

February 7, 2006 Expert Access Program:

Tandem Mass Spectrometry (MS/MS) Screening for Inborn Errors of Metabolism

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Mass Spectrometry in Medicine

GC/MS

- The historical keystone of Mass Spectrometry in Medicine.

Used for more than 20 years for the diagnosis of numerous genetic disorders of metabolism

- Amino Acids
- Organic Acids
- Fatty Acids
- Carbohydrates
- Phospholipids
- Steroids

- Sample matrix was primarily urine.

The Metabolic Profile

- GC/MS introduced the notion of the metabolic profile.
 - Examination of numerous metabolites in a single analyses.
 - One or more metabolites were indicative of a disease.
 - The use of libraries for compound identification utilized extensively.

Limitations of GC/MS

- Require volatile compounds
 - Small molecules
 - Non-polar
 - Polar + derivatization = Non-polar
- Sample Analysis Rate
 - Max is 2-4 samples per hour per instrument
- Extensive Fragmentation
 - Good (structural, spectral identification)
 - Bad (Lose parent ion, MW information)

Newborn Screening Today

- Tandem Mass Spectrometry
 - Fatty Acid Disorders
 - MCAD, VLCAD, LCHAD, MADD, etc.
 - Amino Acid Disorders
 - PKU, MSUD, Tyr, Cit, ASA, etc
 - Organic Acid Disorders
 - PA, MMA, IVA, GA-1, 3-MCC, etc

High Throughput Screening

- Automate patient information.
- Automate specimen sampling.
- Automate specimen preparation.
- Automate specimen injection.
- Automate MS acquisition.
- Automate MS quantitative data processing.
- Automate Clinical Interpretation.
- Automate Clinical Reporting.

METHODOLOGY

Automated Sample Preparation

One 3/16th in. blood disk

Butyl ester derivatization for enhance sensitivity, selectivity, and throughput.

Batched microtiter plate automated sample handling and preparation.

Automated Sample Injection

Two minute analysis time.

Microtiter plate format.

Small sample volume automated injection (10 μ L sample loop)

Low sample carry-over, versatile multiple platforms

High Throughput Electrospray MS/MS

Low Flow Rate (<20 μ L/min) for enhanced sensitivity.

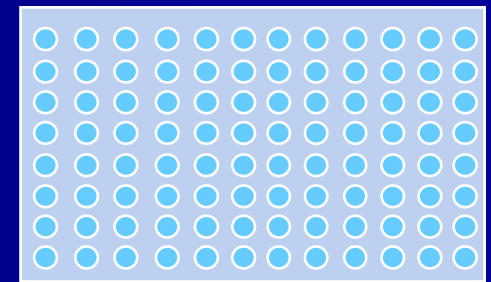
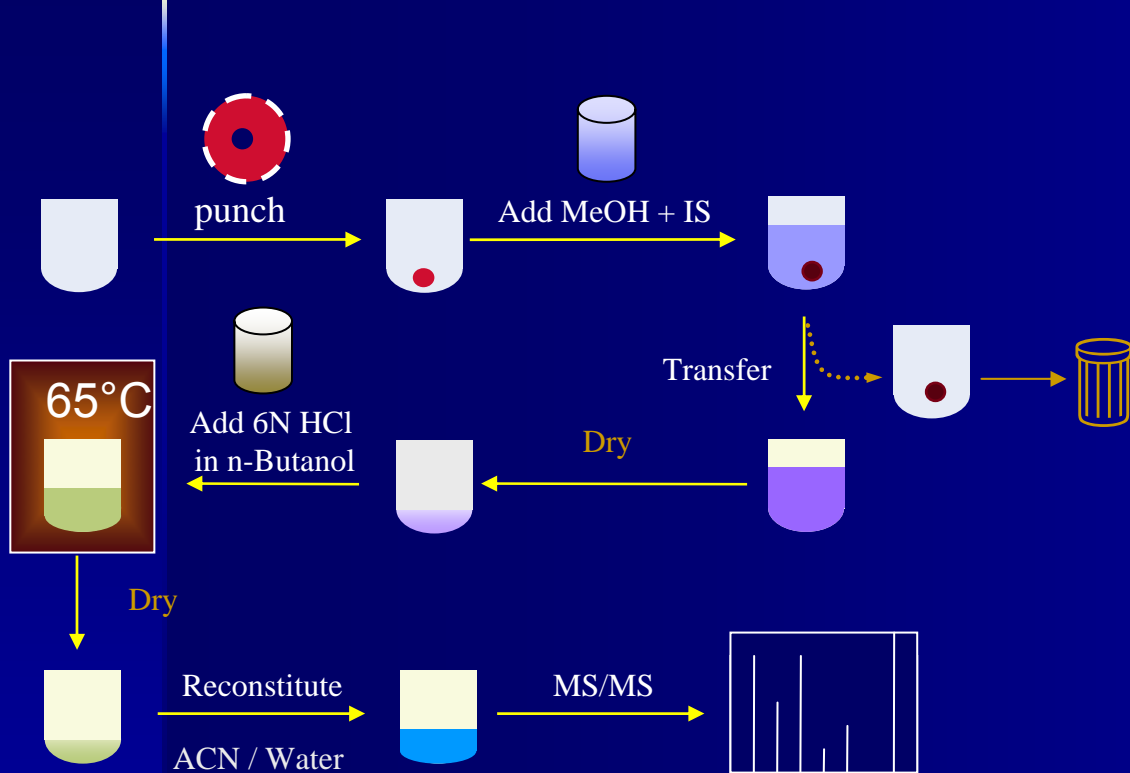
Robust, sensitive, low maintenance systems. 120,000 specimens analyzed per year per instrument.

Comprehensive multi-function MS/MS panel

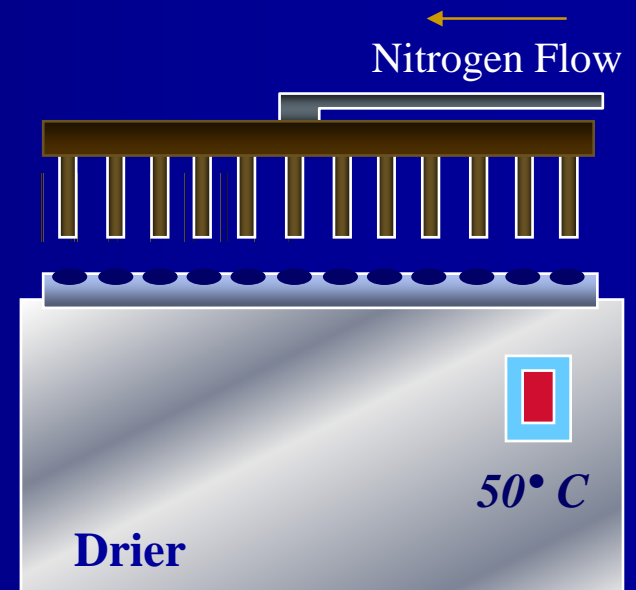
Automated data and result processing.



