Mass Spectrometry in Clinical Chemistry

David Hardy

What is Mass Spectrometry?

- The separation of ions on the basis of mass to charge ratio (m/z)
- Gas phase technique requiring high vacuum
  - Non-volatile compounds require conversion to volatile derivatives

Uses and Problems

- Uses
  - Structural elucidation
    - Masses and fragmentation patterns
  - Identification
    - Fragmentation characteristic of compound
    - Library searches/peak matching
- Problems
  - Isomeric/isobaric interference (esp. with soft ionisation methods)

Instrumentation

- Components of mass spectrometer
  - High vacuum system
    - Rotary backing pump plus turbomolecular or diffusion pump
  - Inlet and ion source (forms ions)
  - Analyser (separates ions)
  - Detector (records ions)
  - Computer control
    - Data acquisition
    - Data manipulation

Pre-analysis Considerations

- Volatile compounds
  - Analyse without modification
- Non-volatile compounds
  - Convert to volatile species
    - Alkylsilyl deriviatives (Me_3Si (TMS); $^tBuMe_2Si$ (tBDMS)
    - Esters
    - More unusual species (hydrazones, etc)

Ion Sources

- Electron impact (EI)
- Chemical ionisation (CI)
- Atmospheric pressure ionisation (API)
  - Electrospray (ESI)
  - Atmospheric pressure chemical ionisation (APCI)
- Fast atom bombardment (FAB) and Liquid secondary ion mass spectrometry (LSIMS)
- Plasma desorption (PD)
- Inductively coupled plasma (ICP)
- Matrix-assisted laser desorption (MALDI)

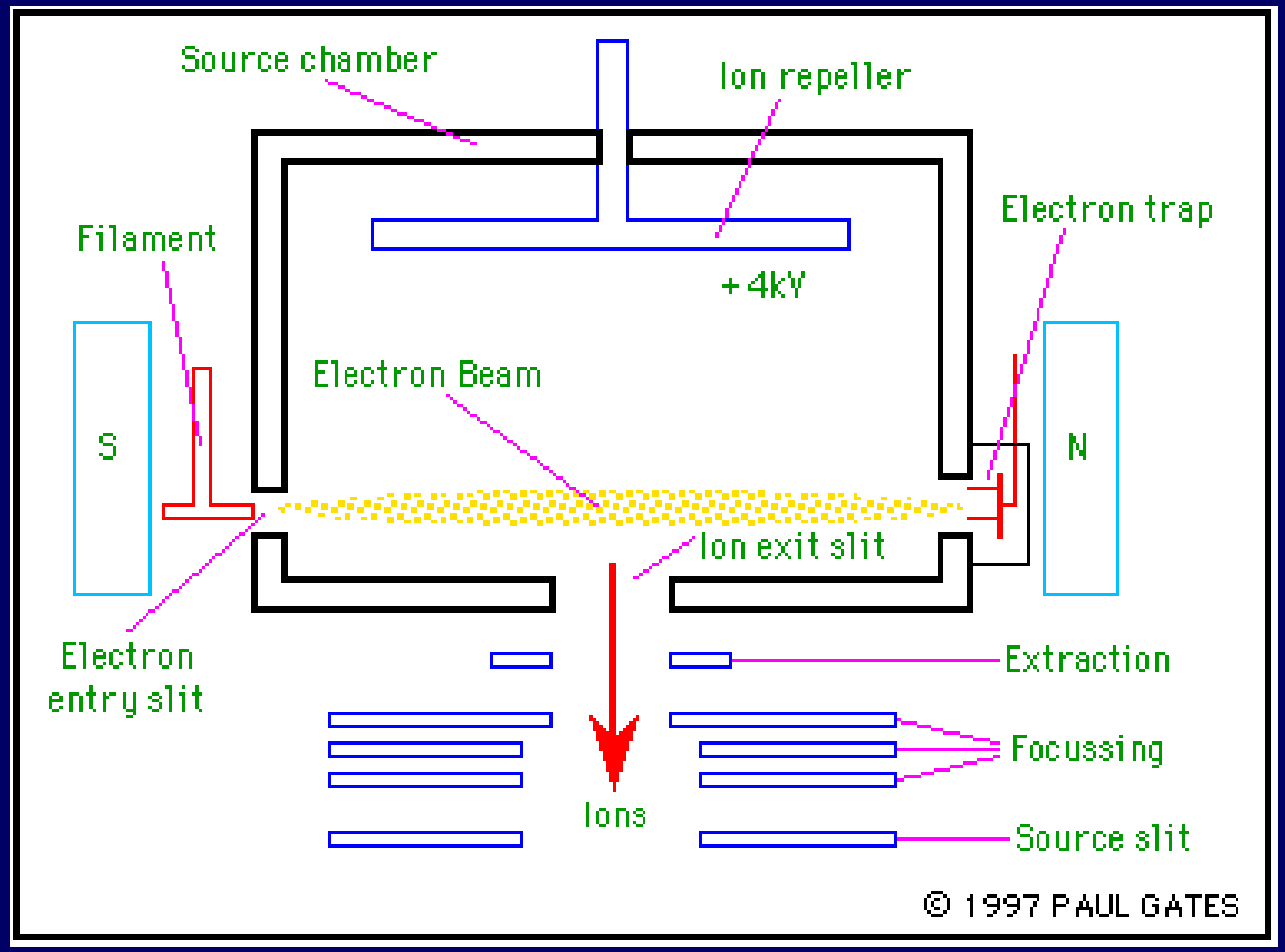
Ion Nomenclature

- Molecular ion
  - Ion formed by loss of electron from molecule
- Pseudomolecular ion
  - Ion formed from molecule by gain of small ion (e.g. H^+, OH^-)
- Precursor ion (a.k.a. “parent ion”)
  - Ion from which a smaller fragment ion has formed
    - Usually molecular or pseudomolecular ion
- Product ion (a.k.a. “daughter ion”)
  - Fragment ion from larger ion

