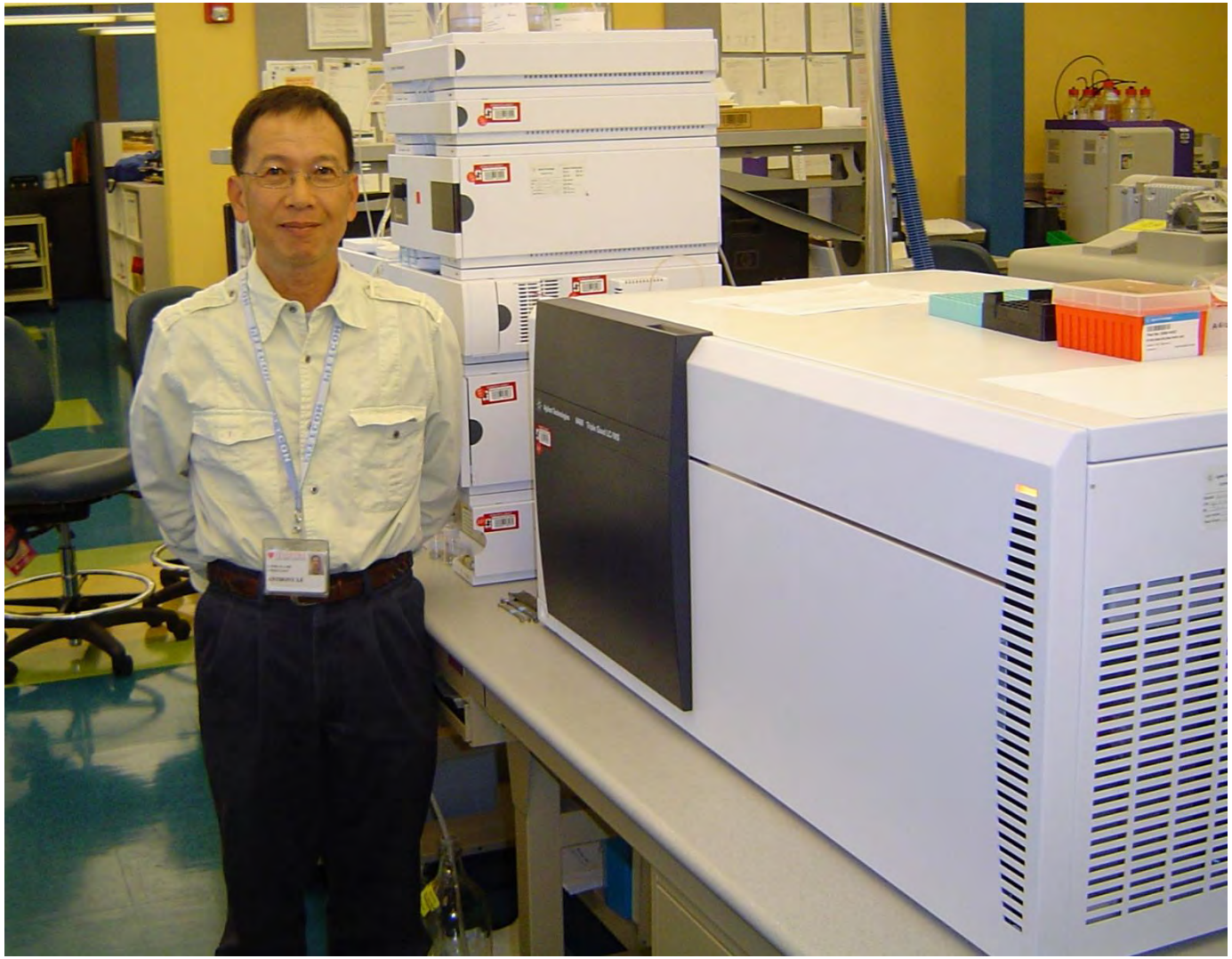
Arginine (Arg, R)
MW: 156.19, pK_a = 12.48

Amino Acid Chromatogram



Amino Acid Analysis by MS/MS: *Challenges*

- Separation of isomers (leu, ileu, alloileu)
- Chromatographic removal of salts
- Availability of standards
- Derivatization
- Ion suppression
  - Sensitivity
  - Imprecision

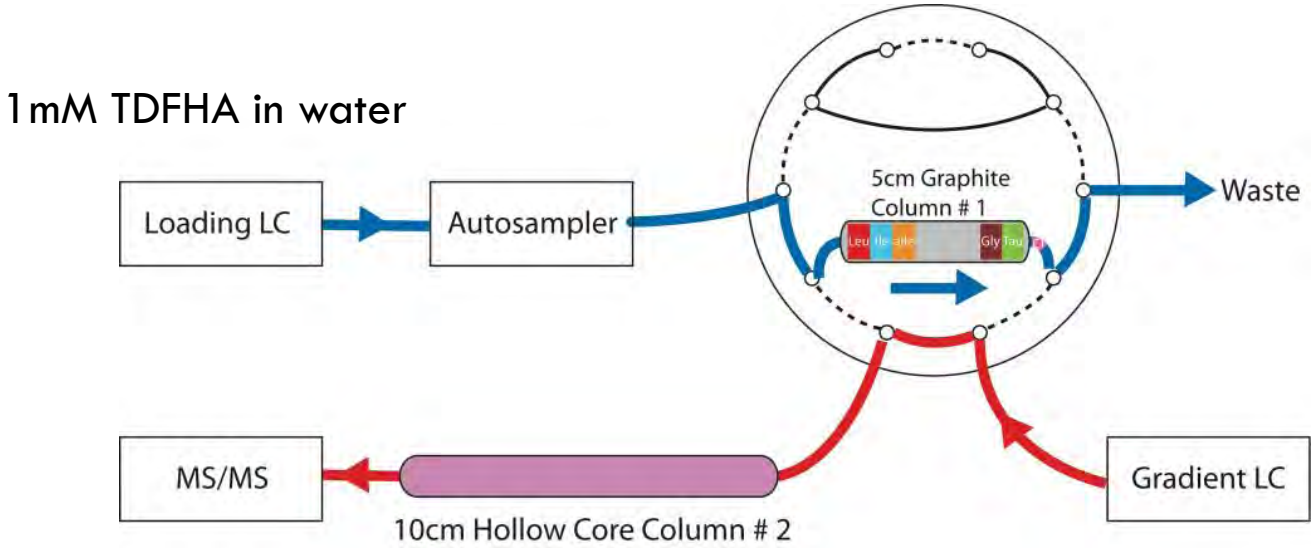


Tony Le, Stanford Biochemical Genetics Laboratory

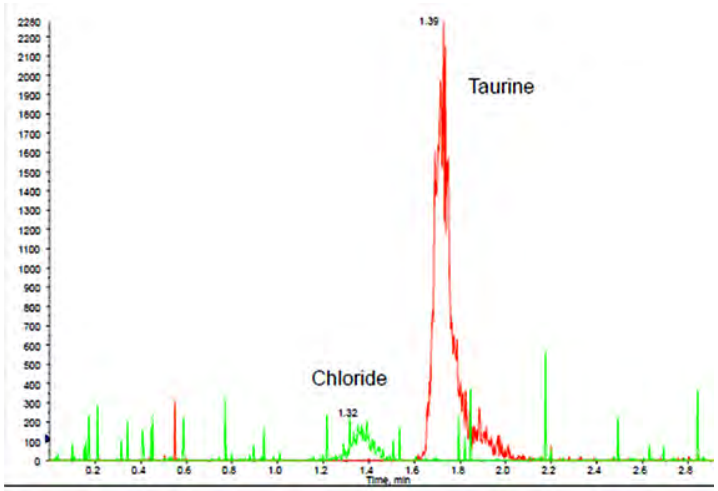
Amino Acid Analysis: Methods

1. Plasma, urine, CSF
2. Deproteinize with 6% sulfosalicylic acid (1:1)
3. Incubate at room temperature, 5min
4. Centrifuge at 13,000rpm, 5min
5. Dilute 10µL of supernatant with 800µL of 2mM tridecafluoroheptanoic acid (TDFHA) containing internal standards glucosaminic acid and s-2-aminoethyl cysteine
6. Transfer to autosampler vials

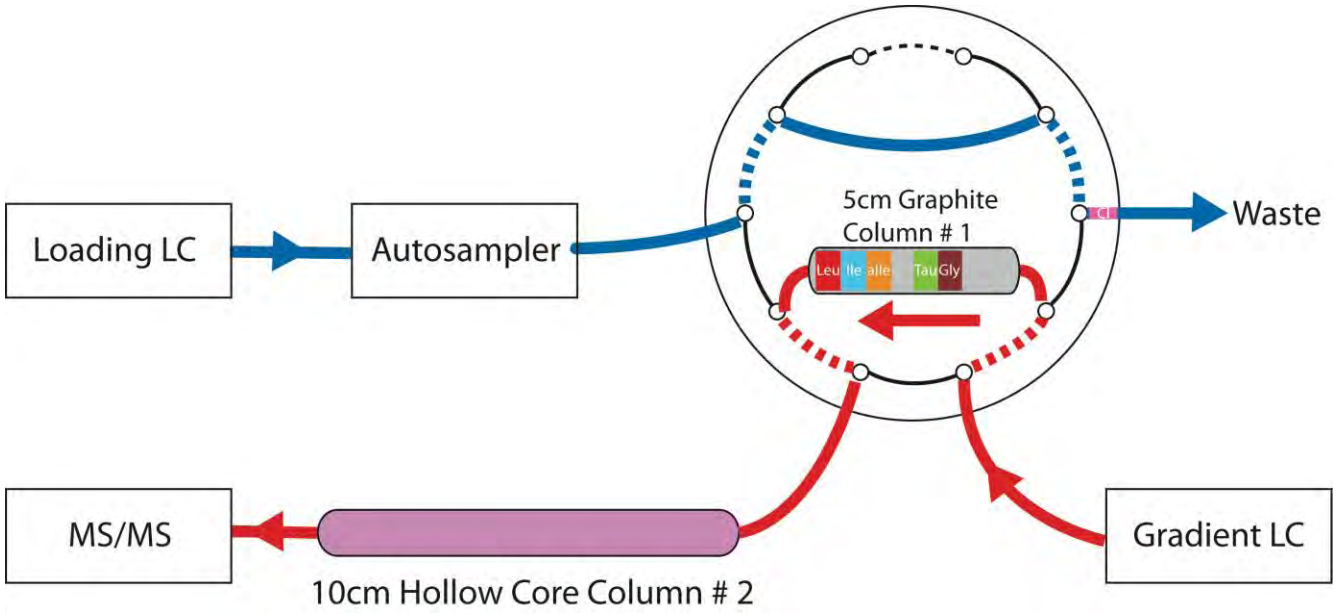
Column 1: Porous graphitic carbon (PGC)
(3µm Hypercarb, 4.6mm ID x 50mm)



