

Amino Acid Analysis by HPLC/Ninhydrin and Tandem Mass Spectrometry Detection

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HPLC with Post-Column Ninhydrin Derivatization

Tried and true, but still slow.....

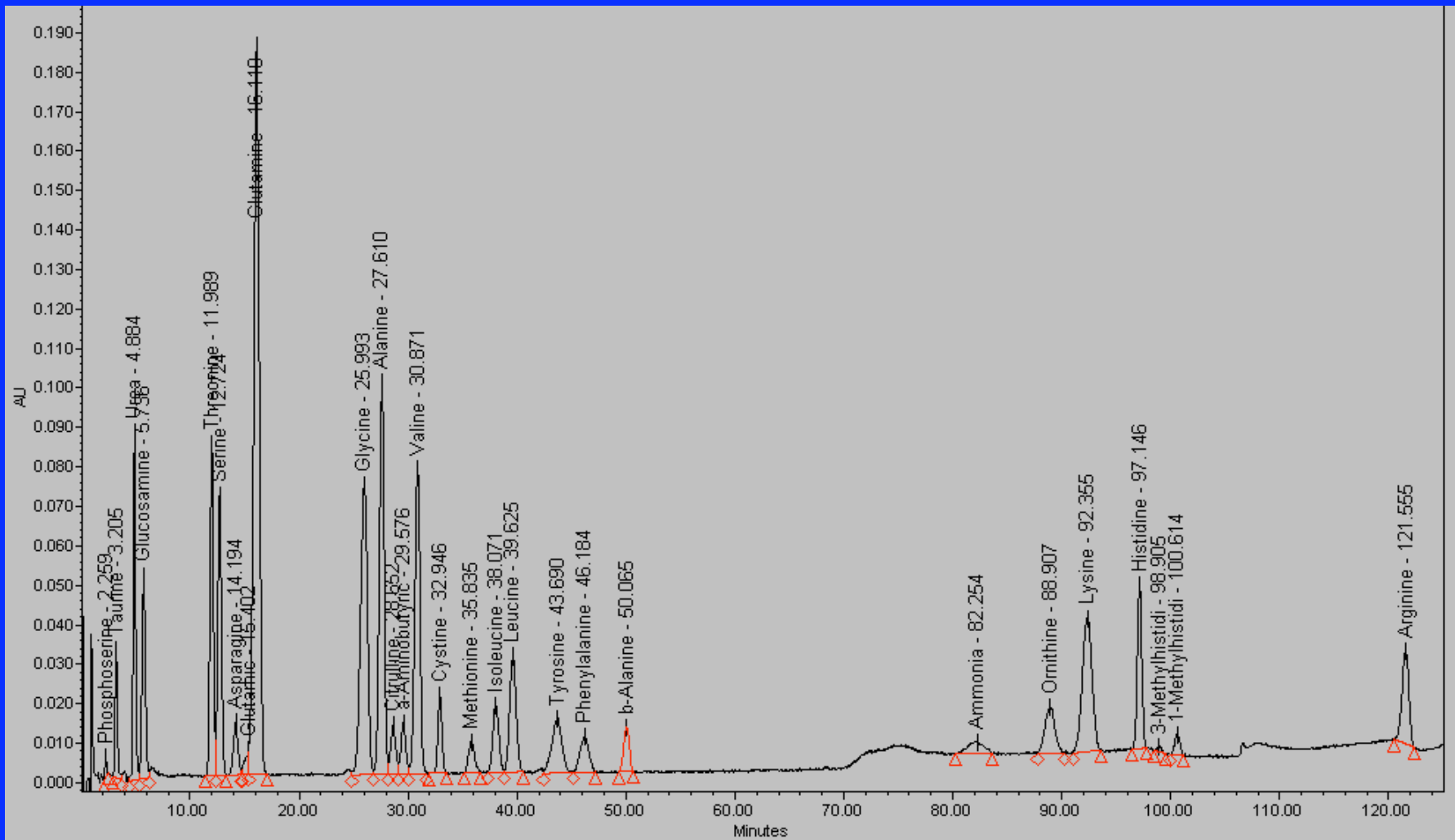
Specifications

- Waters HPLC System for Separation
- Lithium Cation-Exchange Column
- Pickering Post-Column Ninhydrin System
- Detectors
 - 570 nm for 1° amines
 - 440 nm for 2° amines