Electron Impact 1

- Fast electron stream ionised molecules
  - $e^-_{(fast)} + M \rightarrow M^{\cdot +} + 2\ e^-_{(slow)}$
- Very energetic
  - Transfer of electron k.e. to ion may lead to bond dissociation
- Molecular ion may not be seen if easily fragmented
- Many product ions formed

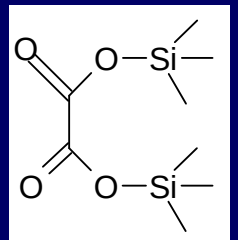
Electron Impact 2



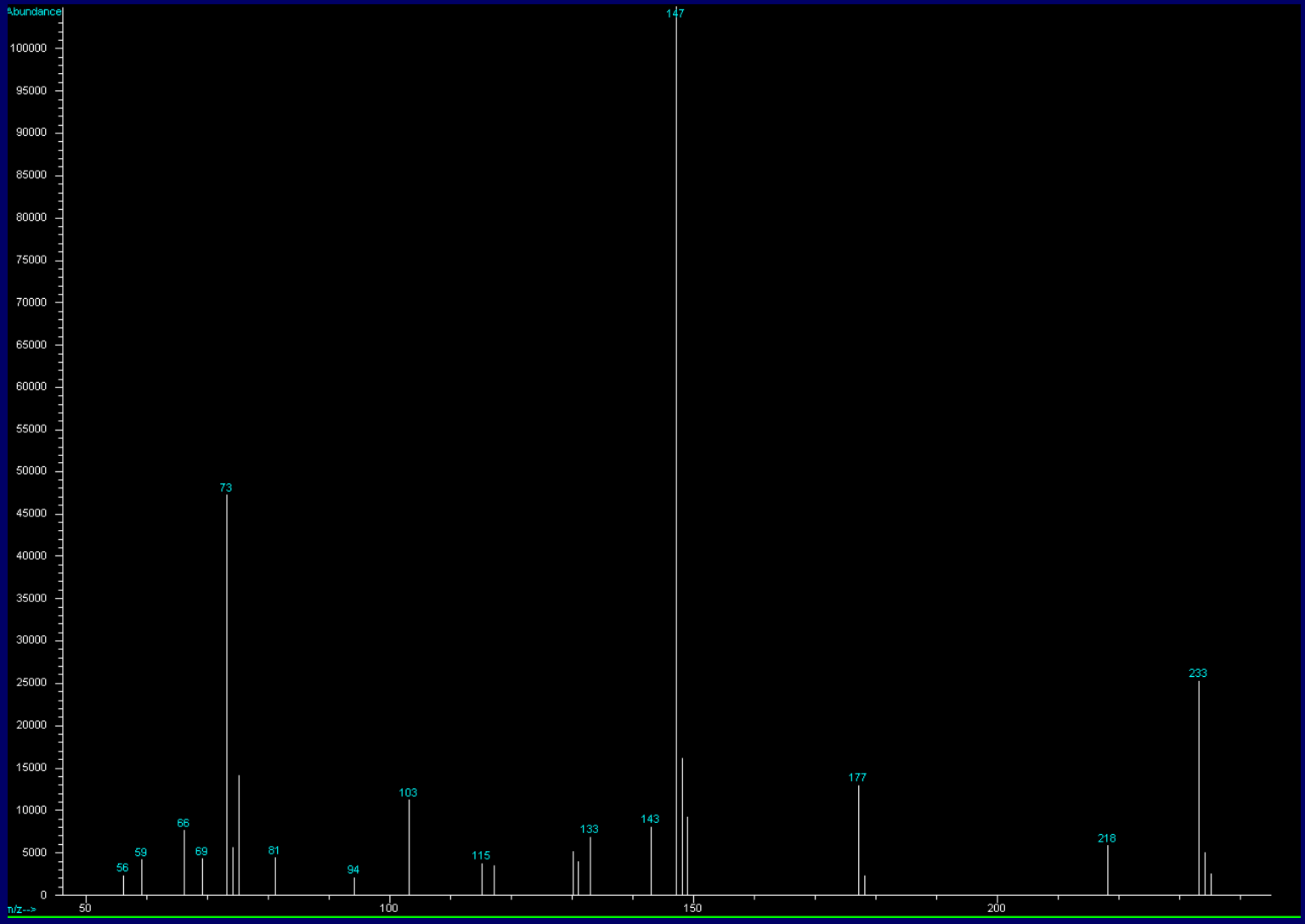
Electron Impact 3

- Fragments may be useful for structure determination
  - Fragments may be ions, neutrals or radicals
  - Certain fragments or losses may be characteristic of functional groups (e.g. loss of 18 (H_2O) suggests OH group)