Sample Preparation



400 µL Round Bottom Plates



Automated Sample Injection

- 10 μL loop injections.
- Low dead volume Rheodyne Valves.
- Microflow Rates at 15-20 $\mu\text{L}/\text{minute}$
 - 50:50 Acetonitrile:Water w/ 0.025% HCOOH
- Capillary from injector valve through tip.
 - Maintains low dead volume.
 - Eliminates particulate blockage of injector.

Ionization

- Started in the early 1990's with Fast Ion Bombardment (FIB).
 - Manual loading with sample dissolved in glycerol/methanol matrix
 - ~40,000 samples analyzed/instrument annually.
 - Dynamic FIB (Flow FIB) problematic after 100 specimens.
- Electrospray adapted in 1994
 - Improved Ionization efficiency
 - Increase sample throughput significantly

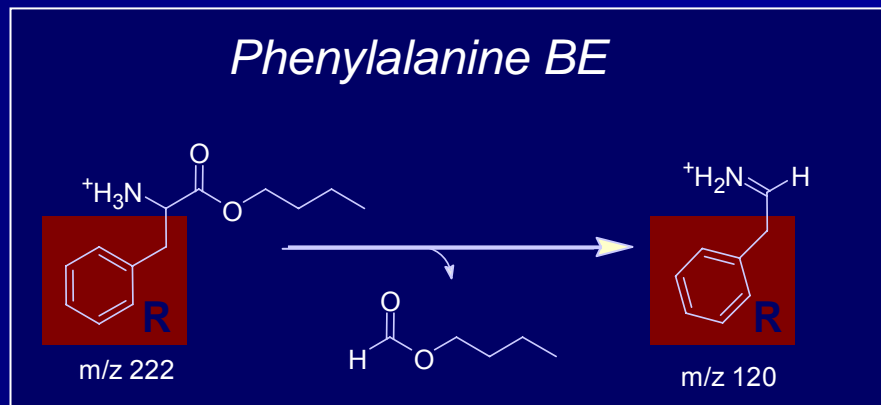
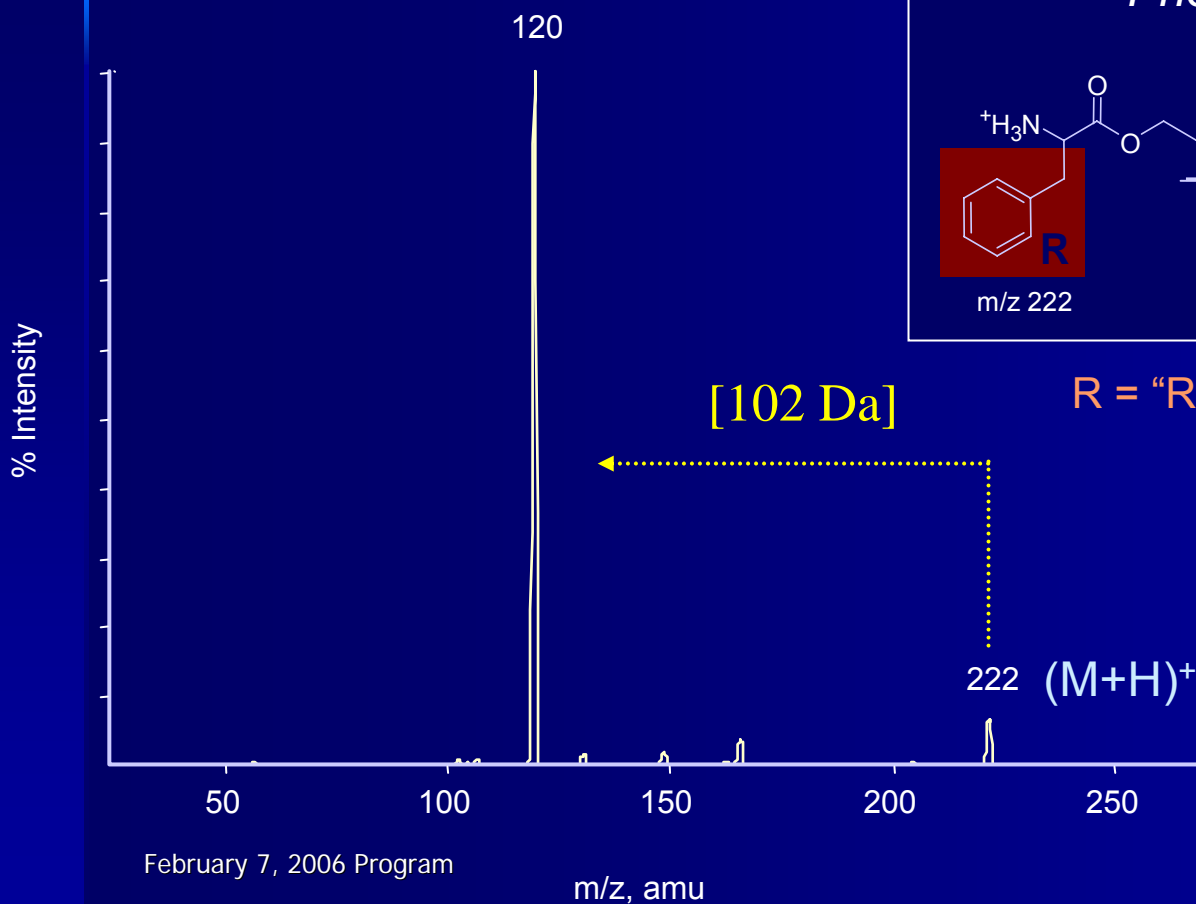
High Impact MS/MS

- High Impact differs from High Throughput
 - One Disease, One Analysis is not cost effective.
 - Many diseases, One Analysis is cost effective
 - Multiple Metabolite, Multiple Disease Screening.
 - Identify amino acid disorders from amino acid profile.
 - Add different disorder groups, Fatty Acid and Organic Acid Metabolism Disorders.