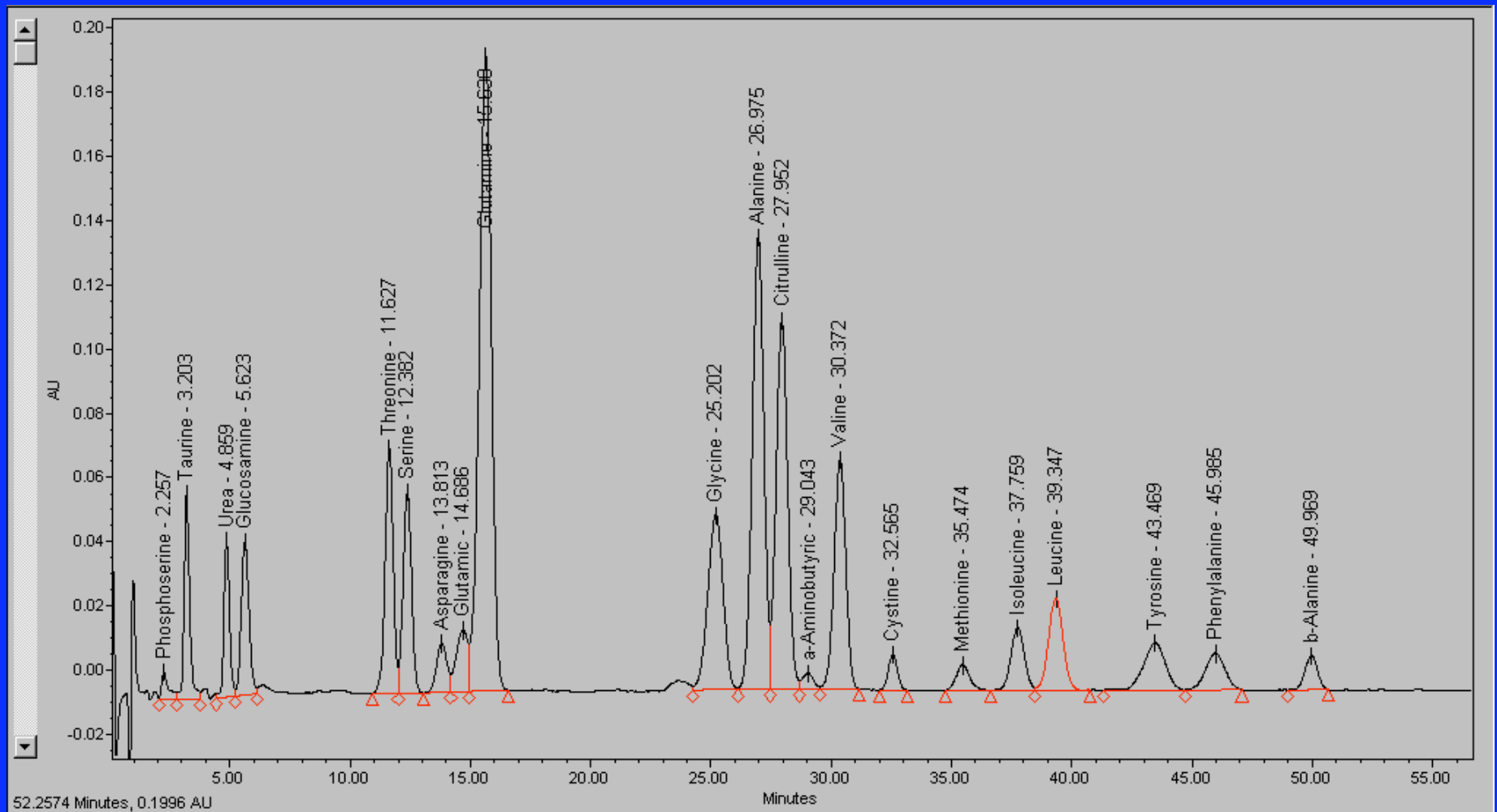
Parameters-Phe

- Plasma dynamic range 10-500 mM (calibrated)
- Normal range (0-18 yrs), 16-105 mM
- LOQ, LOD both ~ 5 mM
- 100 mM calibrator
- Accuracy 92.8 %
- Precision (CV) 6.1 %