Oxalic Acid (bis TMS ester)



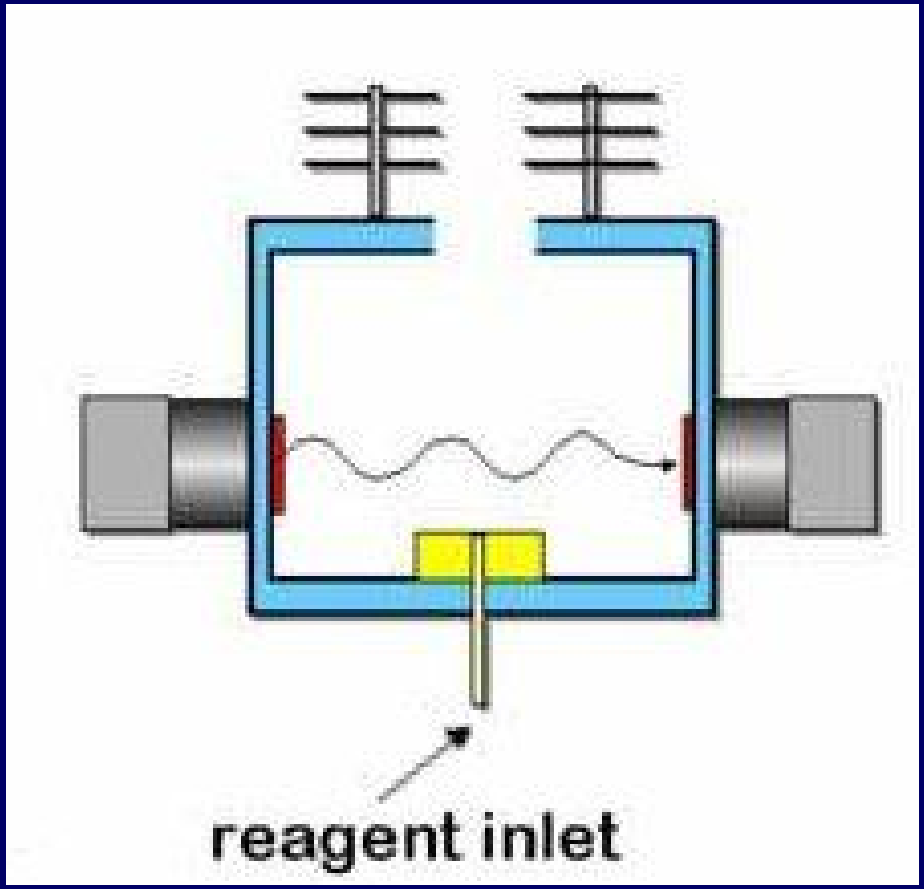
Mw = 234



Chemical Ionisation 1

- Uses EI source with a “cage” to trap ions with a reagent gas (CH_4 or NH_3)
- Electrons ionise reagent gas which reacts to form reactive ions (e.g. CH_3^+ and CH_5^+ from methane)

Chemical Ionisation 2



Chemical Ionisation 3

- Different types of compound react differently with reagent gas ions
  - Generally proton transfer
    - $M + CH_5^+ \rightarrow [M+H]^+ + CH_4$
  - Saturated hydrocarbons
    - $RH + CH_5^+ \rightarrow R^+ + CH_4 + H_2$
  - Polar molecules
    - $M + CH_3^+ \rightarrow [M+CH_3]^+$

Chemical Ionisation 4

- Mass spectrum shows few fragments usually
  - Main peak is $[M+H]^+$ pseudomolecular ion
  - Soft ionisation technique

Chemical Ionisation 5

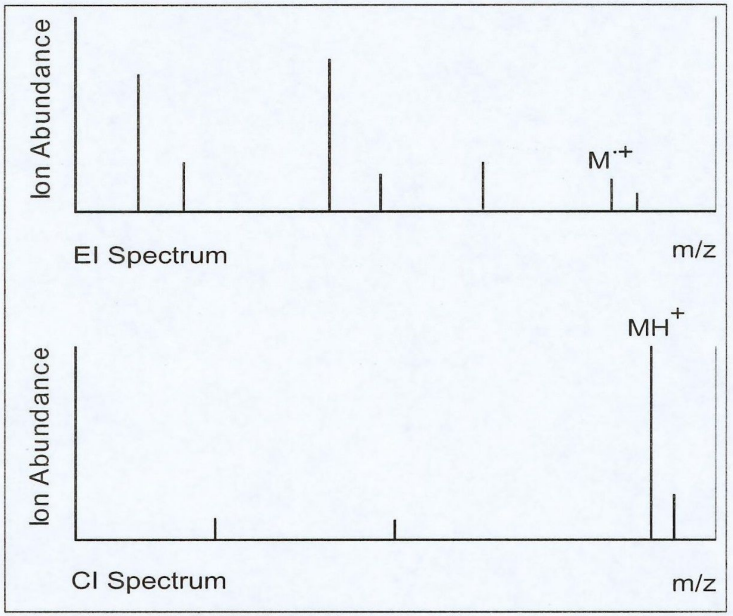


Figure 3 Comparison of EI and CI mass spectra illustrating the greater degree of fragmentation in the former and their greater abundance of quasimolecular ions in the latter.