Tandem MS of Amino Acids

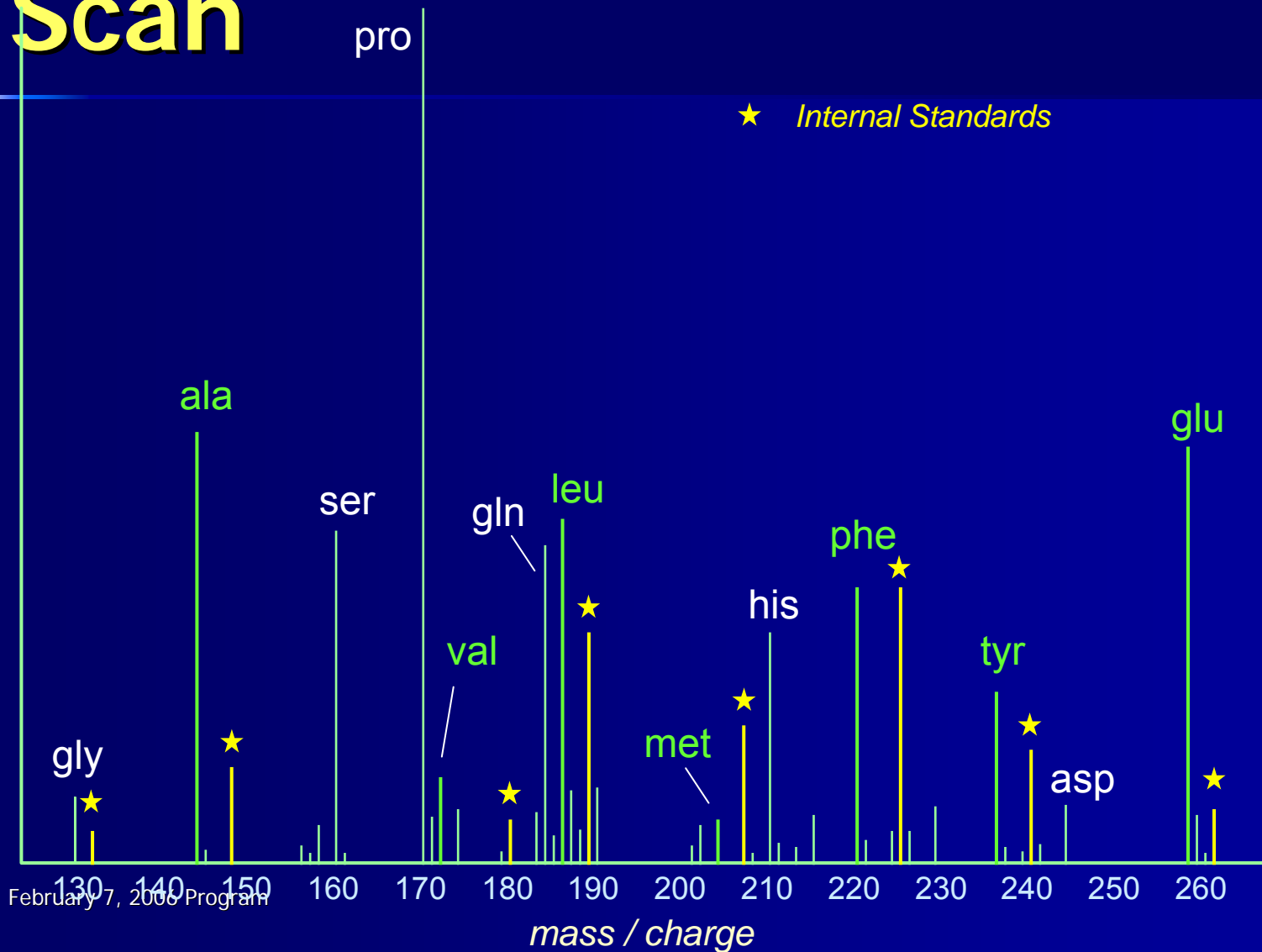
- Challenge:
 - Analyze diagnostically important amino acids from a few μL of blood that is dried on filter paper.
 - Analyze 500 specimens per instrument per day.
 - Minimize False Results
 - No False Negative Results
 - Minimal False Positive Results