Separation of negatively charged salts from first-eluting amino acid

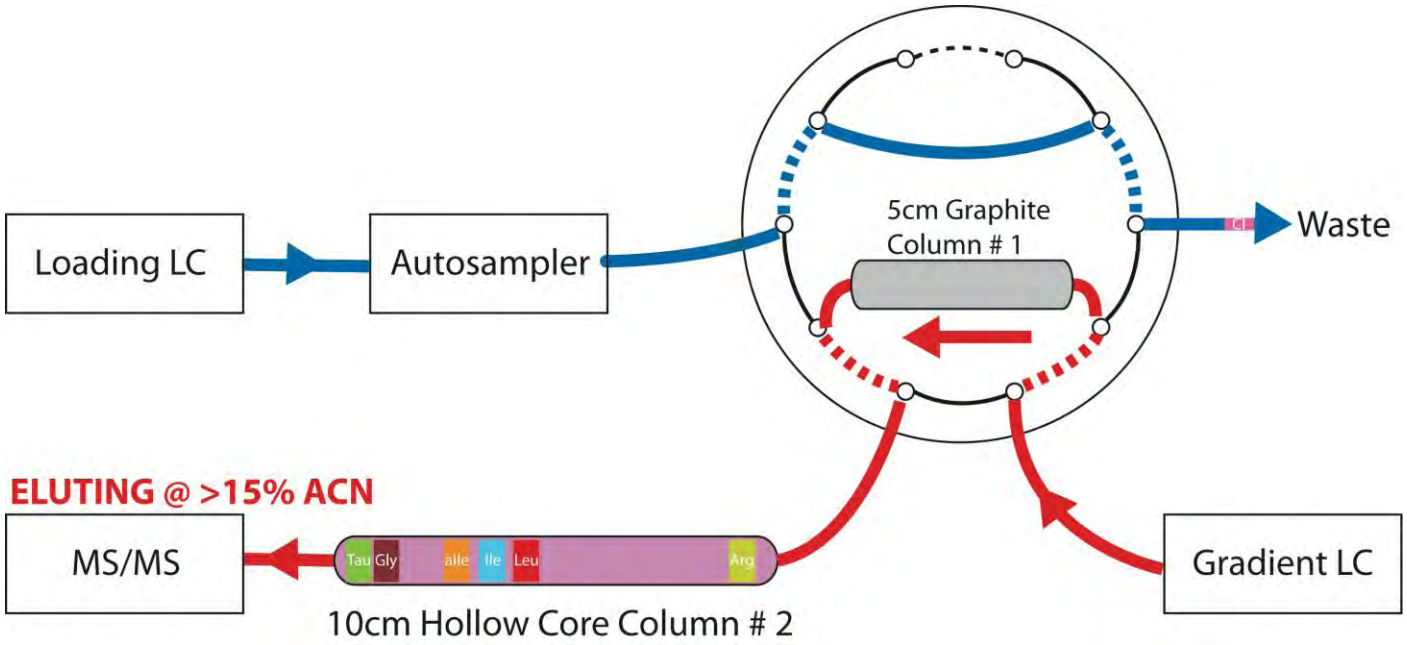


Flow-Reversal of Column 1



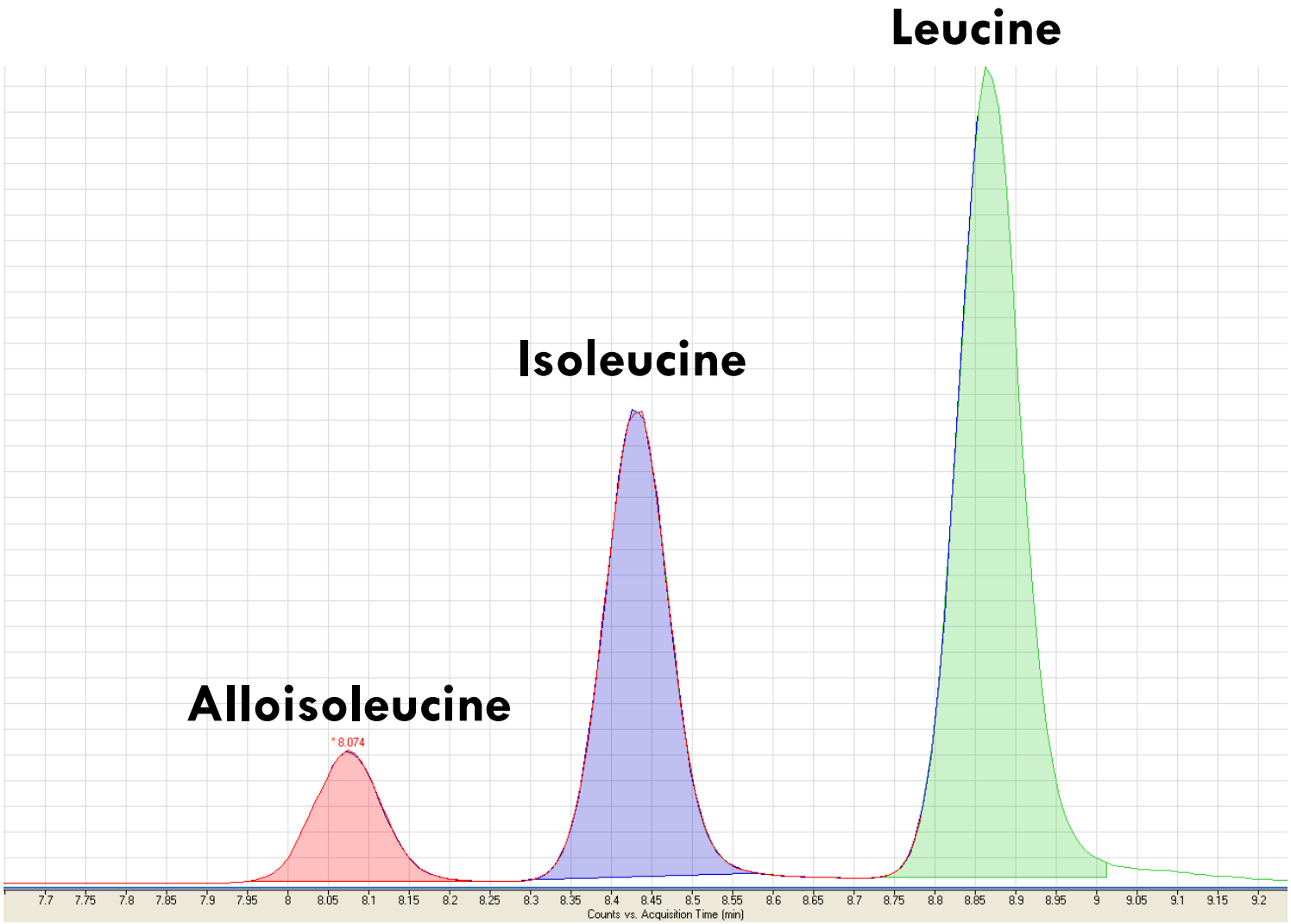
Column 2: Fused-core

(2.7µm Halo C18, 2.1mm IDx 100mm), 50°C



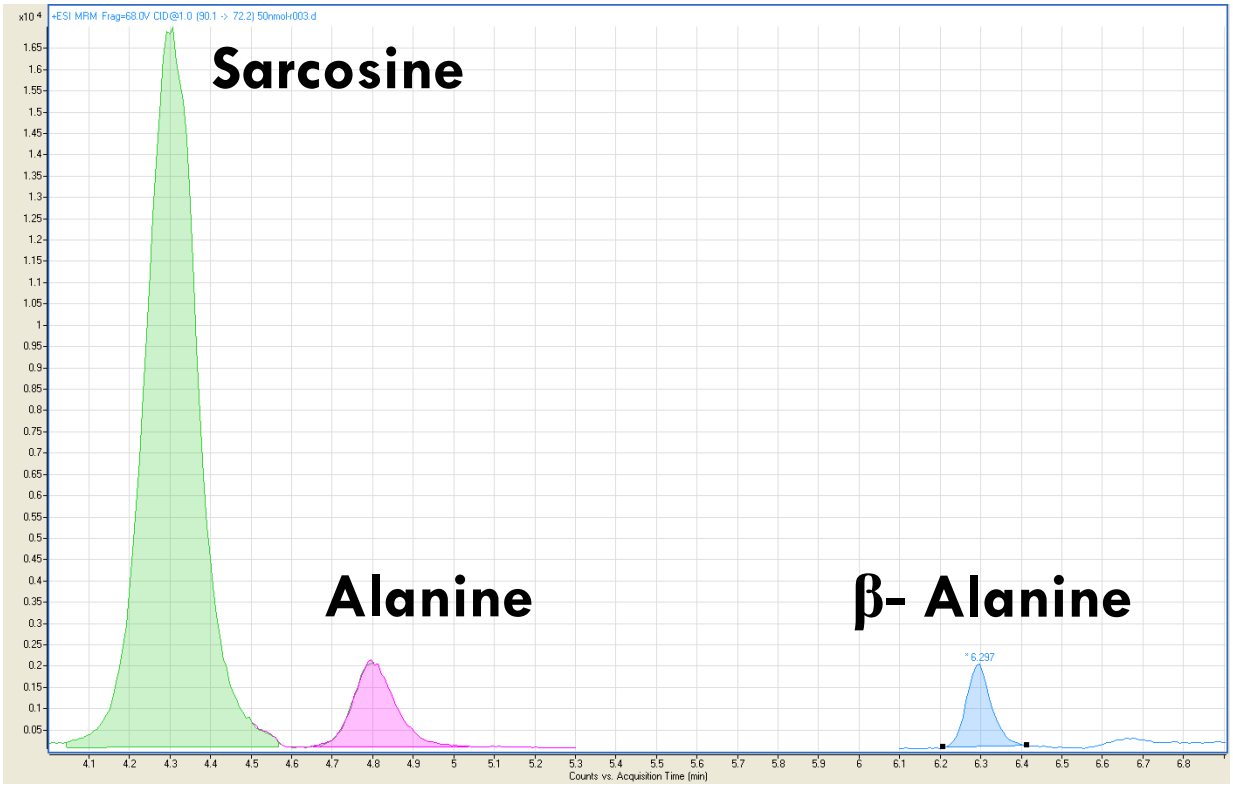
Baseline resolution of leucine isomers

(m/z 132.1>86.1)