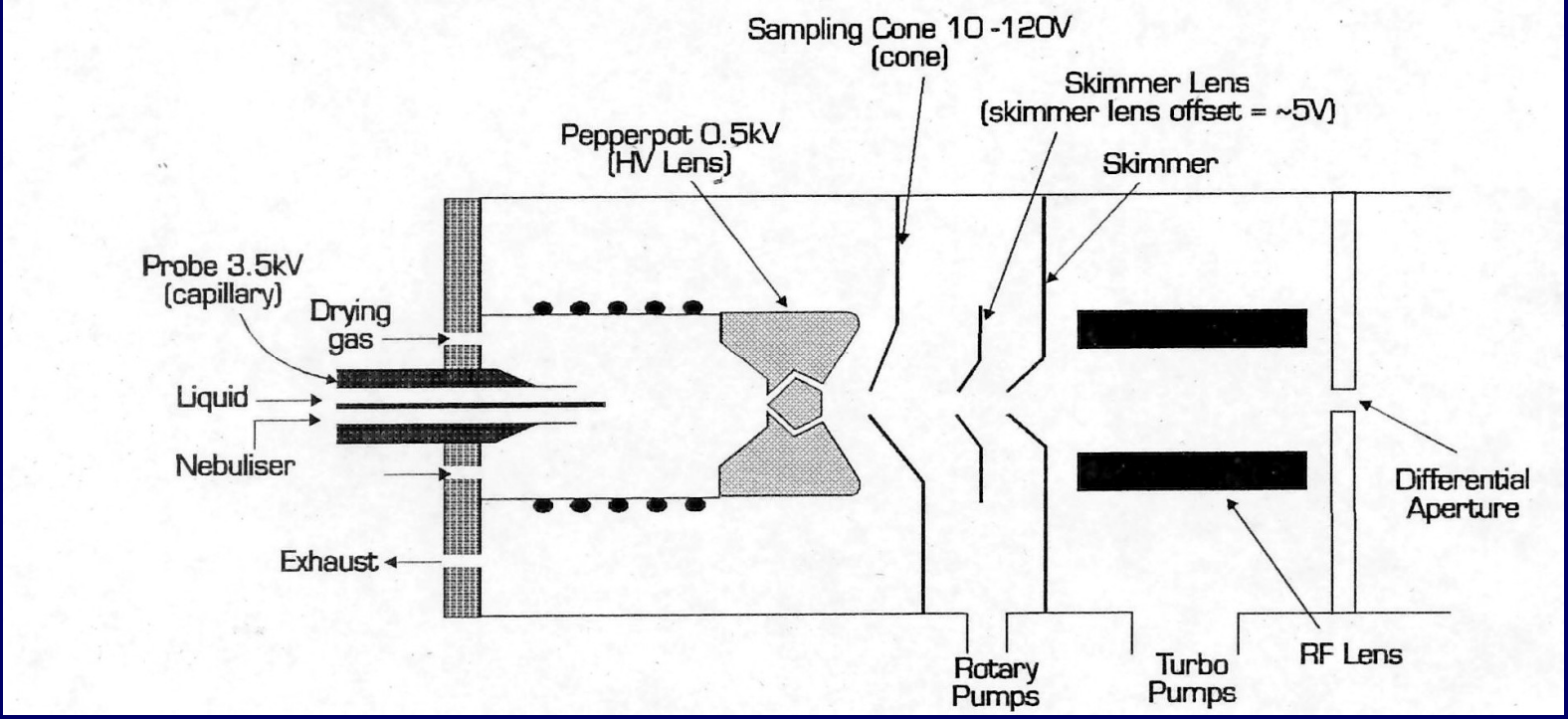
Reagent gas	Molecular ion	Reactive reagent ion
H_2	$H_2^{\bullet+}$	H_3^+
C_4H_{10}	$C_4H_{10}^{\bullet+}$	$C_4H_{11}^+$
NH_3	$NH_3^{\bullet+}$	NH_4^+
CH_3OH	$CH_3OH^{\bullet+}$	$CH_3OH_2^+$
NO	$NO^{\bullet+}$	NO^+

Figure 4 Some types of reagent gases and their reactive ions.