Product Ion Scans of Butyl Esters of Amino Acids



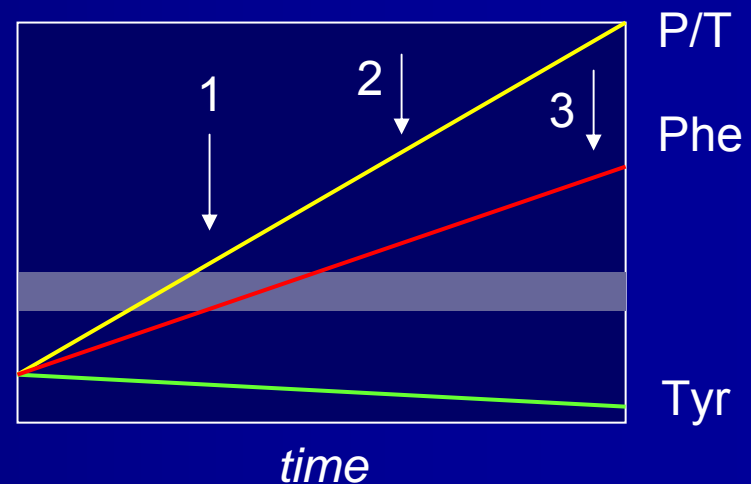
R = "R" group for Phenylalanine

Neutral Loss 102 MS/MS Scan



Tandem MS and PKU Detection

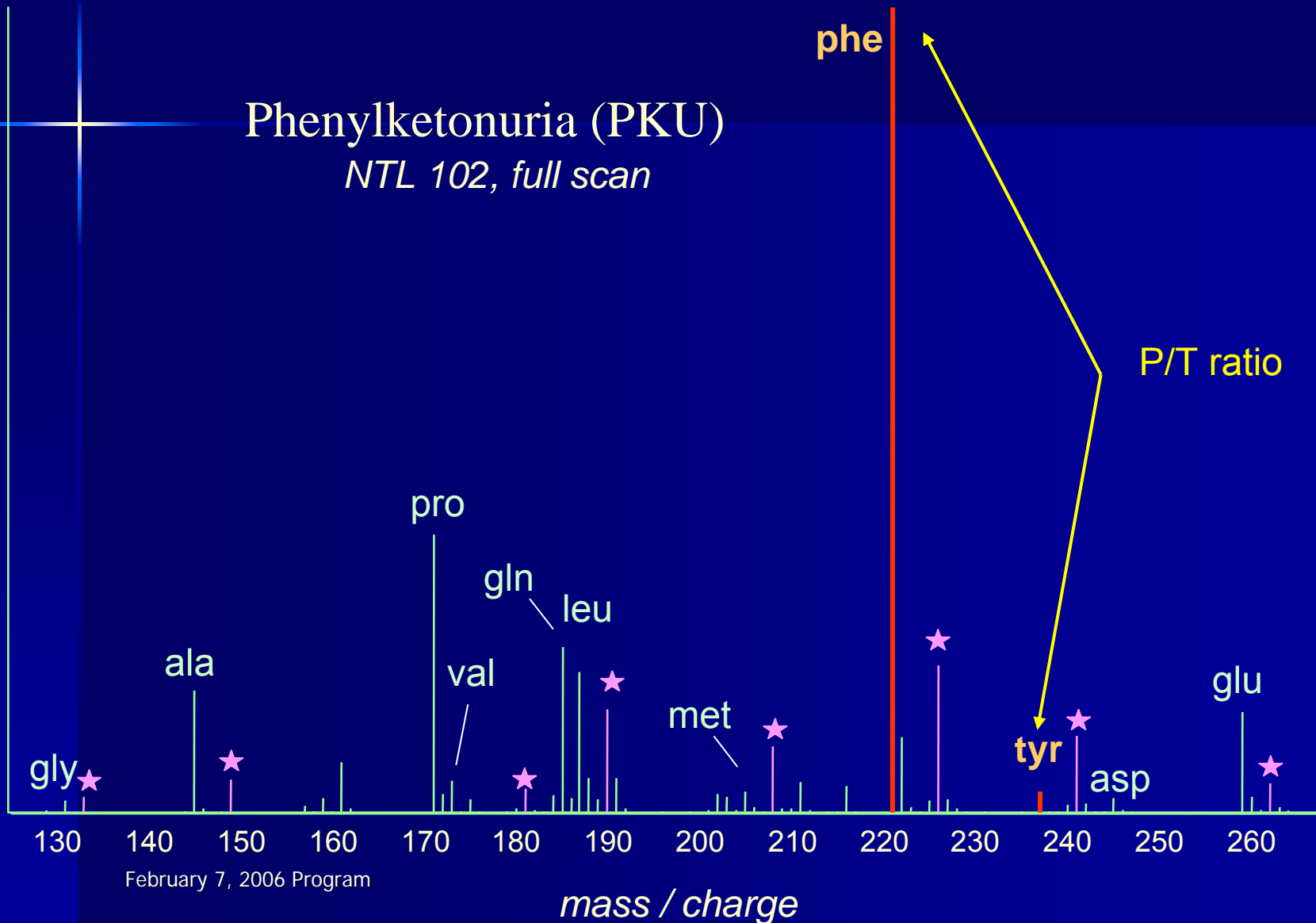
- Measure both Phenylalanine and Tyrosine
- Obtain a "Molar" Ratio for Diagnosis.
- Higher Precision and accuracy enables detection of PKU less than 24 hours.
- False + rate <0.01%



Diagnosis: PKU

Phenylketonuria (PKU)