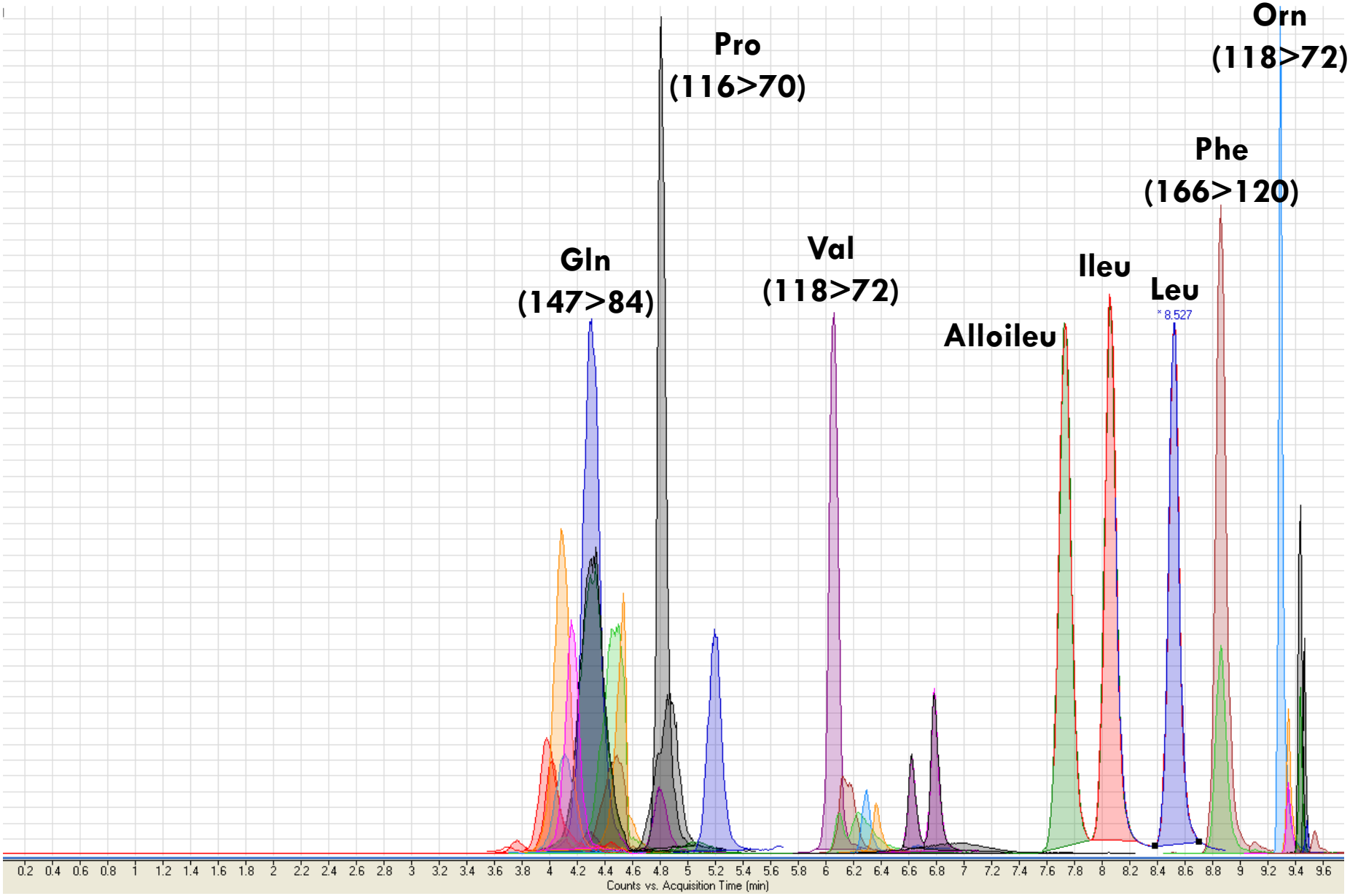


Baseline resolution of sarc and ala

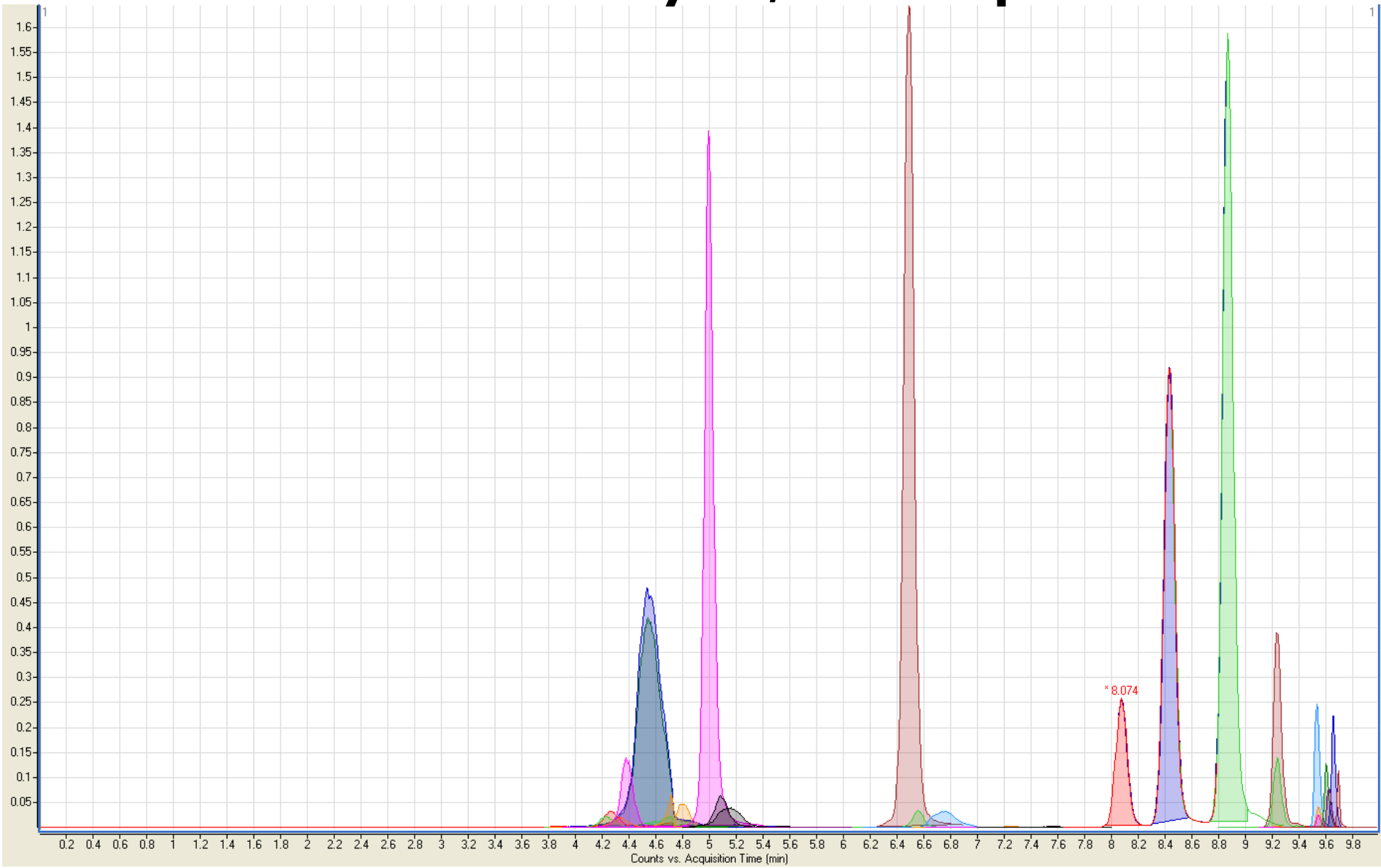
(m/z 90.0>44.2)