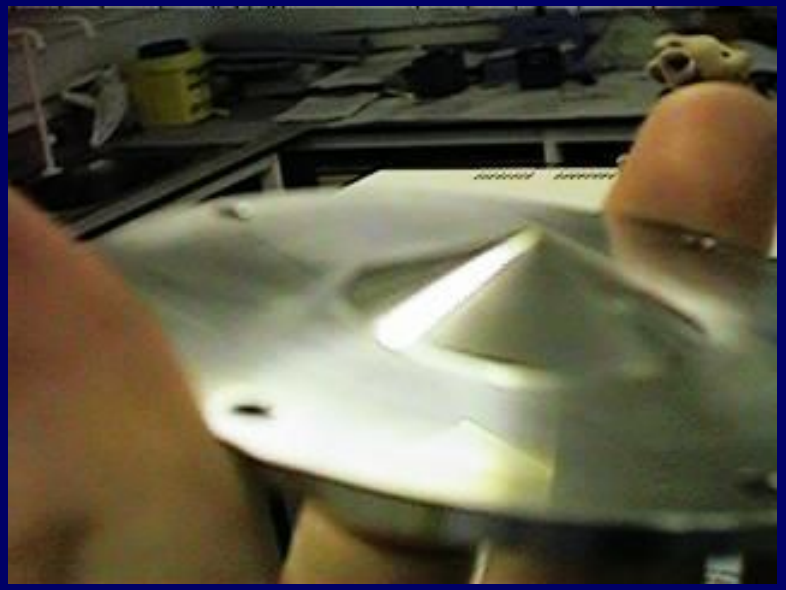
Electrospray 1

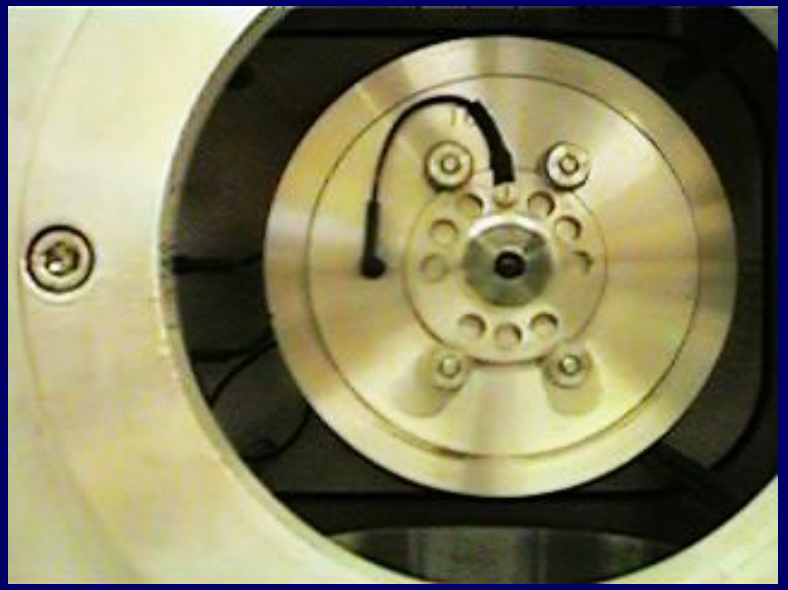
- Stream of solution sprayed out of capillary at high voltage (ca. 3 – 5 kV)
- Charged droplets formed by spray (“Taylor cone”)
- Solvent evaporated by stream of warm N_2
- As droplet shrinks, charge density increases until analyte ions ejected
  - Ions may be formed by proton transfer in spray or from reactions prior to analysis (i.e. may already be in solution)
  - Pseudomolecular $[M+H]^+$ ions formed
- Solvent pumped away and ions admitted to mass spectrometer