NTL 102, full scan

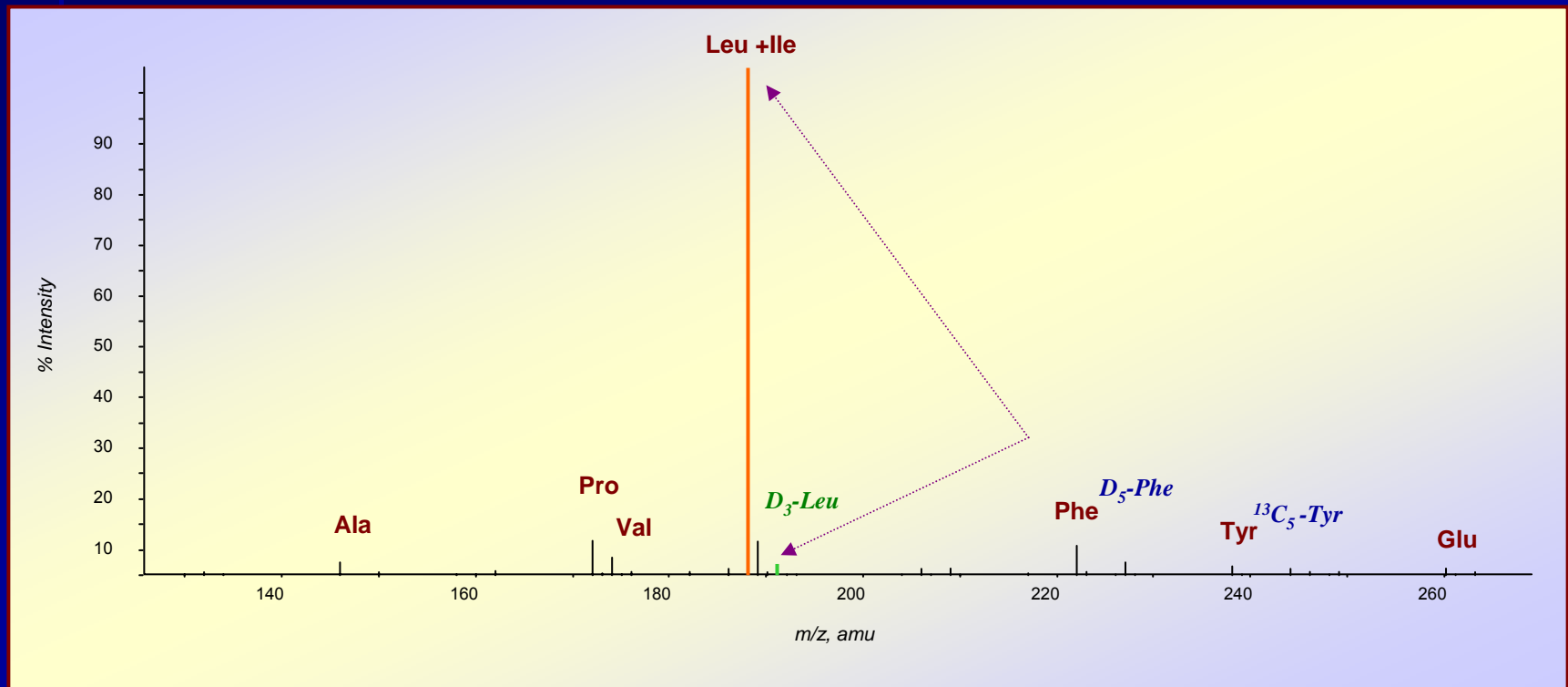


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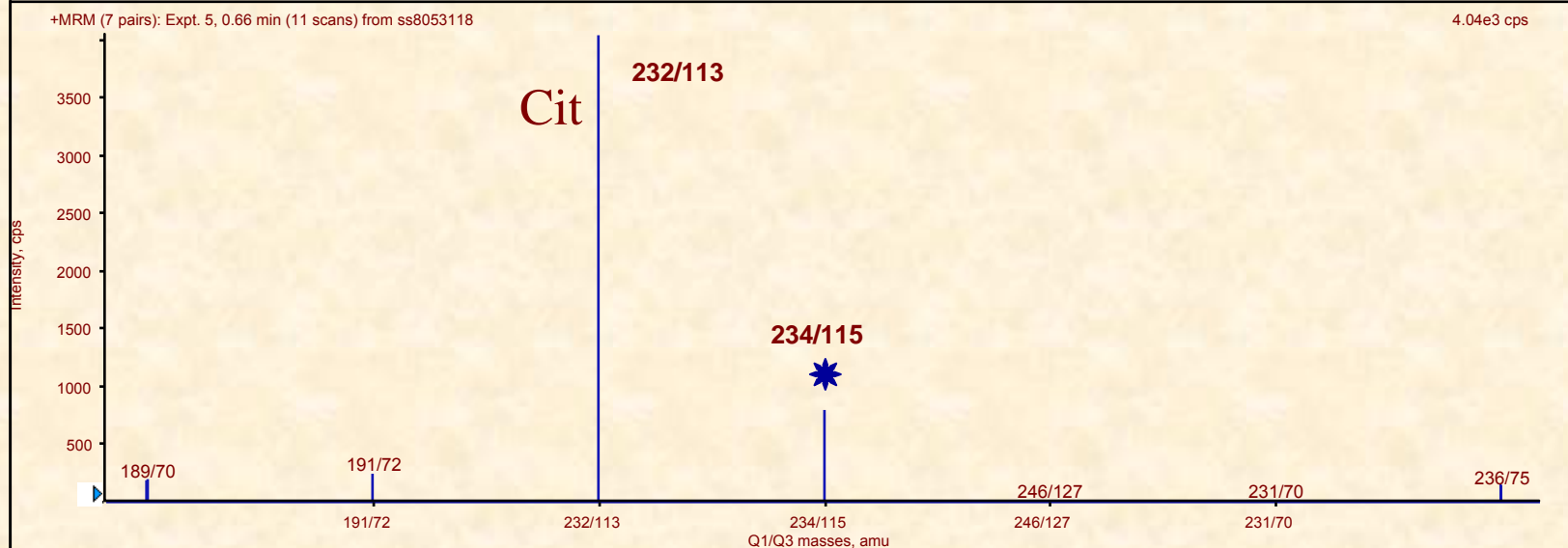
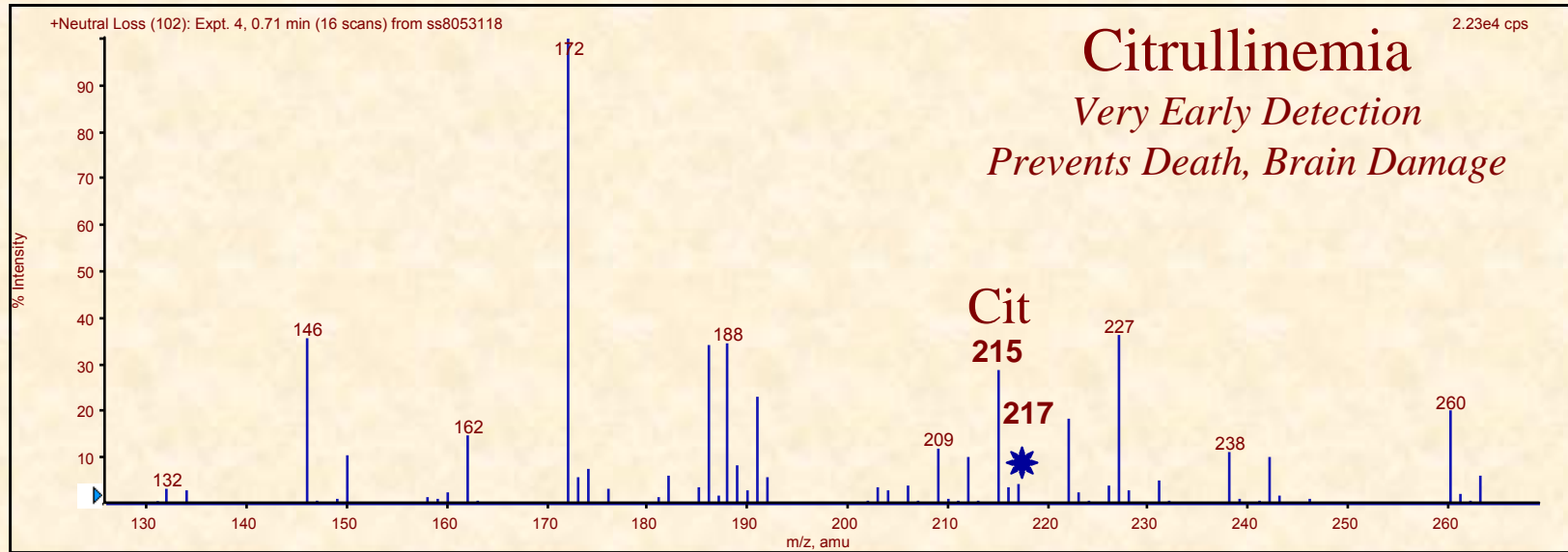
An Example of Other Amino Acid Disorders