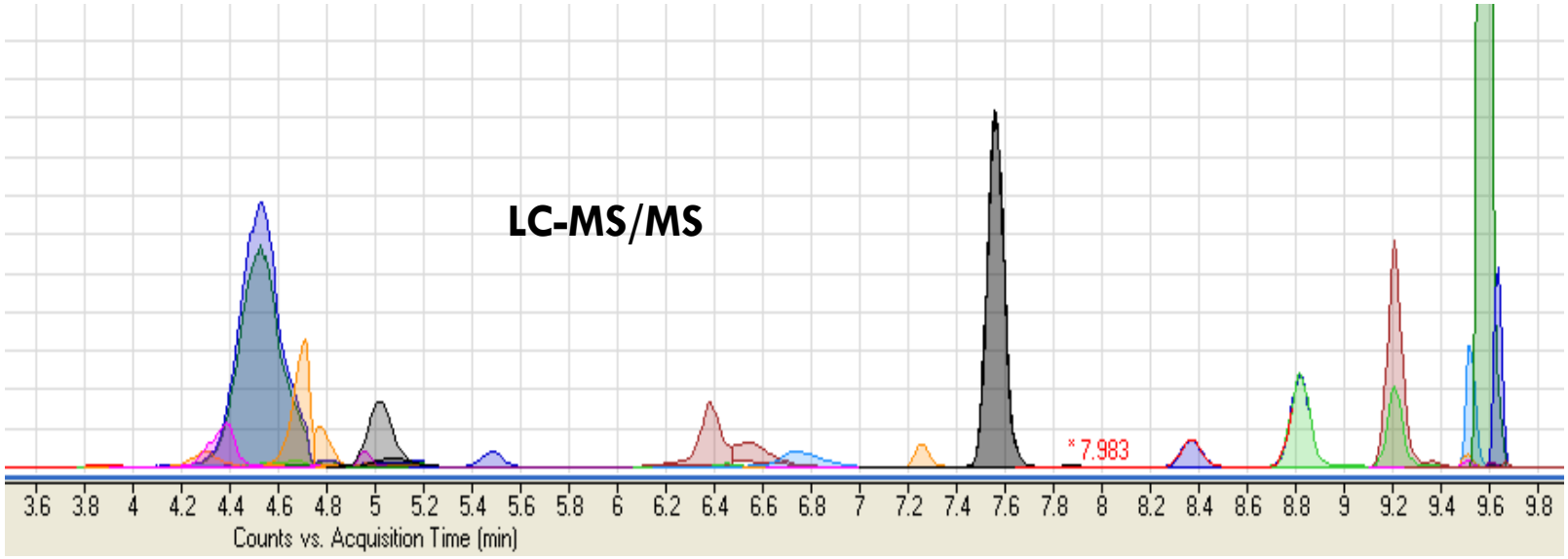
Amino Acid Analysis, 50 nmol standard

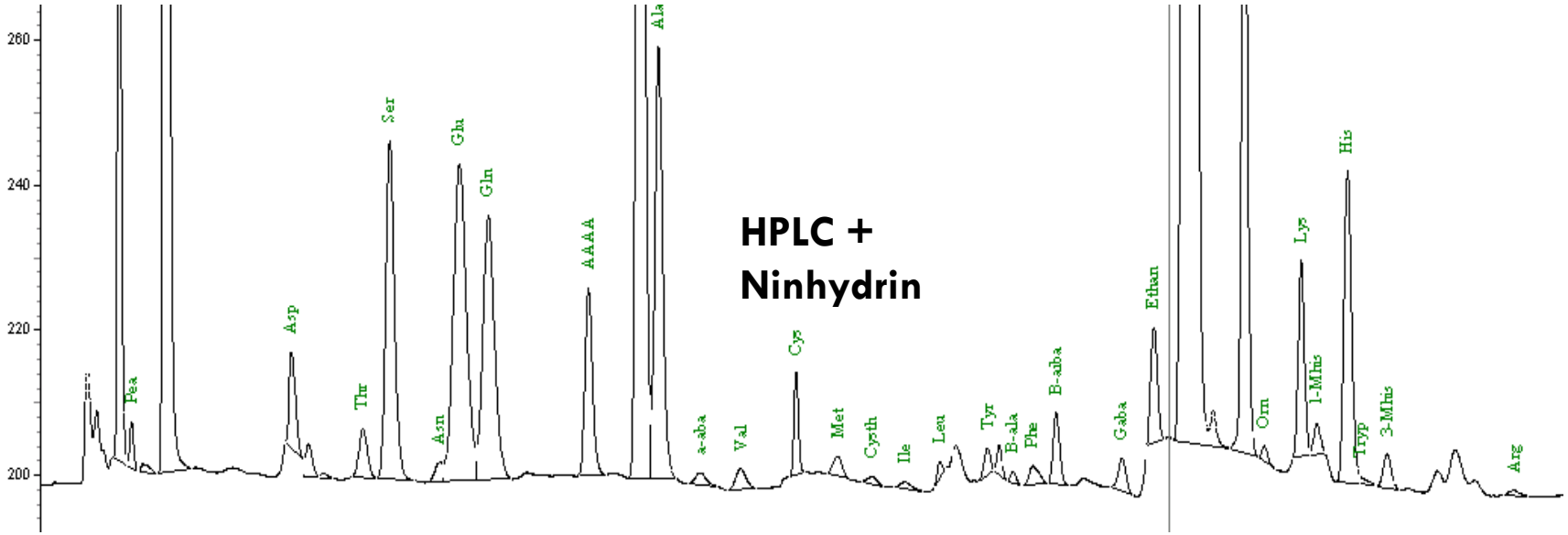


Amino Acid Analysis, MSUD plasma

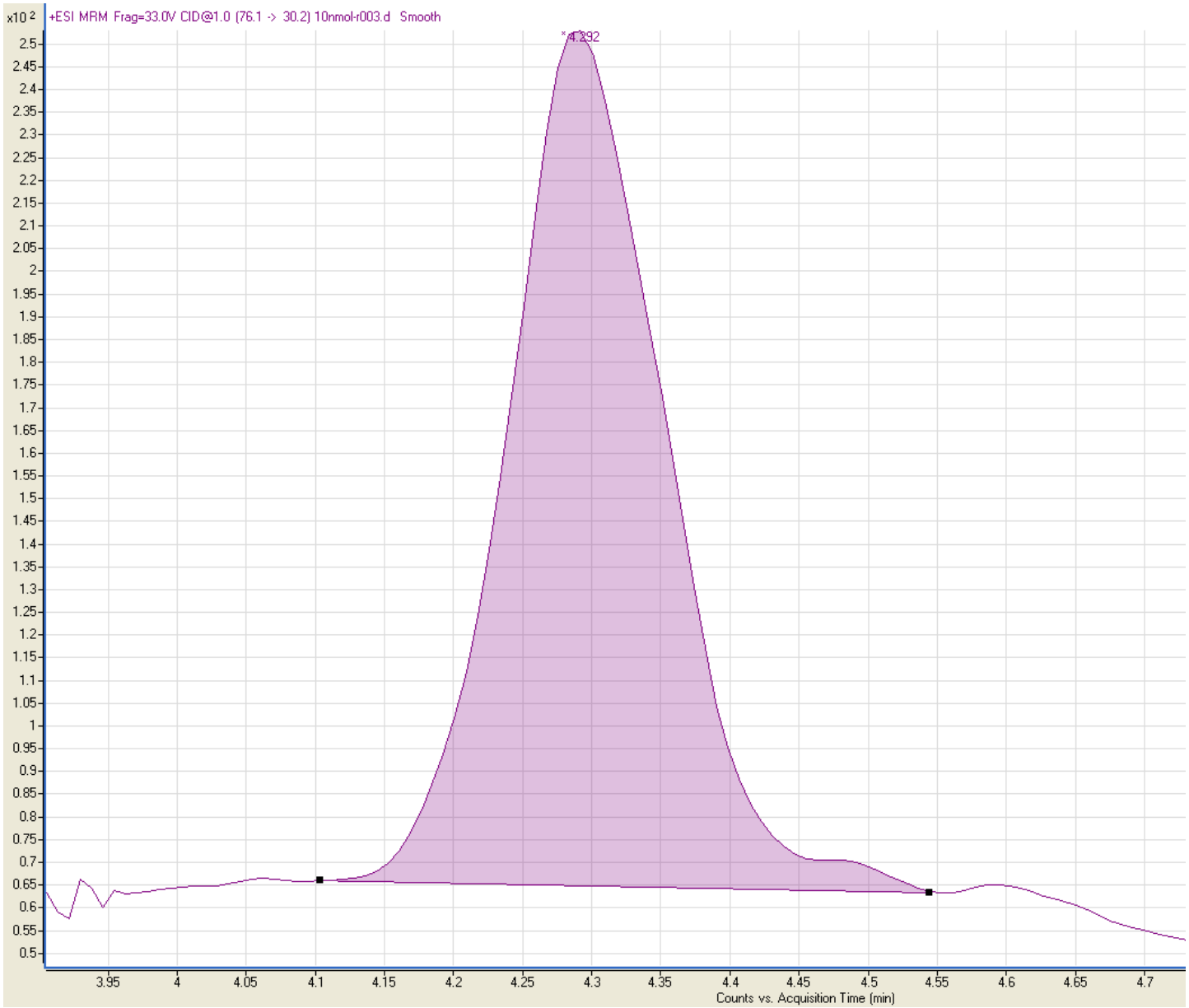


Urine Amino Acid Analysis

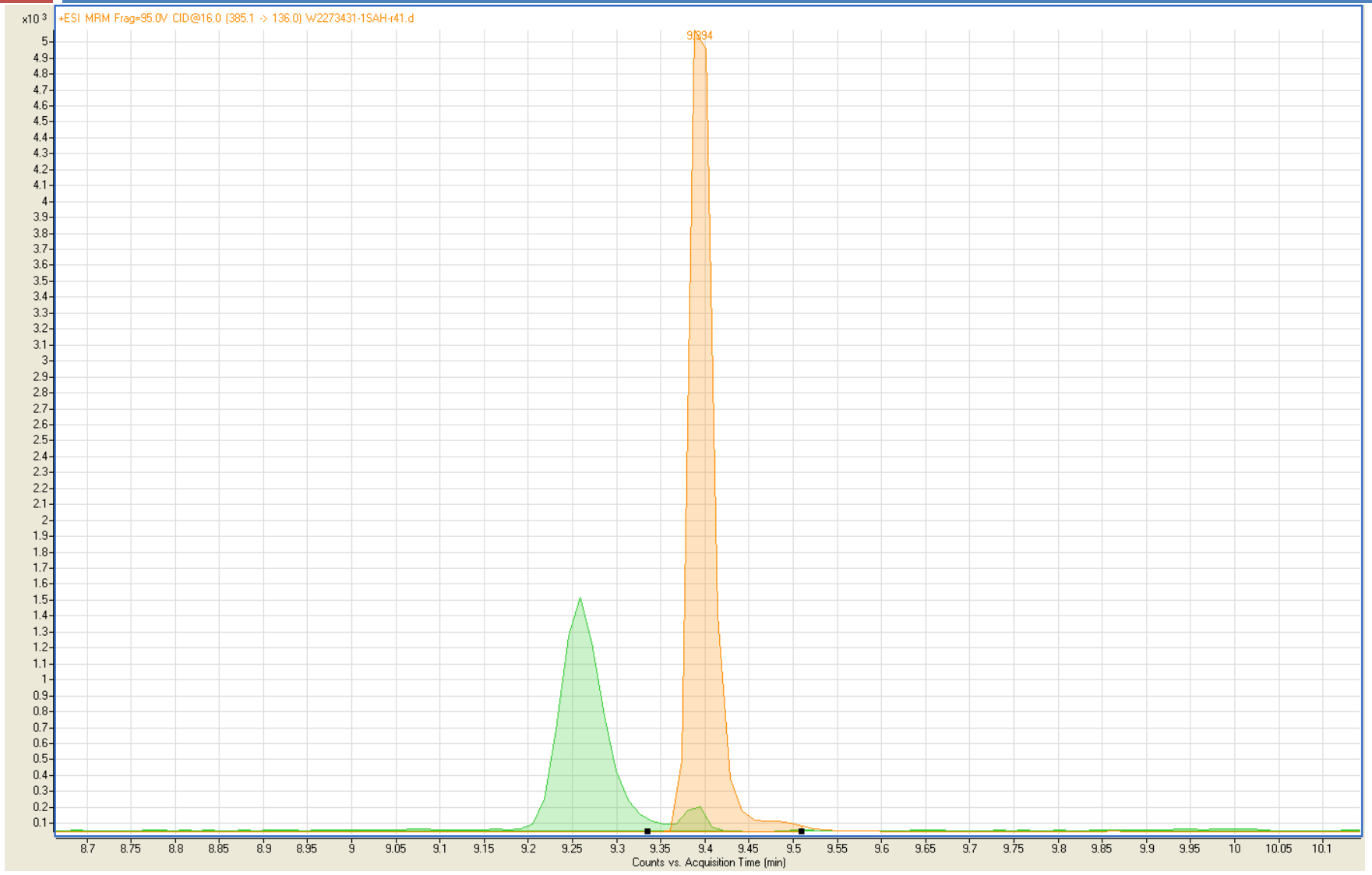




Glycine 10 nmol/ml



Adding other compounds: S-adenosylmethionine/S-adenosylhomocysteine