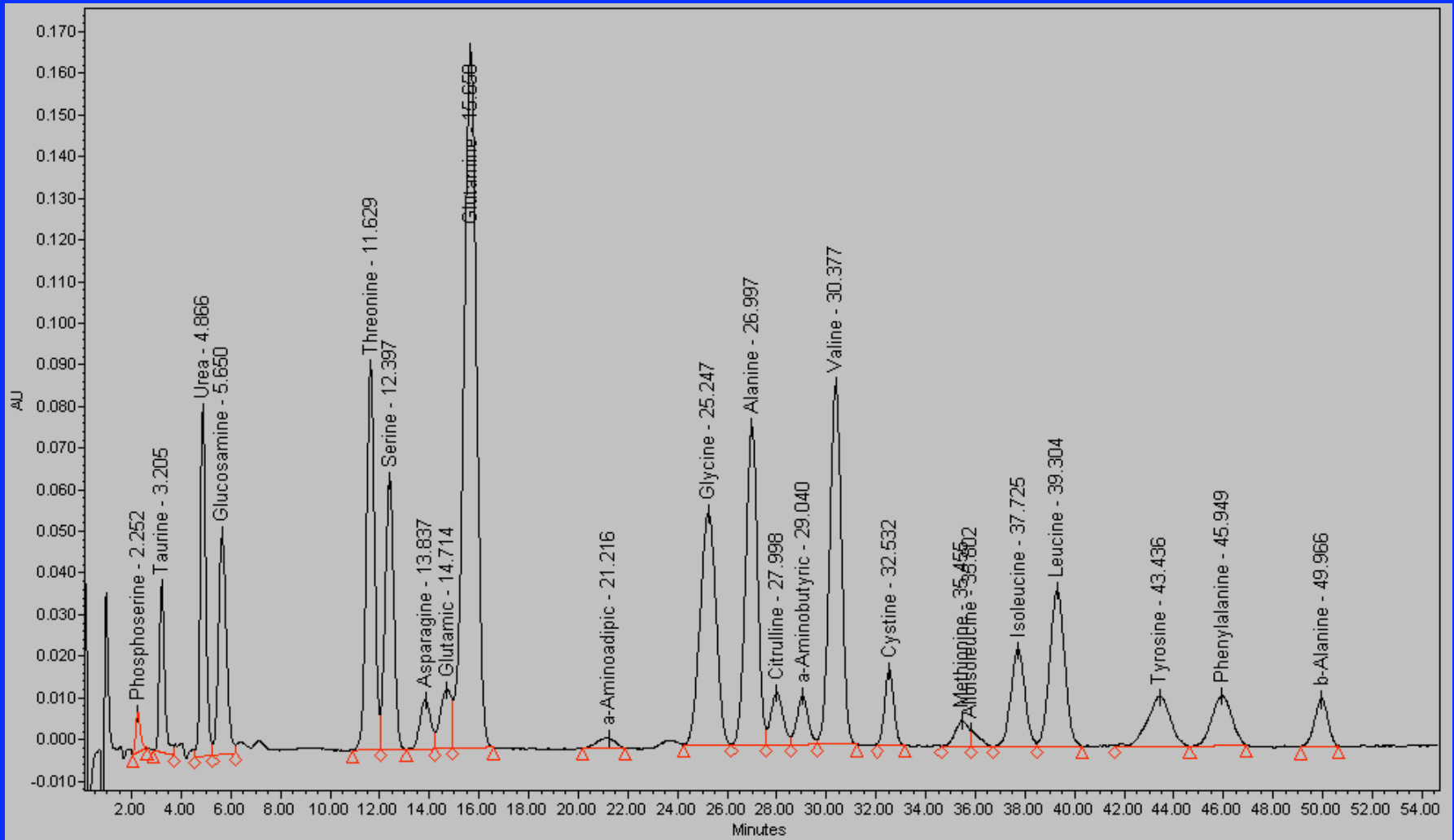
Normal



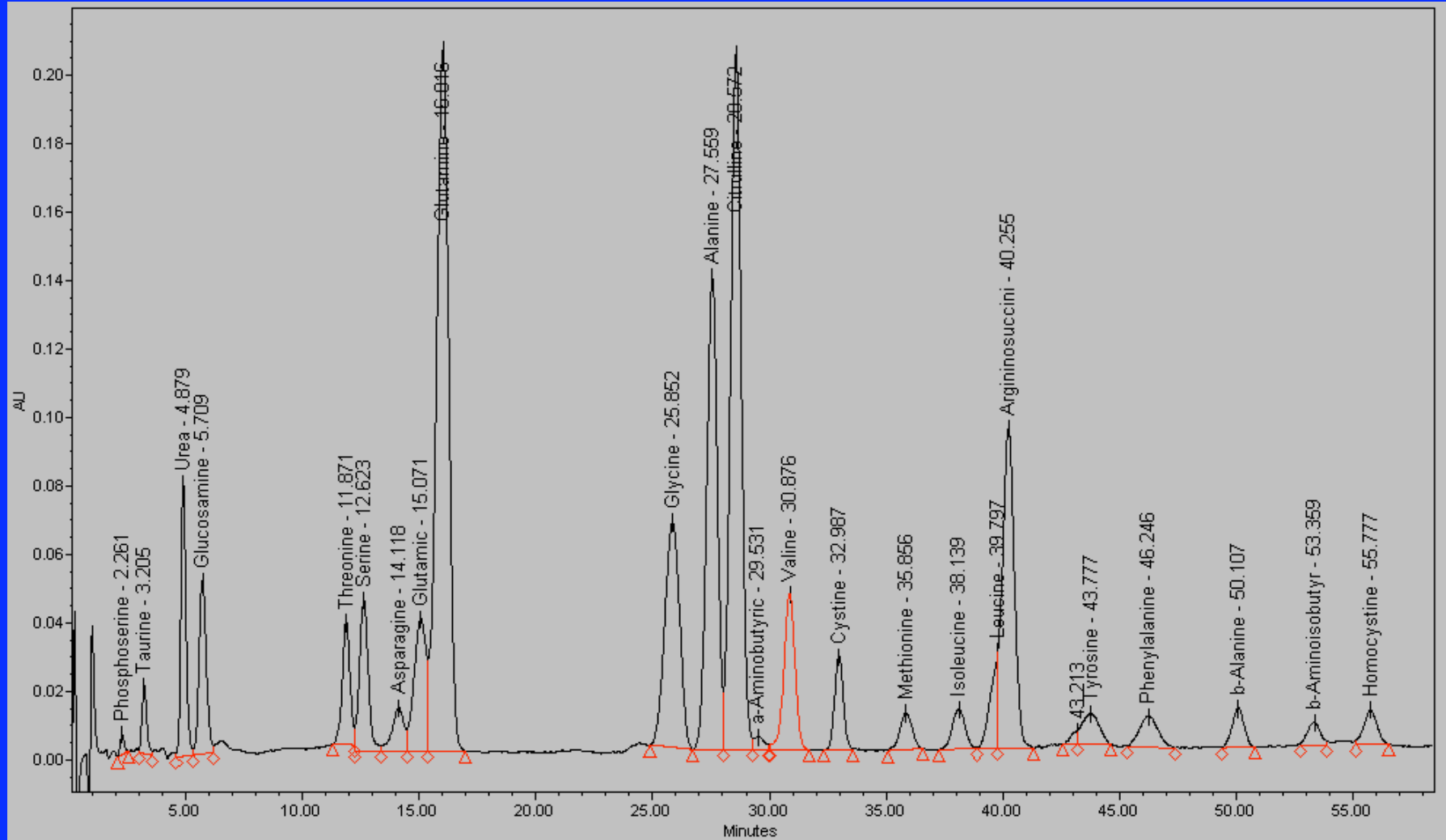
Citrullinemia



MSUD



ASA



Reagents/Maintenance