Electrospray 2

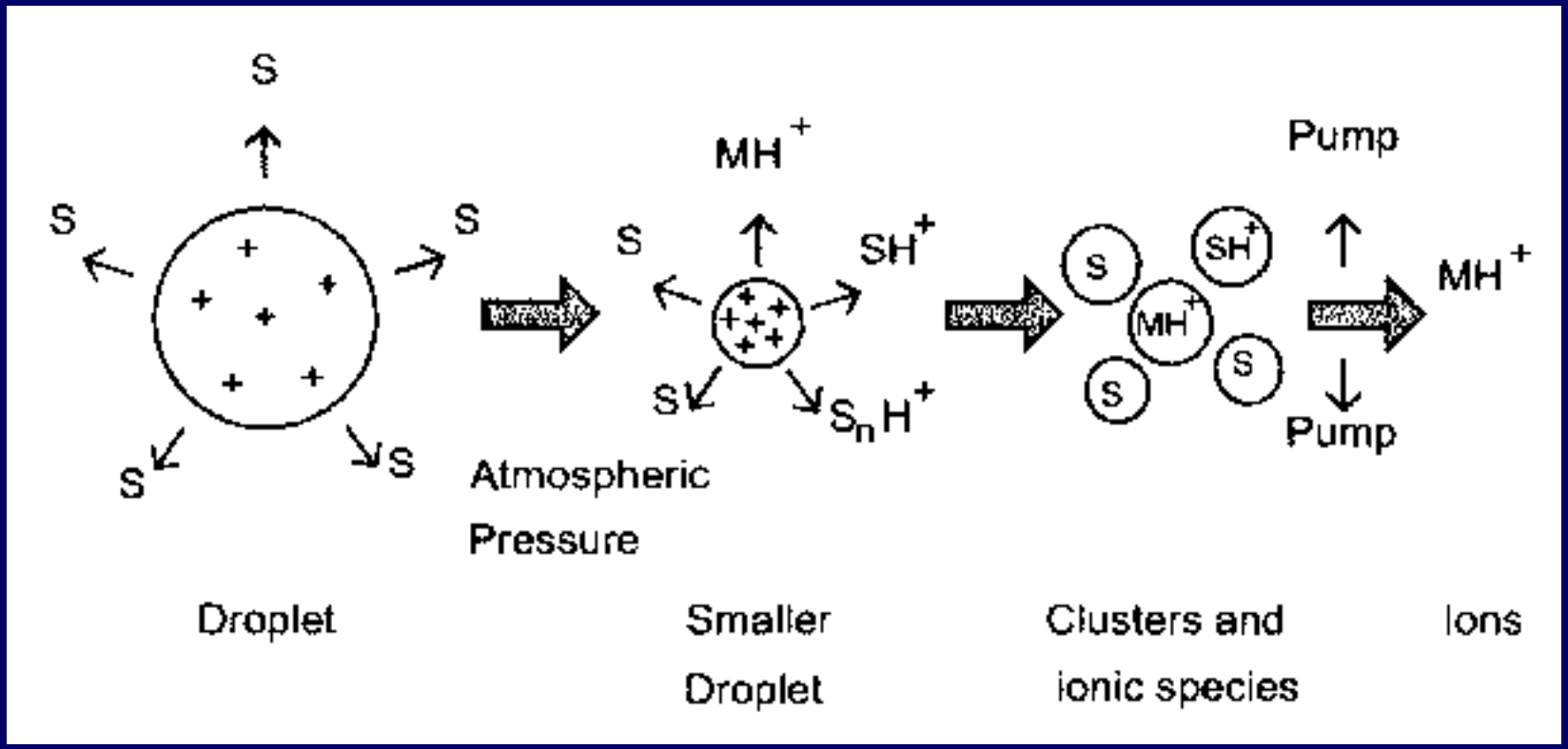


Electrospray 3





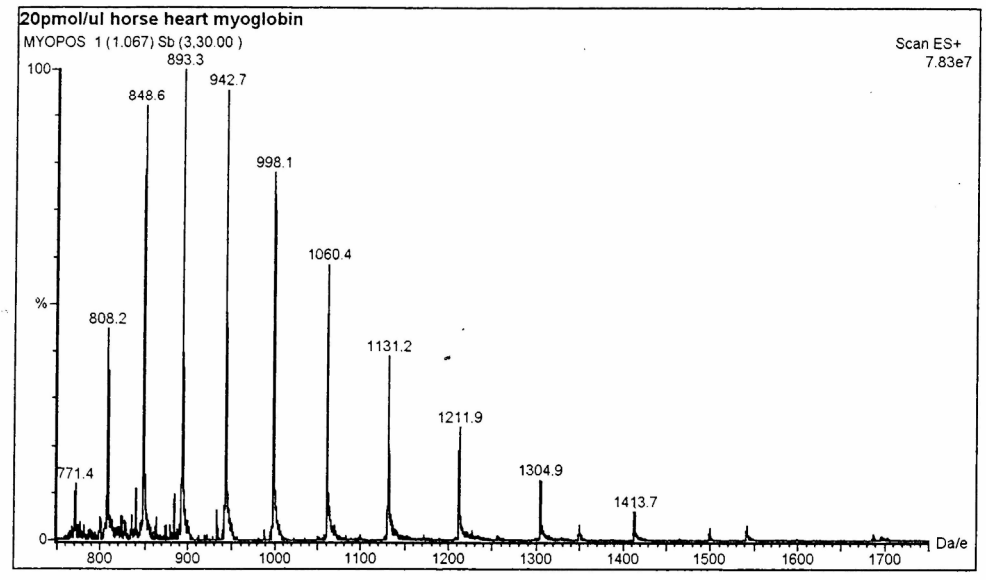
Electrospray 4

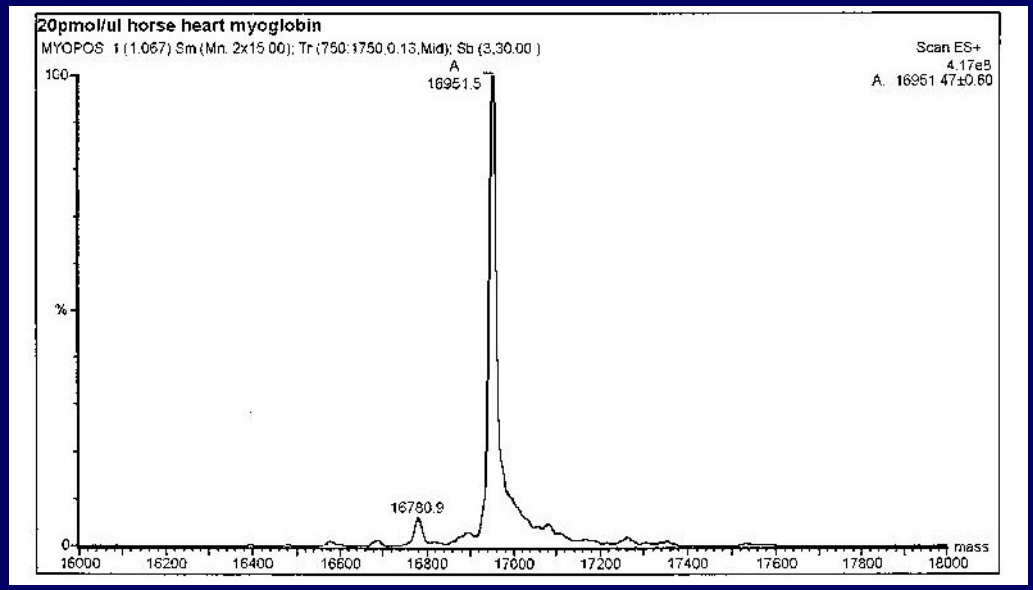


Electrospray 5

- Soft ionisation technique; little fragmentation
- Large molecules may be protonated more than once
  - Peaks seen for same compound with different m/z ratios
  - “Deconvolution” of peaks allows representation of species with effectively m/z for one charge
    - Allows determination of molecular weight