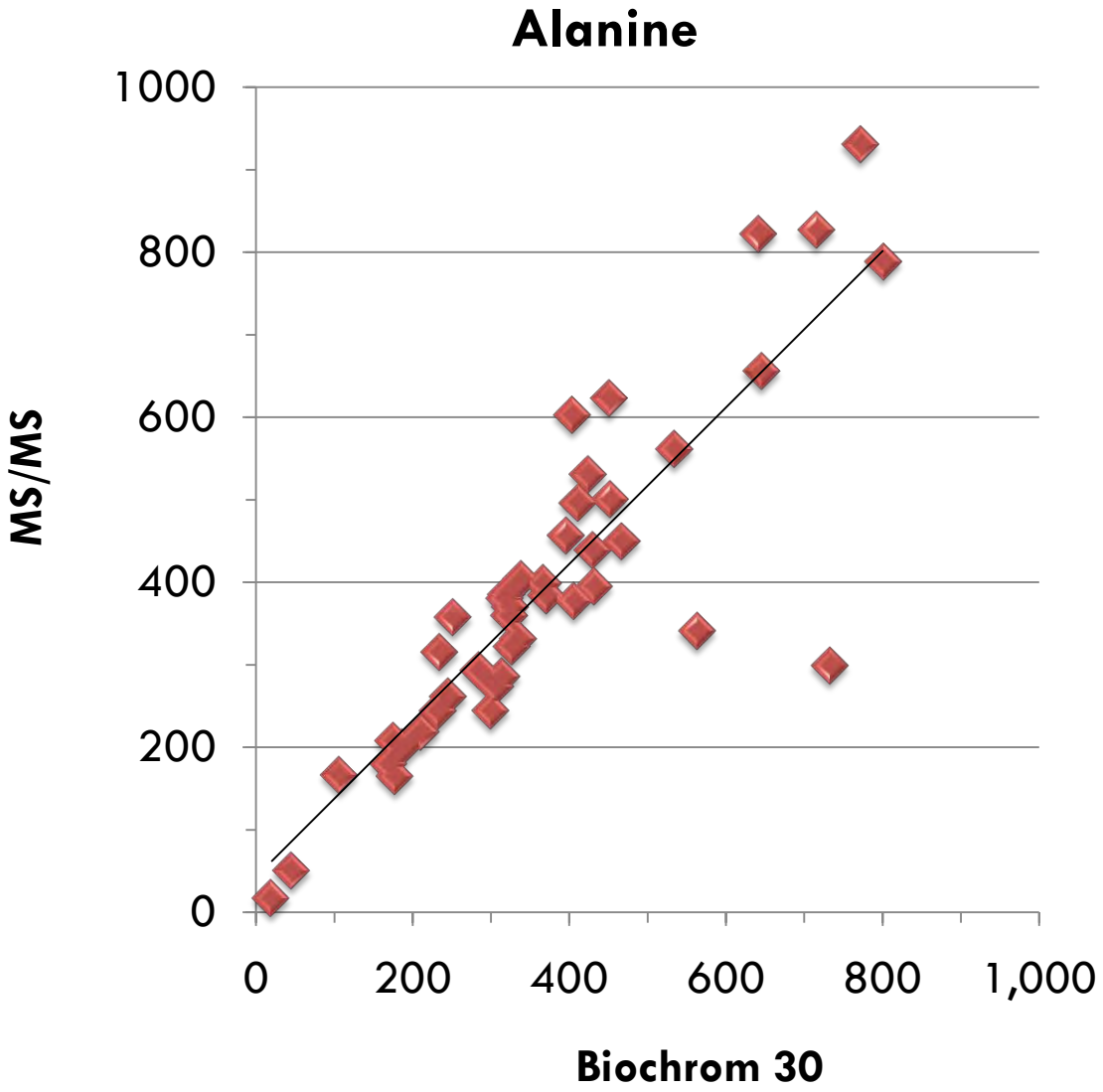
Lessons Learned Along the Way…

- Sample preparation matters
- Ion suppression where you least expect it
- Choice of calibration material
- Choice of internal standards

Not All Sample Preps Are Created Equal

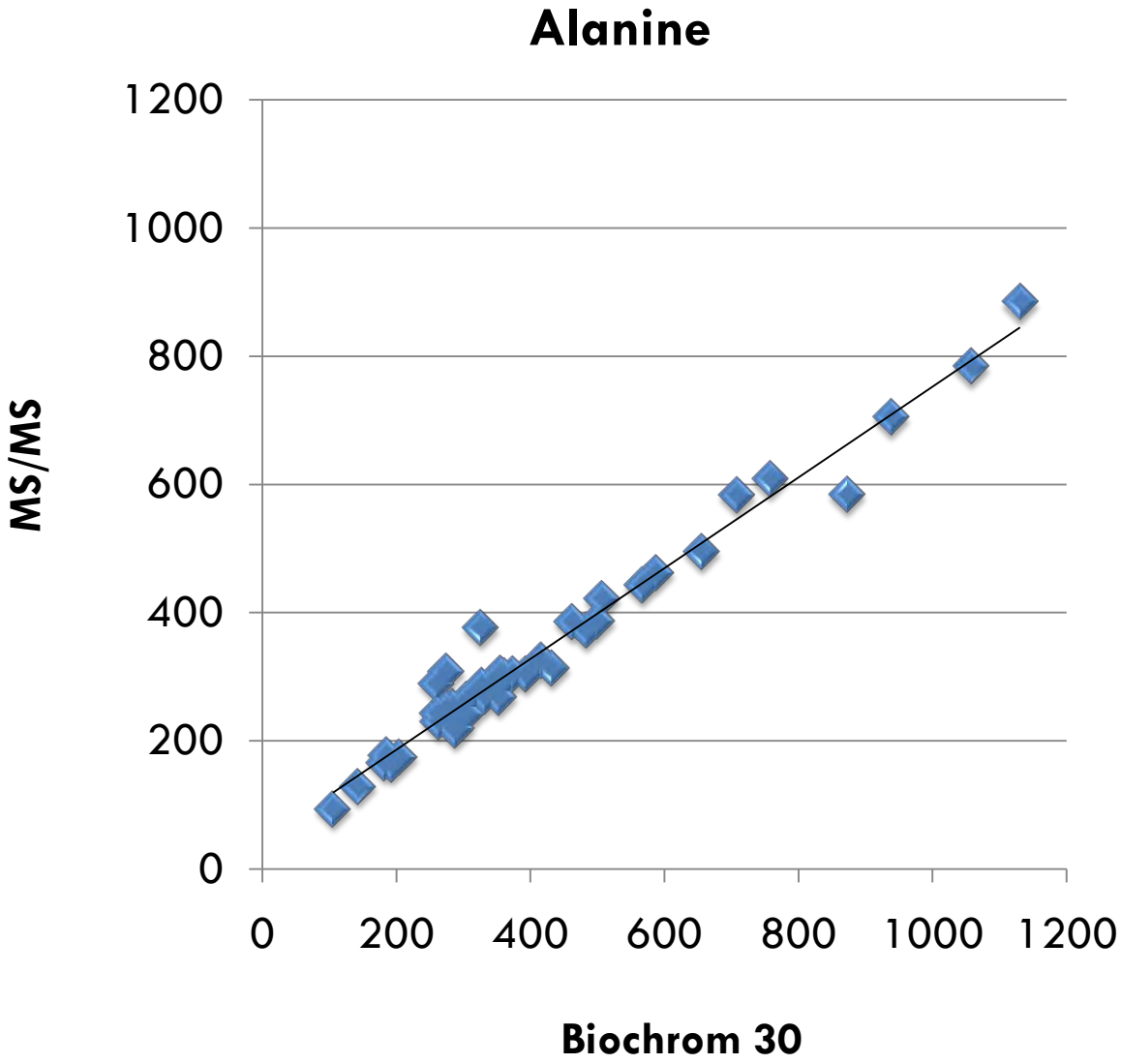
Plasma Prep 1: Methanol:Acetonitrile (3:1)