MSUD

Maple Syrup Urine Disease

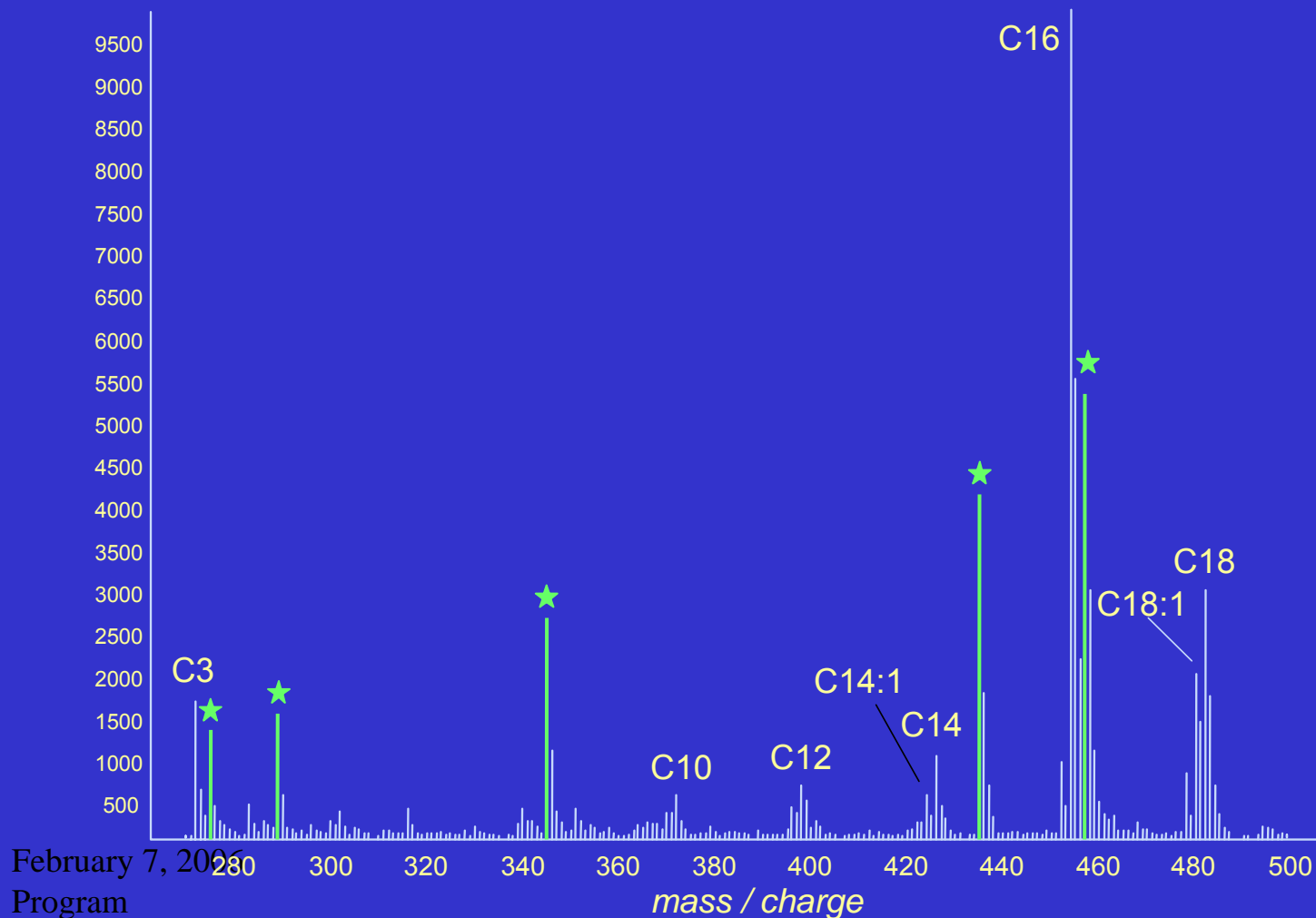


Citrullinemia



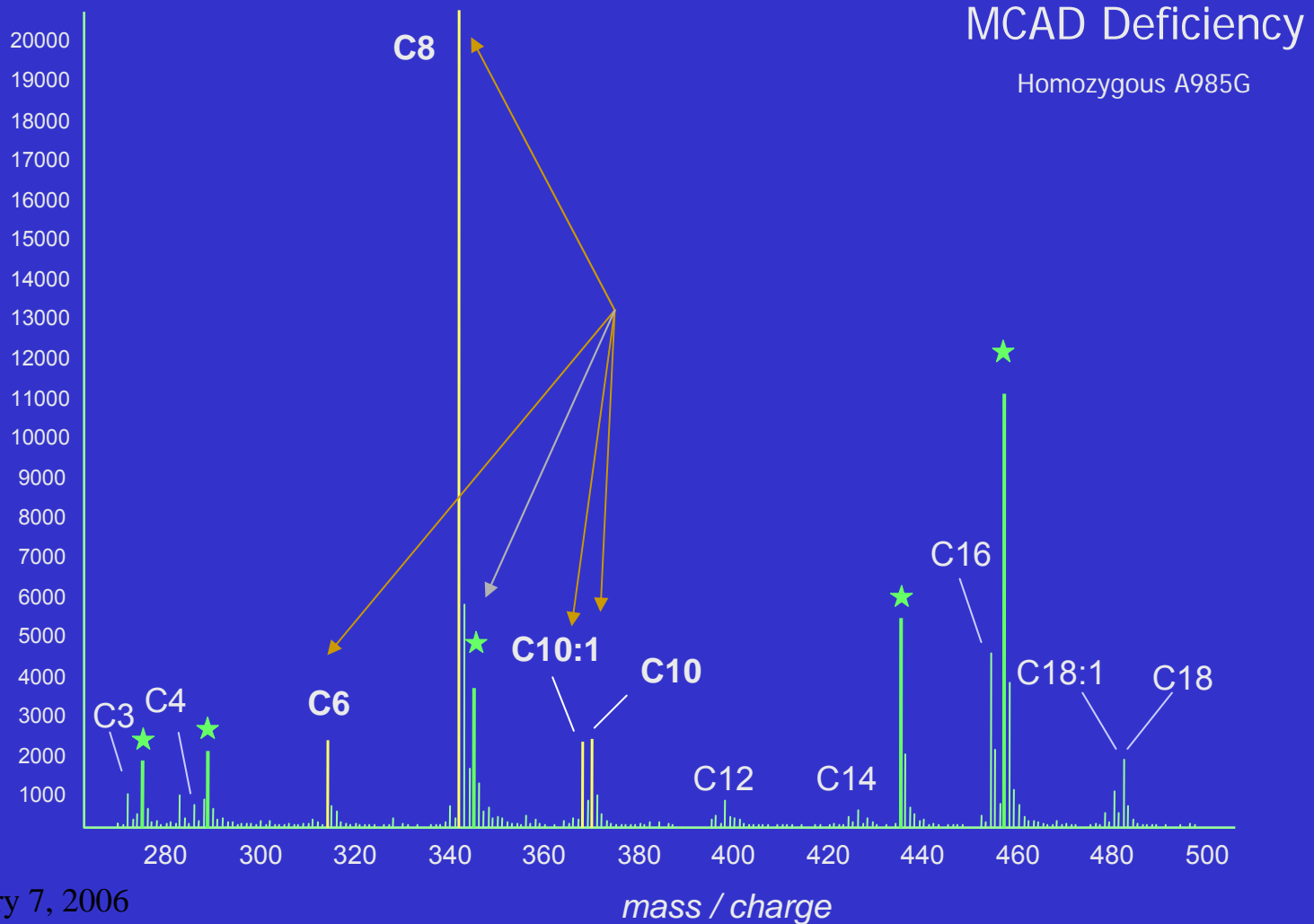
Acylcarnitine Profile (normal)