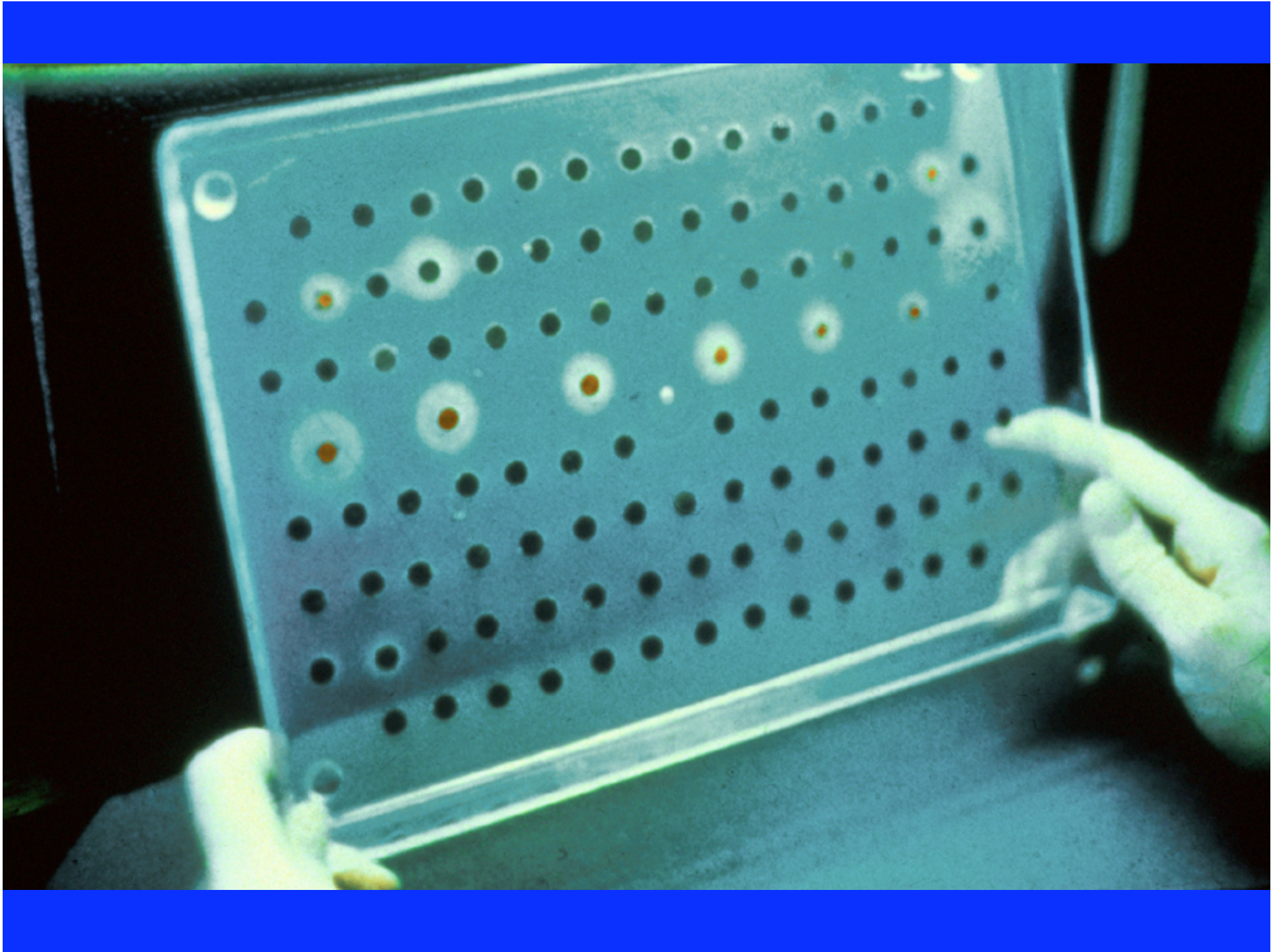
- Tech time @ \$17.35/hour=0.5 hour
- Run time 140 min.
- Reagents per run
 - Ninhydrin, buffers, seraprep or uriprep, standards, etc=\$8.15
- Instrument ~ \$80,000 total
- Maintenance contract ~ \$4,090/yr (PM, any breakage)-critical due to clinical samples