R^2=0.7488



Plasma Prep 2: 6% SSA in H_2O

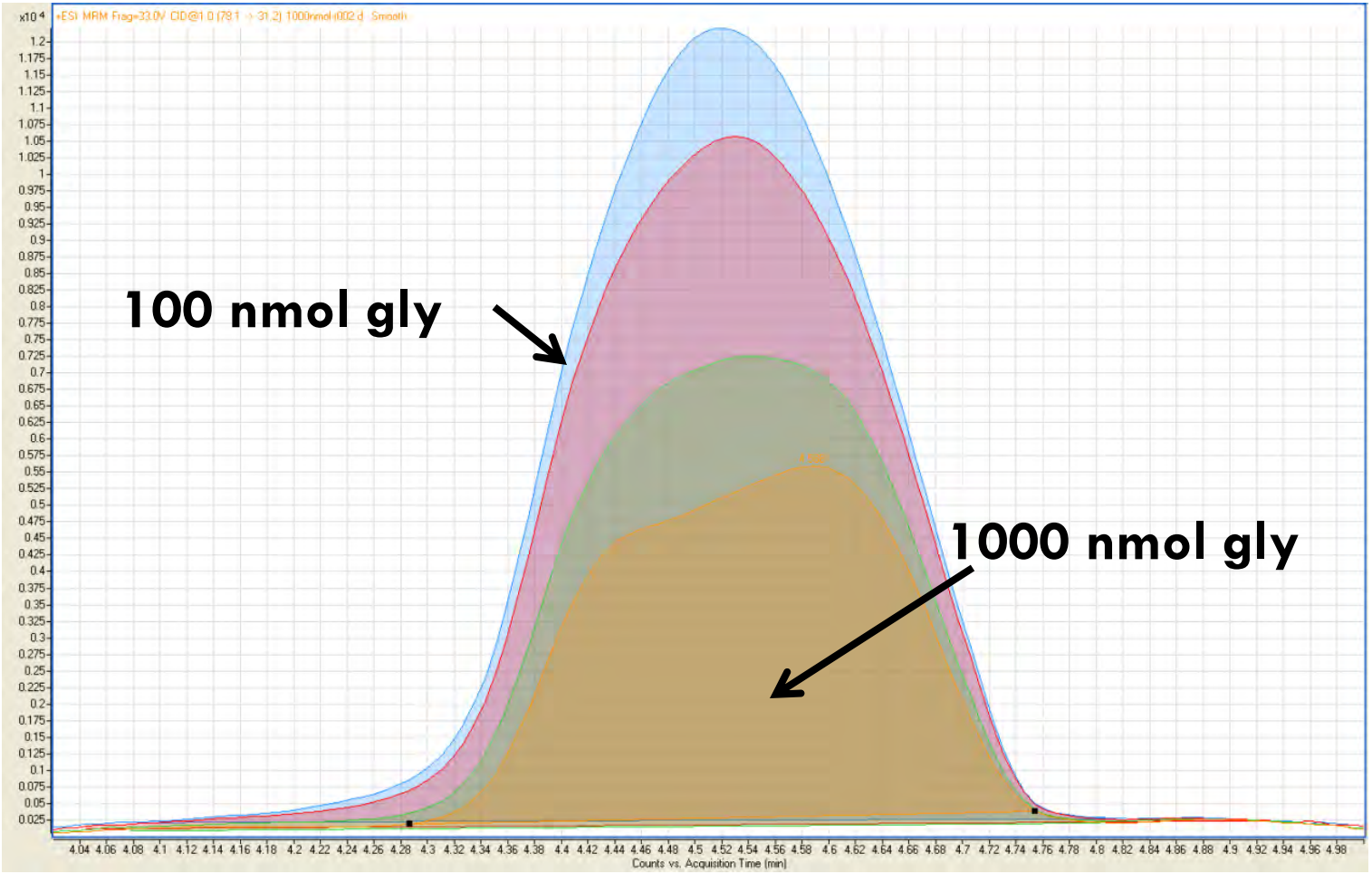
R^2=0.9702



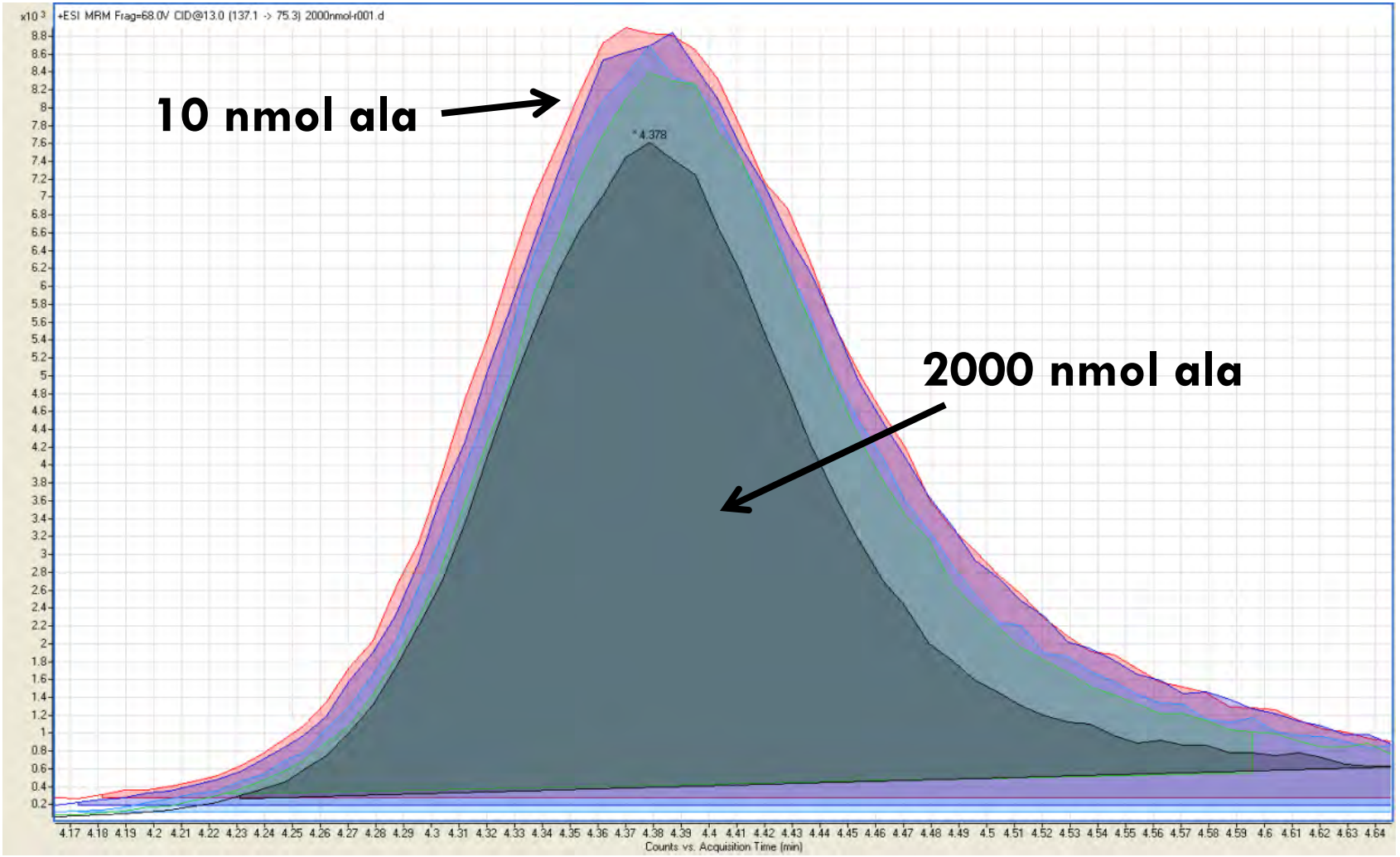
Ion Suppression Where You Least Expect It

Or: Calibration Matters

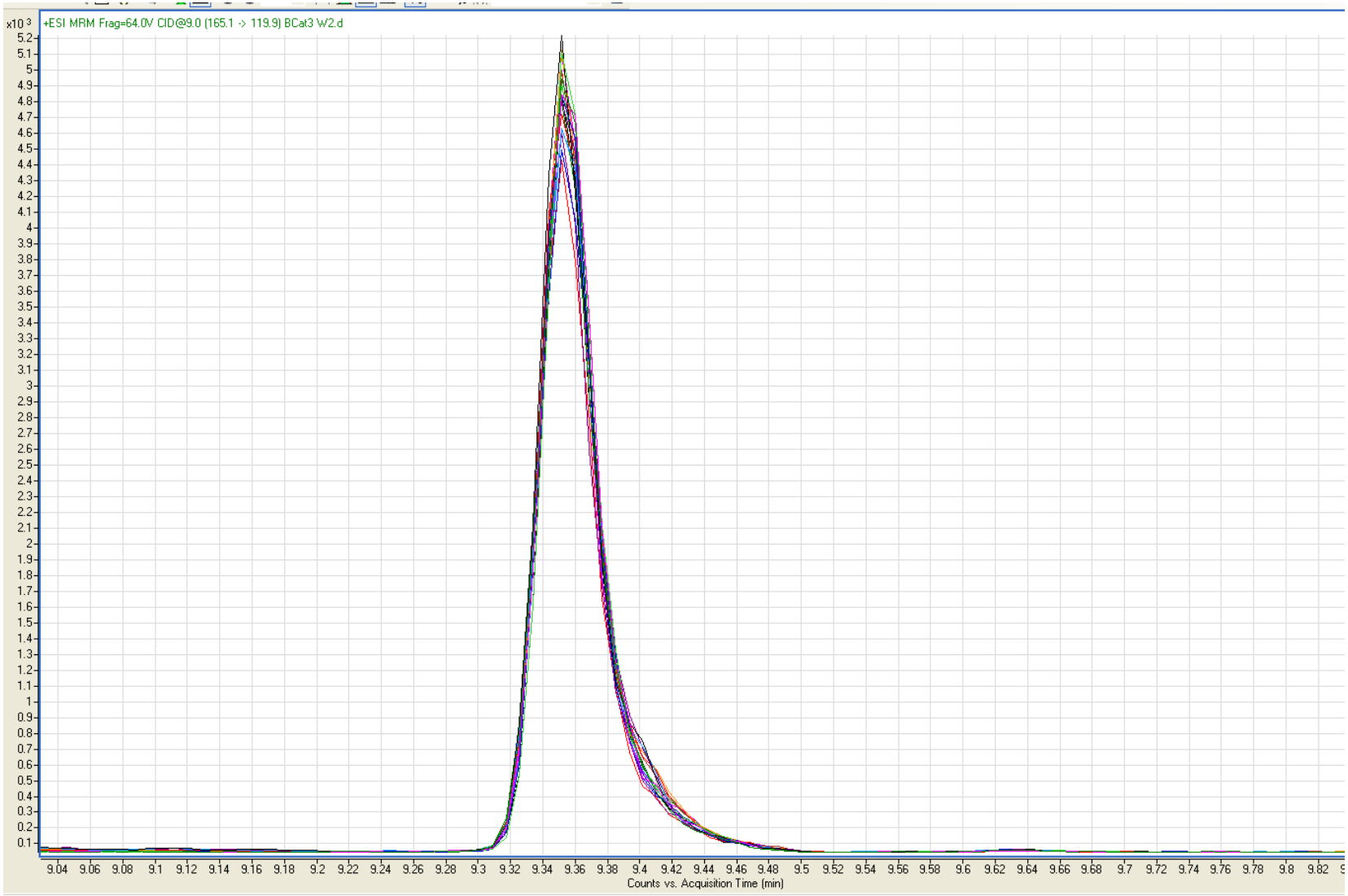
^{15}N-^{13}C-Glycine Response at Varying Glycine Concentrations