Electrospray 6

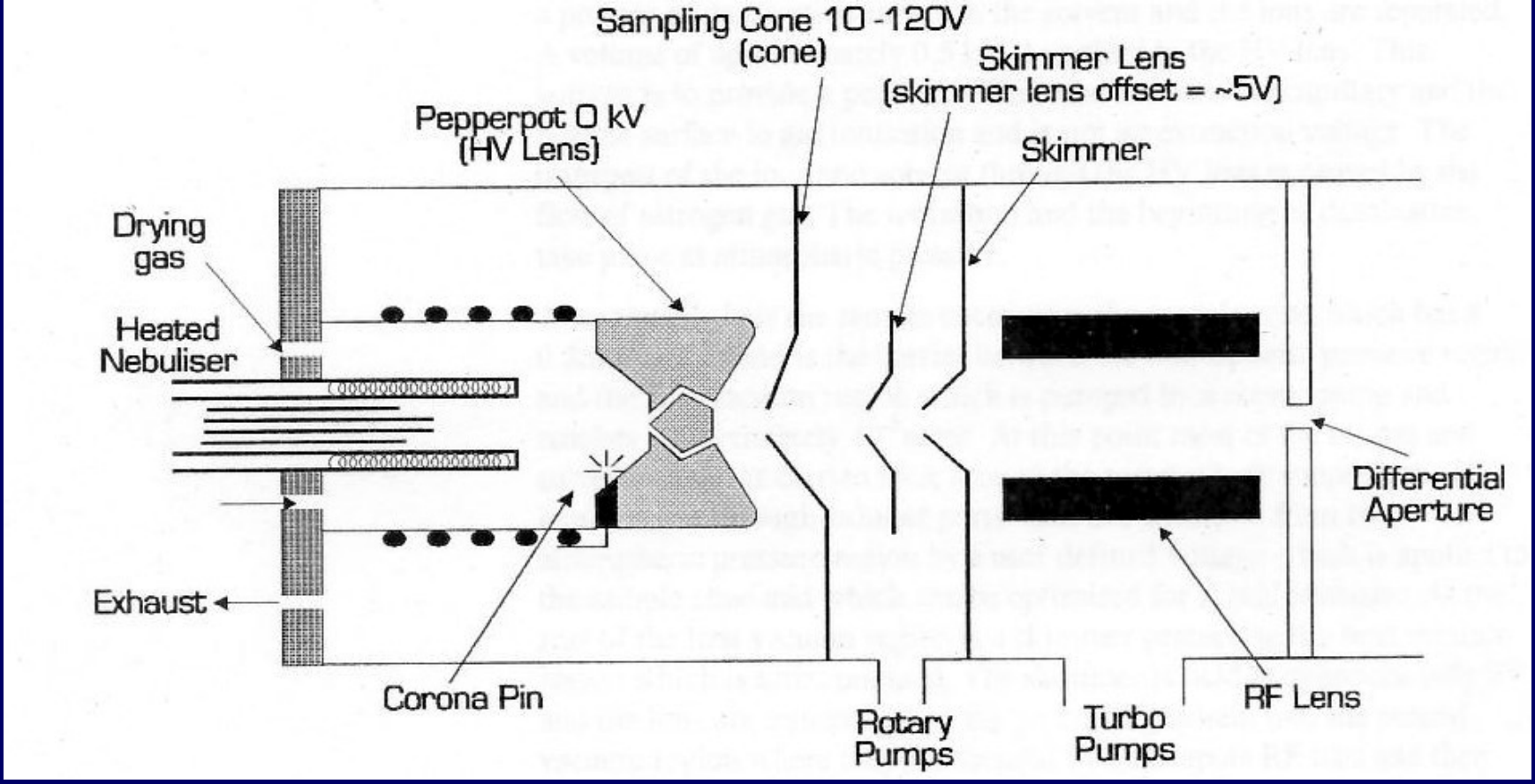




APCI 1

- Similar source to ESI but higher flow rate (ca. 2 mL/min)
- Discharge pin placed in the solvent spray
  - Causes solvent molecules to ionise and behave like reagent gas
  - Solvent ions transfer protons to analyte
- Soft ionisation technique
  - Pseudomolecular ion formed
  - Little fragmentation

APCI 2


Sampling Cone 10 -120V
(cone)
Skimmer Lens
(skimmer lens offset = ~5V)
Pepperpot 0 kV
(HV Lens)
Skimmer
Drying
gas
Heated
Nebuliser
Differential
Aperture
Exhaust
Corona Pin
Rotary
Pumps
Turbo
Pumps
RF Lens