LiCitrate Pros/Cons

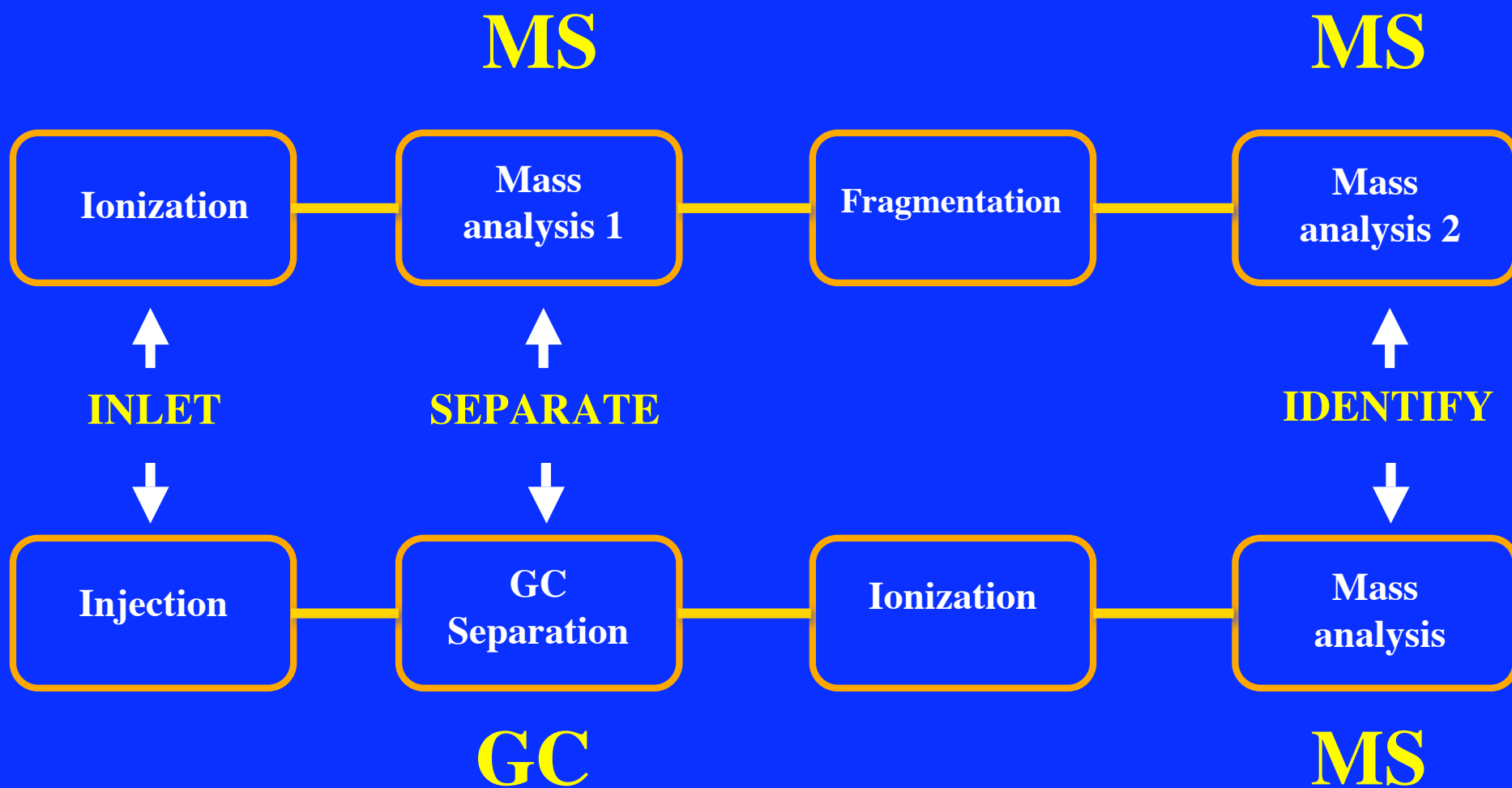
- Pros
 - All physiological amino acids, Good Specificity, Semi-Automated, Ability to Monitor Treatments
- Cons
 - Slow (2 hour run time), blood draw required, spin for separation of plasma
 - Li⁺ corrosive-replace lots of fittings, etc.