$^2H_4$-Alanine Response at Varying Alanine Concentrations

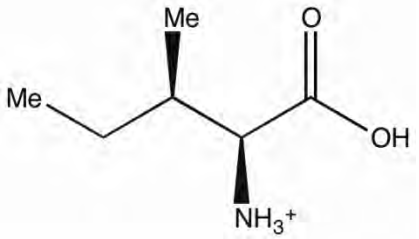


S-aminoethyl-cysteine response at varying amino acid concentrations

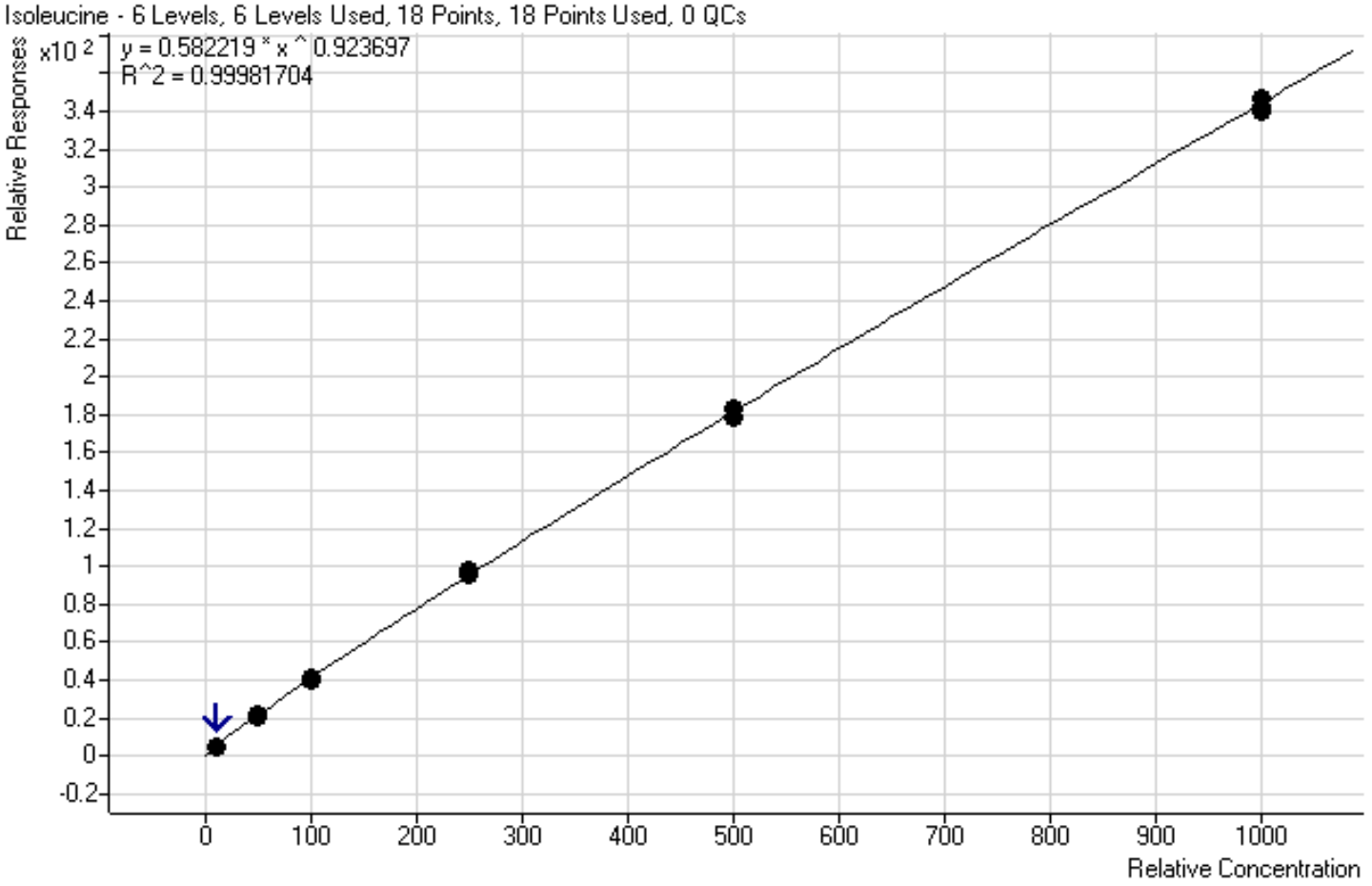


The Standard Curve

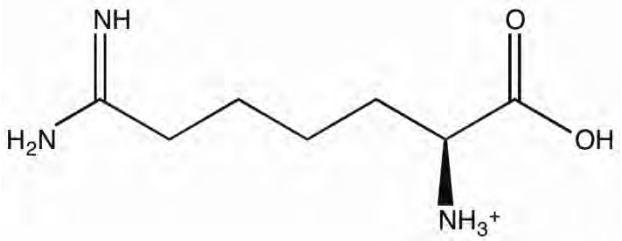
Isoleucine



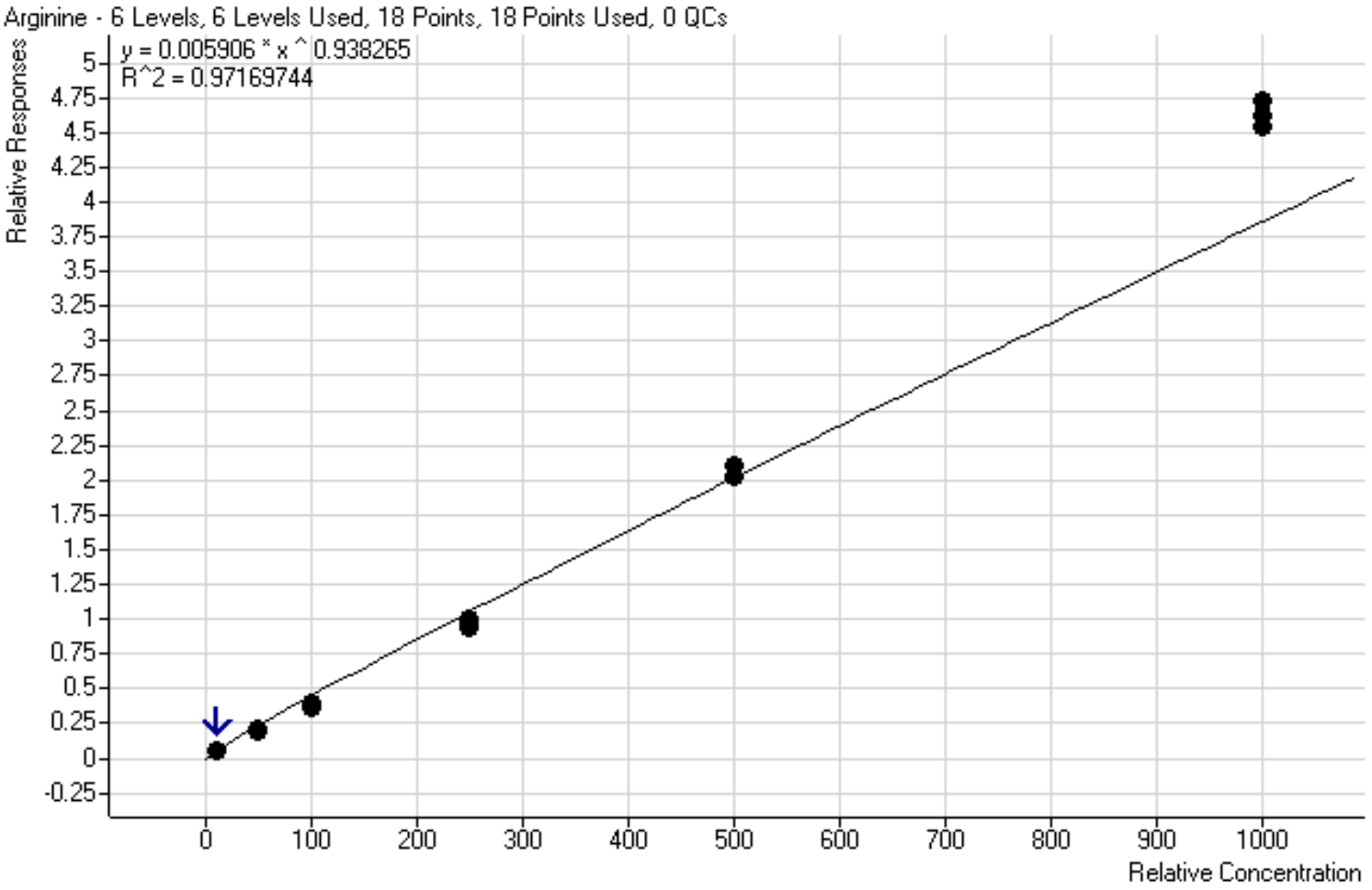
Curve fit: $1/x^2$



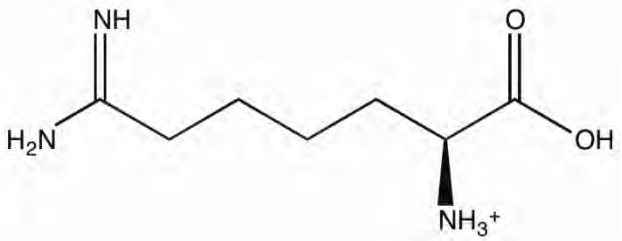
Arginine



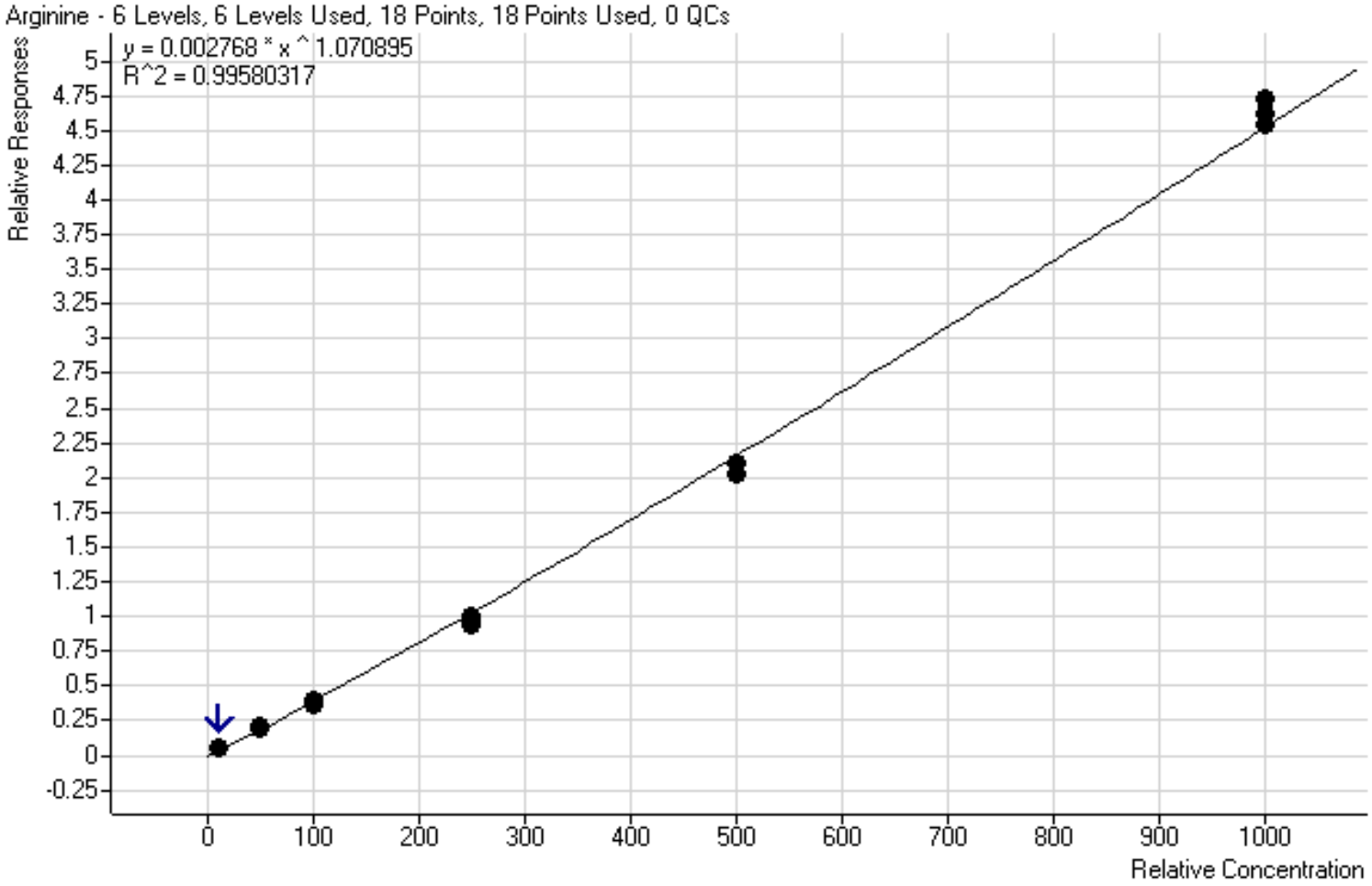
Curve fit: $1/x^2$



Arginine

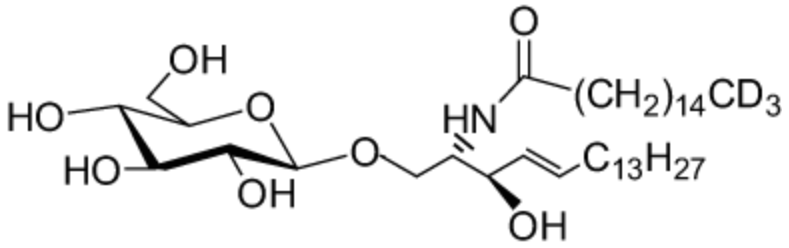


Curve fit: 1/x

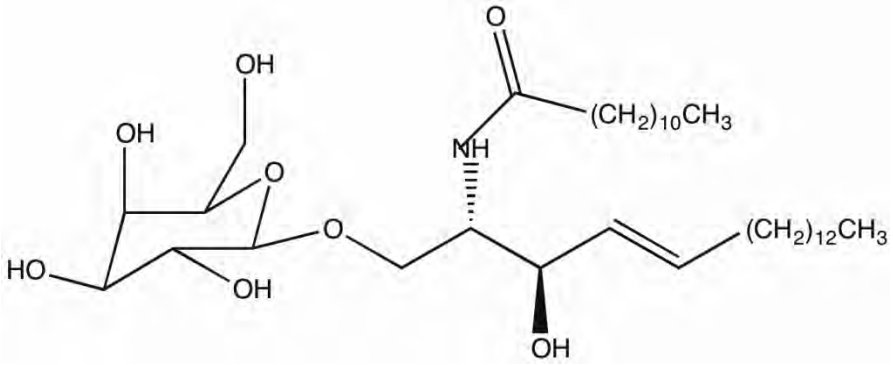


Lysosomal Enzyme Testing by MS/MS
Gaucher disease

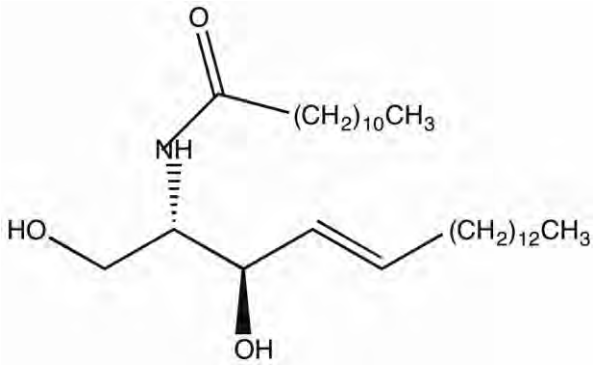
- Inherited deficiency of β-glucocerebrosidase
- Abnormal accumulation of lipids
- Enzyme replacement therapy
- Candidate for newborn screening?