* Deuterated Internal Standards



Diagnosis: MCAD Deficiency **

* Deuterated Internal Standards

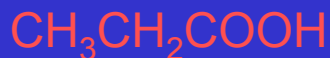


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Organic Acid Metabolism

- Disorders of organic acids – metabolites of amino acid and other endogenous substances.

Propionic Acid

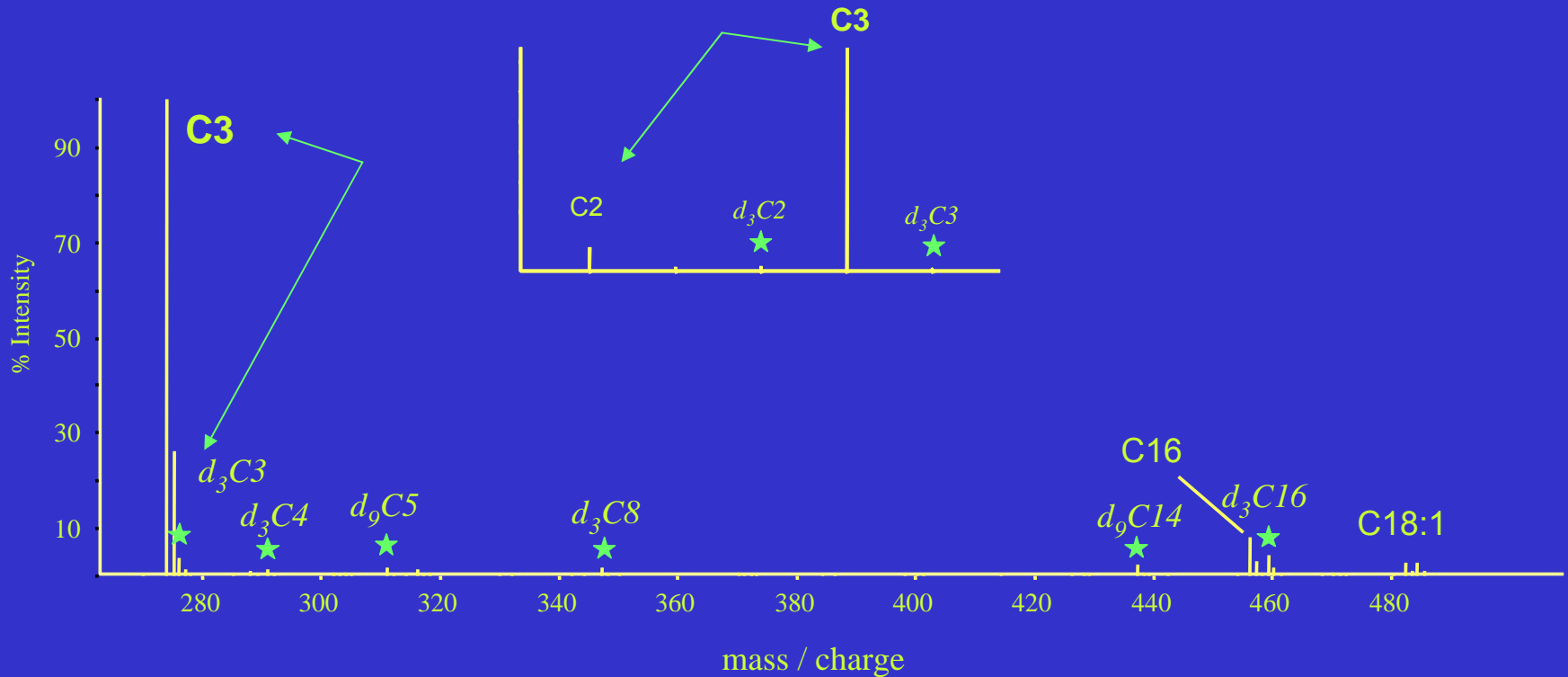


Glutaric Acid



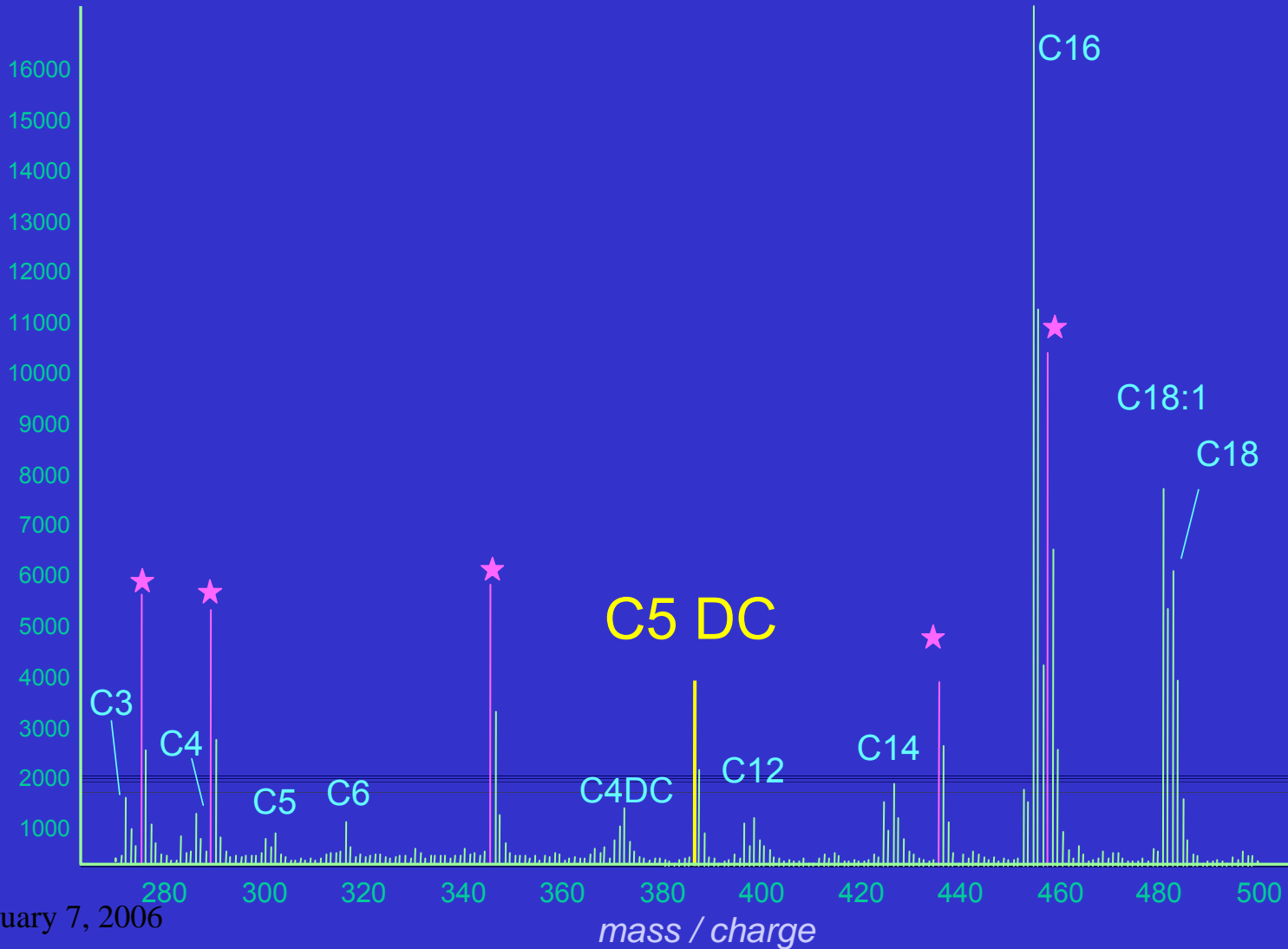
Diagnosis: Propionic Acidemia

* Deuterated Internal Standards



Diagnosis: Glutaric Acidemia

* Deuterated Internal Standards



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Multi-Component Analyses

6 Profiles, 410 masses, 60+ concentrations, 20+ diseases

Free Carnitine Profile

Precursors (Parents) of 103 Da

MRM

Acetylcarnitine Profile

Precursors (Parents) of 85 Da

MRM

Acylcarnitine Profile

Precursors (Parents) of 85 Da

Full Scan

Neutral Amino Acid Profile

Neutral Loss of 102 Da

Full Scan

Basic AA Profile

Neutral Loss of 119 Da

MRM

Arginine Profile

Neutral Loss of 161 Da

MRM

Why MS can make a difference.

Not Screened
Affected
GA-I

Screened
Well
GA-I

Screened
Well
GA-I



Amnio Acid Disorder Totals and Frequency (MS/MS)

■ **Amino Acid Disorders** **170**

n = 1,250,000

Frequency 1/7406

Organic Acid Disorder Total and Frequency (MS/MS)