Tandem Mass Spectrometry

*The new wave of newborn screening
for inborn errors of metabolism....*

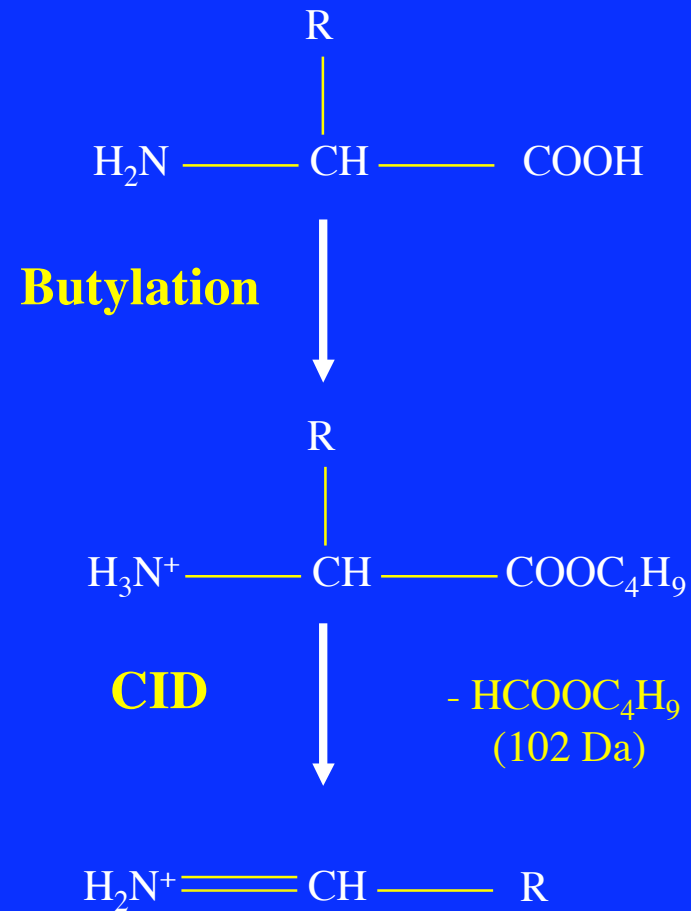


Tandem mass spectrometry vs GC-mass spectrometry

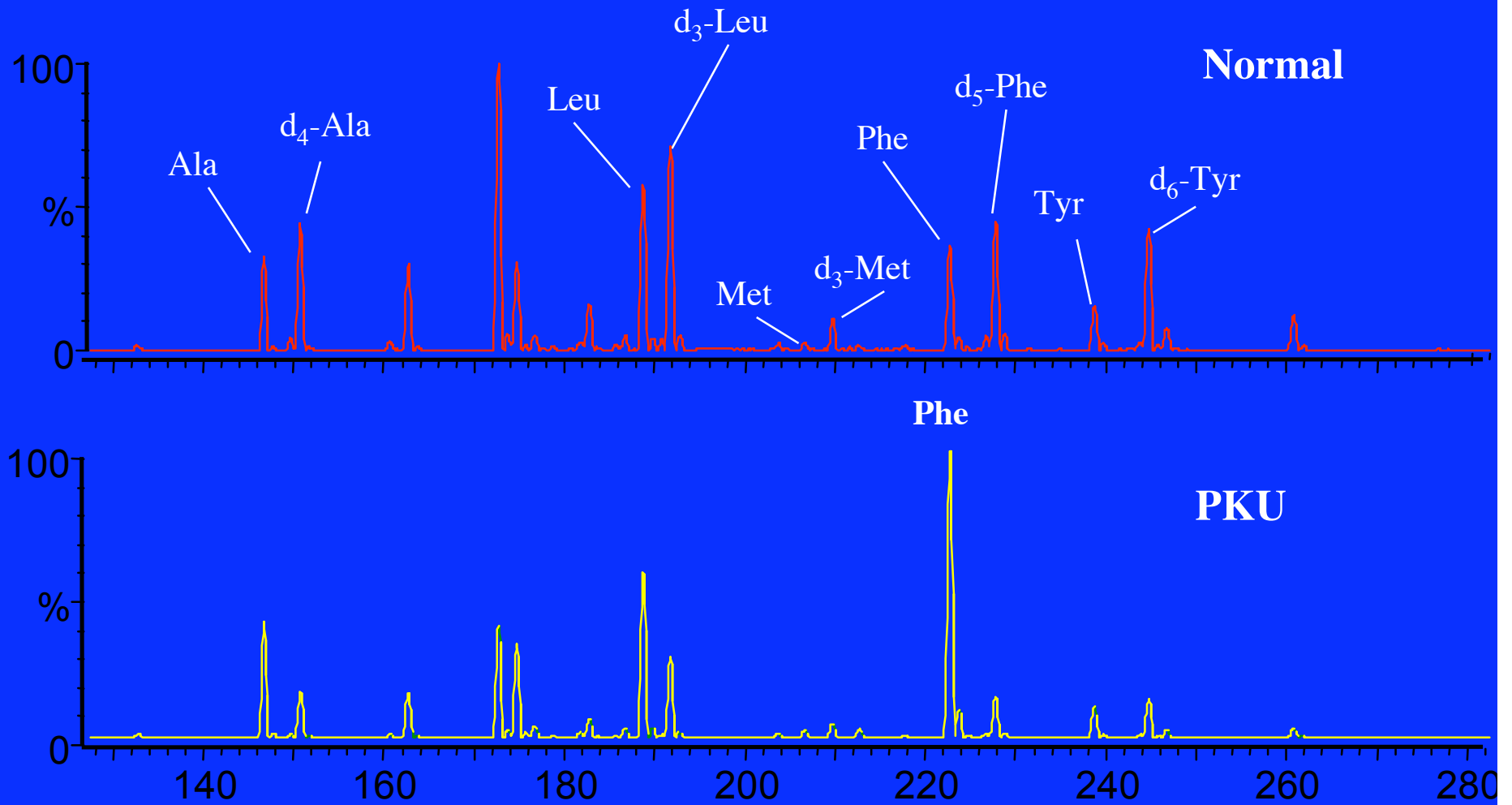


Neutral and Acidic Amino Acids

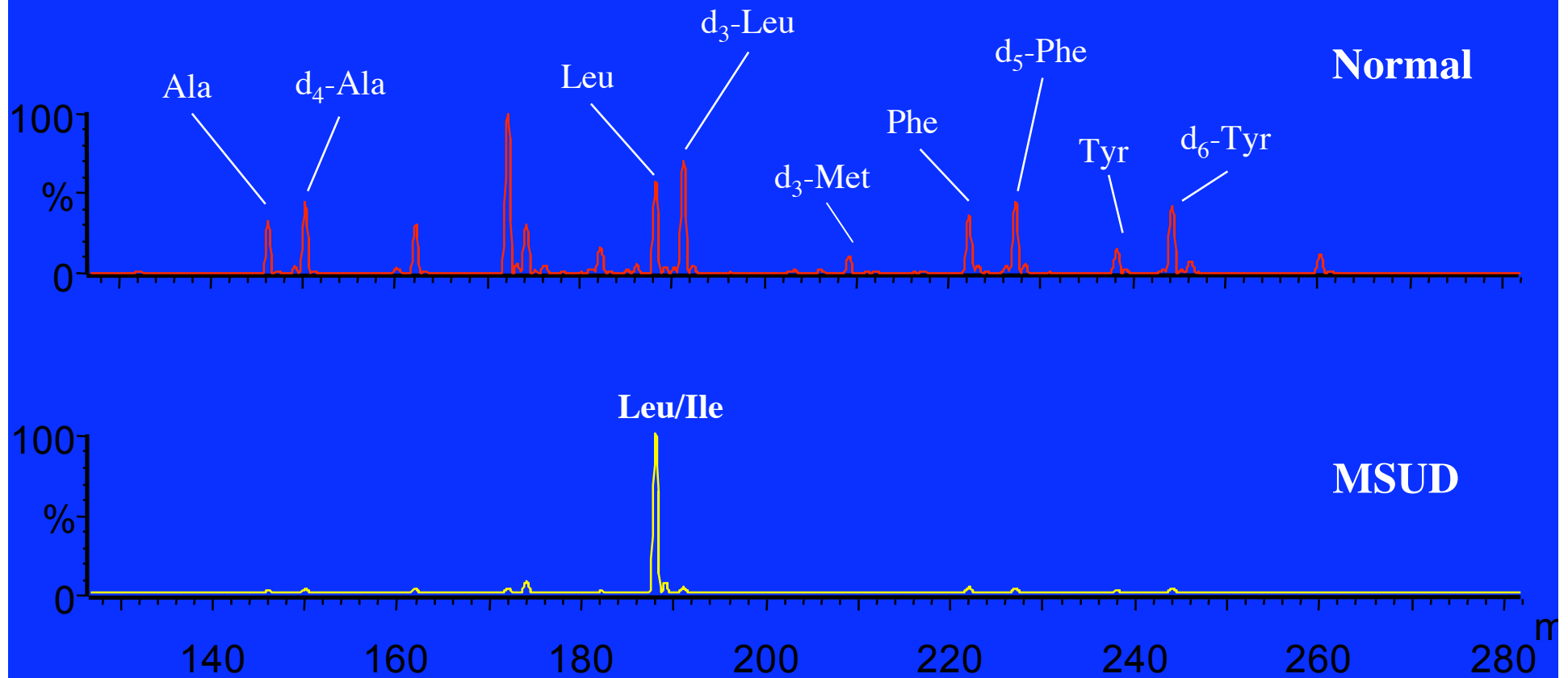
Derivatisation and Fragmentation



Normal vs PKU



Normal vs MSUD



MSMS

- Cost/specimen-(labor, lease, consultants, rent, utilities, reagents)-\$7.53
- OPHL Leases ~ \$70,000/yr lease (includes all repairs and PM).
- Only currently looking at Arg, Cit, Leu, Phe, Tyr, Met
- Blood spot-1/8 in (~3.1-3.2 mL whole blood)

Parameters-Phe

- Dynamic range 1-1265 mM (calibrated)
- LOD~0.1 mM, LOQ~0.2 mM
- 250 mM calibrator
- Accuracy 98.6 %
- Precision (CV) 11.8 %

Labor

- Labor for 1044 specimens=3 MT and 1 MLT/day
- 1 MT can process 3 “96” well plates (261 specimens) in 2 hours
- MSMS can read one plate in 2 hours
- Gilson 215 autosampler-holds 10 plates (960 samples) for 20 hours run time

MSMS Pros/Cons

- Pros
 - Rapid (2 min analysis), Sensitive, Specific, Semi-Automated
- Cons
 - Expensive equipment (\$215,000), limited number of amino acids detected, lack of isomer separation (I.e., leucine/isoleucine)