Gaucher Disease Testing

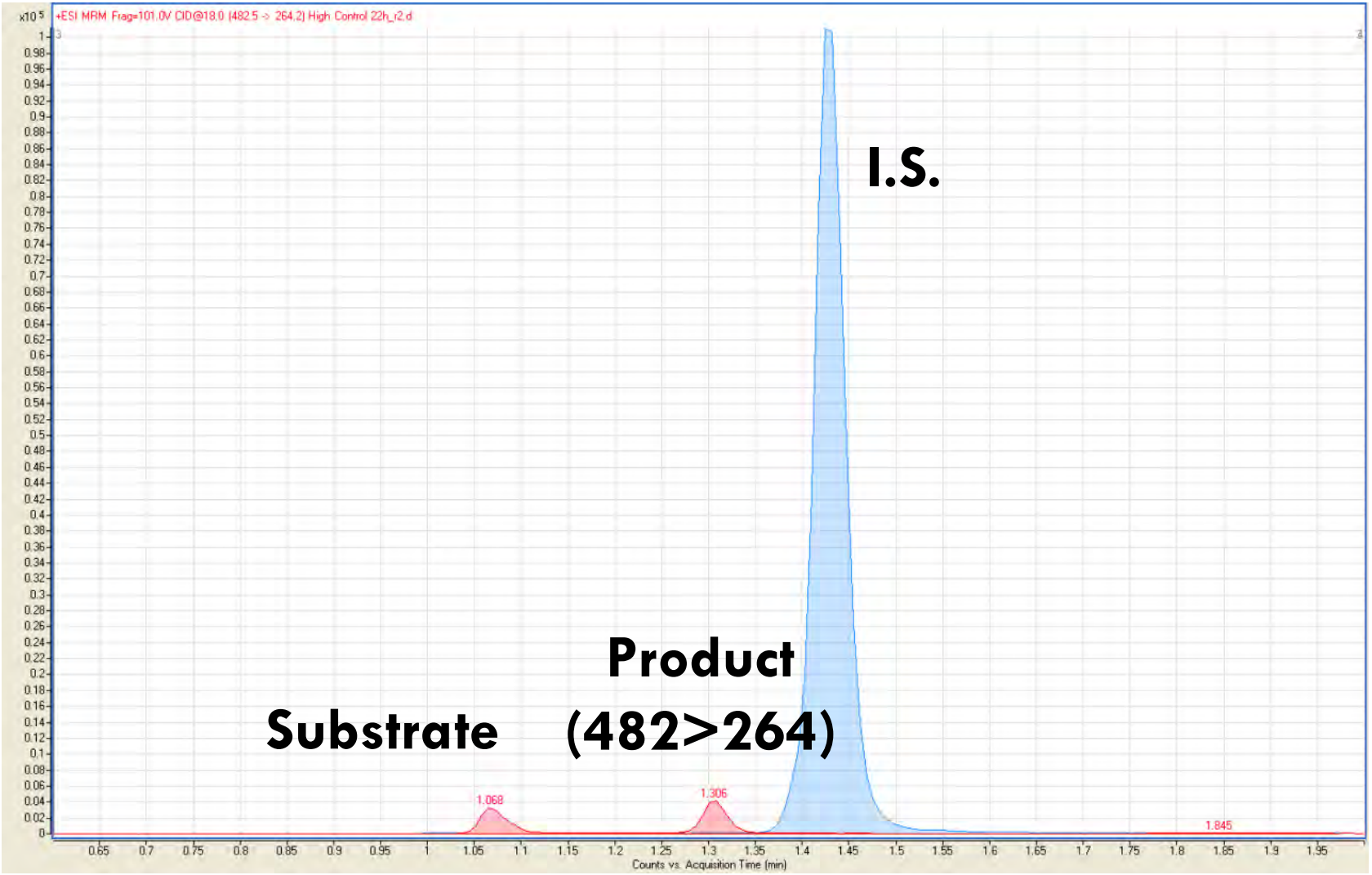


substrate



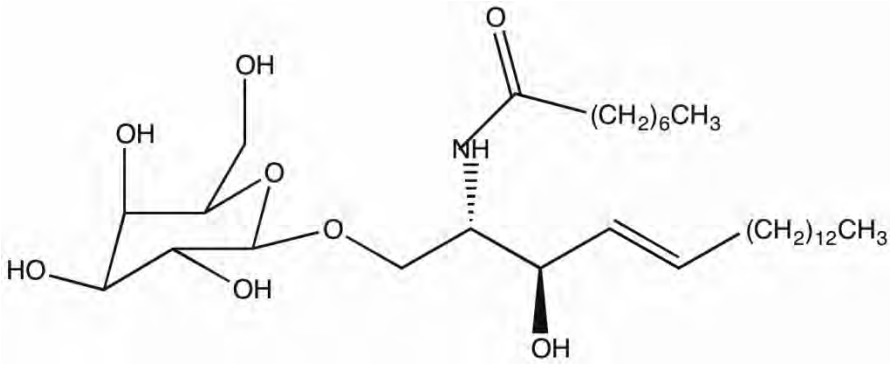
product

Gaucher Disease Testing: Results

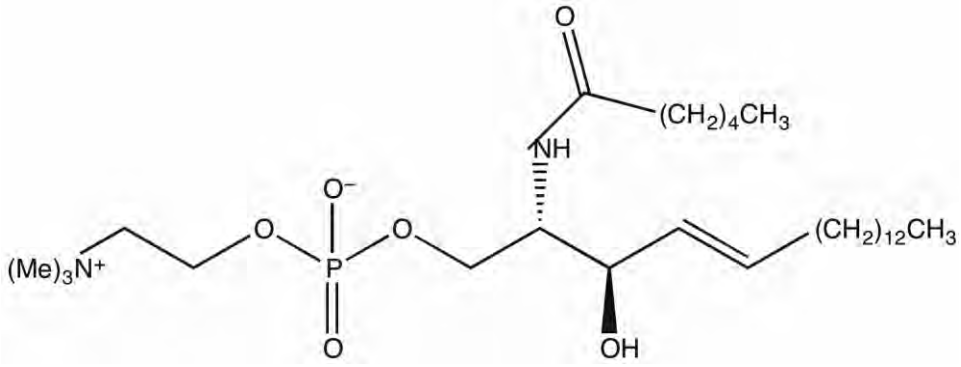


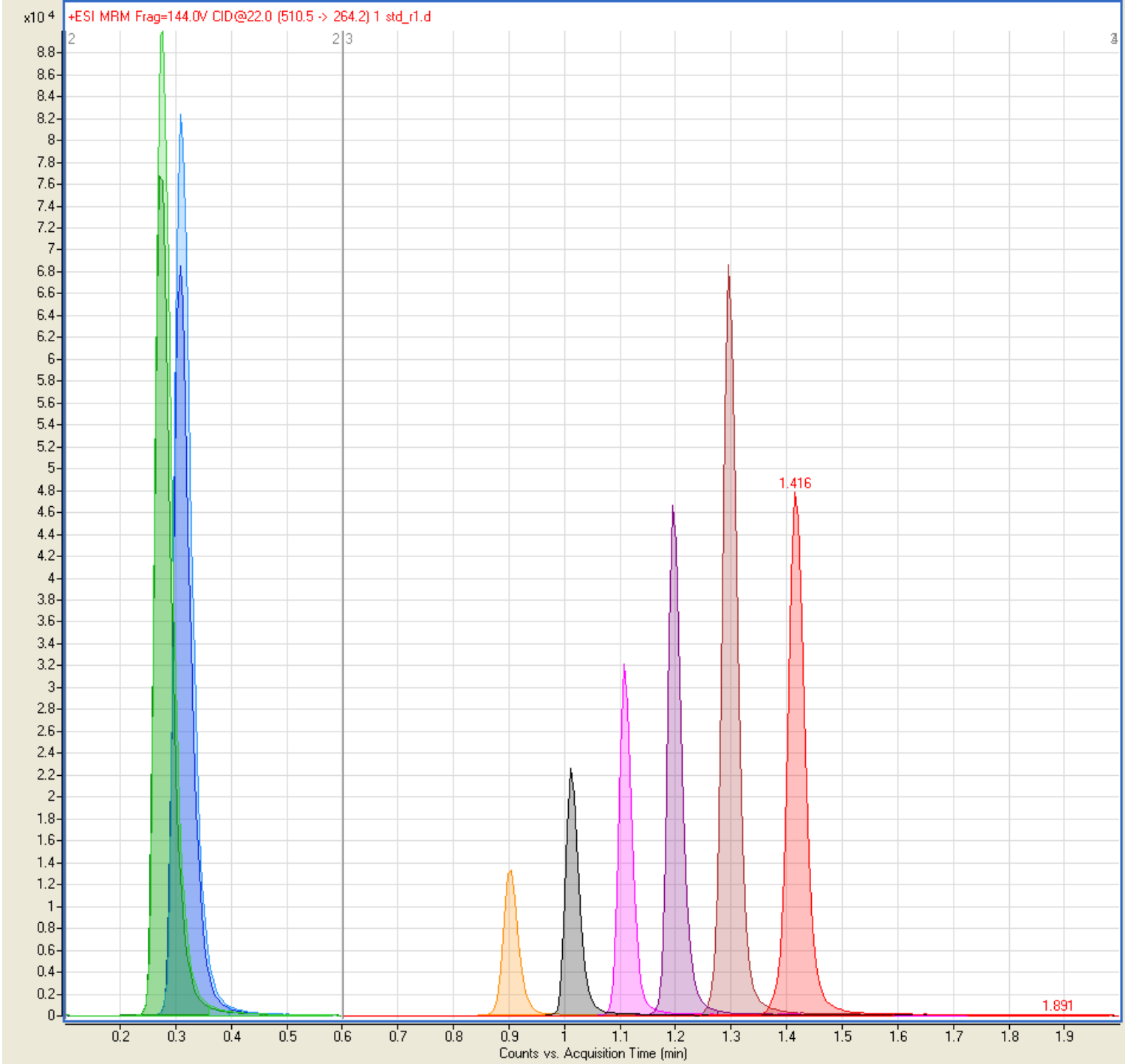
Other Lysosomal Enzymes

Krabbe disease



Niemann-Pick A&B




+ESI MRM Frag=144.0V CID@22.0 (510.5 -> 264.2) 1 std_r1.d
x10 4
1.416
1.891
Counts vs. Acquisition Time (min)

MS/MS in the Clincial Lab: *Ongoing challenges*

- Sample prep
- Choice of standards
- Chromatographic conditions
- Ease of operator use
- Software
  - Clinical lab workflow
  - Peak review
  - Report generation, interface

Thanks to…..
Tony Le
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Tony Kway
Kristina Cusmano-Ozog