■ Organic Acid Disorders

52

n = 1,250,000

Frequency 1/21,332

Fatty Acid Oxidation Disorder Totals and Frequency (MS/MS)

- **Fatty Acid Oxidation Disorders** **76**
n = 1,250,000

Frequency 1/14,596

Totals and Combined Frequency (MS/MS)

■ Amino Acid Disorders	170
■ Organic Acid Disorders	52
■ Fatty Acid Oxidation Disorders	76
TOTAL	298

Combined Frequency:

1 positive case per 4,225 newborns

Treatment Strategies

- Dietary change, avoidance
- Pharmacological
(drugs, vitamins, cofactors)
- Dialysis, glucose
- Enzyme replacement

MS/MS Detection of Untreatable Disorders

- Mutase⁰ Methylmalonic Aciduria
- Carnitine Palmitoyltransferase II

Newborn Screening Tomorrow

- Primary DNA Screening
- High Throughput Screening
Robotics
- Multiplexing of Screening Tests

Tenets of Newborn Screening

- Detectable in the newborn period
- Detectable in dried blood spots
- Low False Positive and False Negative rate
- Treatable or manageable condition
- Parents, Genetics, Family Planning

Expansion of MS/MS

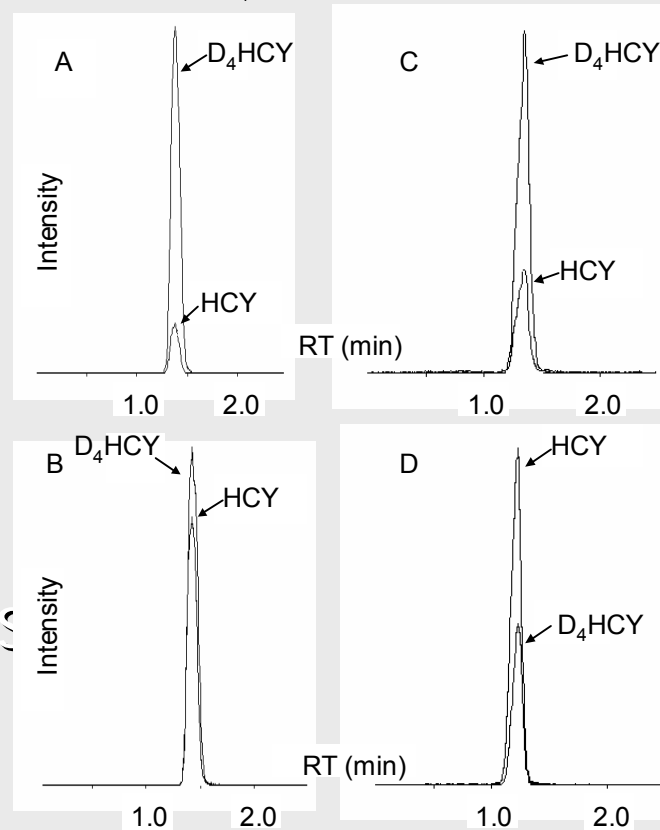
- Metabolome
 - Homocysteine
 - Bile Acids
 - Steroids
 - Very Long Chain Fatty Acids
 - Complex Carbohydrates
 - Thyroid Hormones

Courtesy of P. Rinaldo et al.

SRM (Precursor / Product)

HCY (136 / 90)

D₄-HCY (140 / 94)



Chemical Reviews **101**, 2001