Fast Atom Bomdardment

- Fast Ar (or Xe) atoms formed by
  - Accelerating Ar^+ or Xe^+ ions by electric field then
    - Colliding with Ar or Xe atoms
      - $Ar^+_{(fast)} + Ar_{(slow)} \rightarrow Ar_{(fast)} + Ar^+_{(slow)}$
    - Or firing through electron cloud
      - $Ar^+_{(fast)} + e^- \rightarrow Ar_{(fast)}$
- Atoms fired at compound in matrix and knock analyte ions out
  - Glycerol or m-nitrobenzylic acid commonly used for positive ions
  - Me_2NH and Me_3N used for negative ions
- Fast atoms cause little ionisation – essentially just ion displacement

LSIMS

- Similar to FAB but uses Cs^+ ions instead of Ar atoms
  - Analyte ions emitted *secondary* to primary Cs^+ ion collisions
  - Better sensitivity than atom beam for high MW compounds
  - Charge accumulation at surface can be problematic
- Use of matrix in FAB and LSIMS can complicate spectrum
  - FAB and LSIMS not “soft” ionisation techniques
    - Pseudomolecular ions and fragments formed
    - Exact pattern depends on amount of analyte
    - Fragments may be masked by matrix ions
- Useful for polar compounds up to 10 kDa

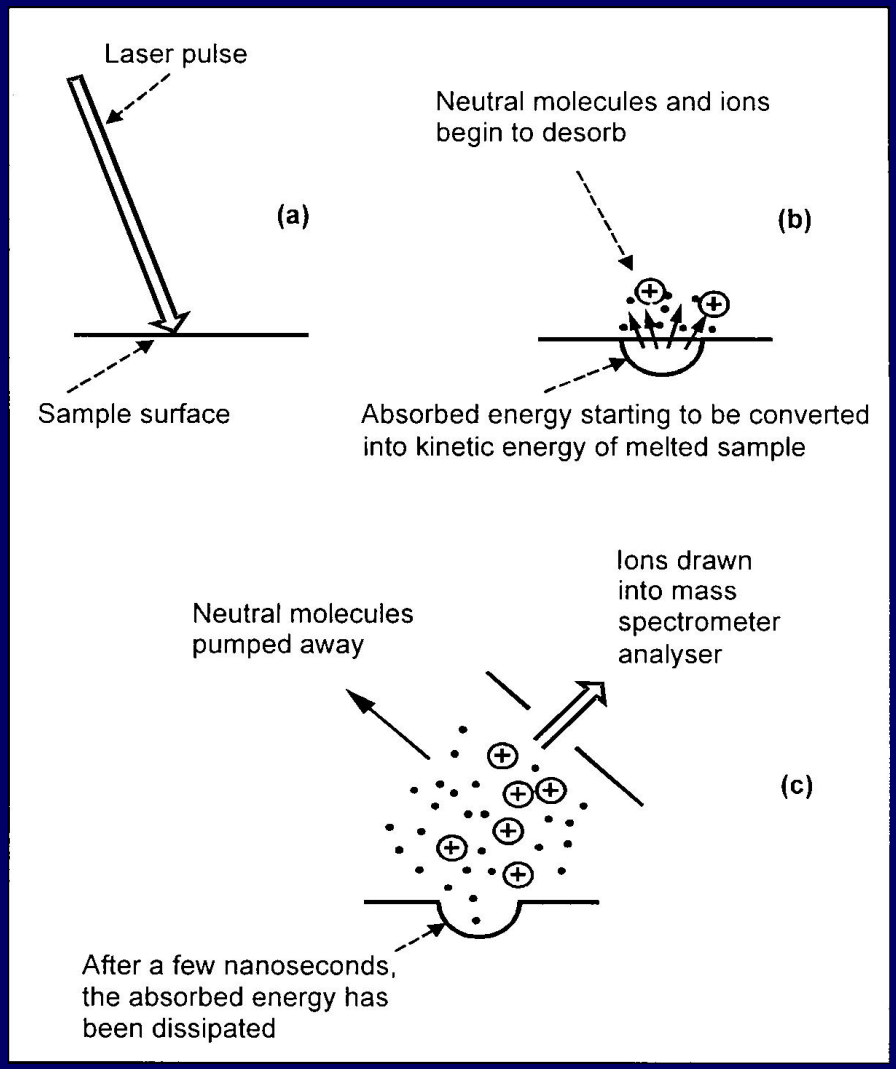
Plasma Desorption

- Fission fragments of ^{252}Cf impact on sample deposited on Al/nylon mesh
  - High energy impact
    - Fission fragments have energy of several MeV
  - Shockwave of impact ejects neutrals and ions
  - Useful for masses up to 10 kDa
- Rarely used, replaced by MALDI

MALDI 1

- Analyte dissolved in matrix containing dye
- Laser irradiation causes ion formation/ejection
  - Complex process; not well understood
  - Energy from laser pulse absorbed by matrix
  - Clusters of matrix/analyte desorbed from surface
  - Proton transfer from matrix to analyte produces pseudomolecular ions; matrix species evaporate off
    - Transfer can occur at any stage of process
    - Exact mechanism unknown
- Useful for analytes up to 100 kDa
  - Has been extended to 300 kDa
- Multiply charged ions may be formed from larger molecules

MALDI 2


Laser pulse
(a)
Sample surface
Neutral molecules and ions begin to desorb
(b)
Absorbed energy starting to be converted into kinetic energy of melted sample
Ions drawn into mass spectrometer analyser
Neutral molecules pumped away
(c)
After a few nanoseconds, the absorbed energy has been dissipated

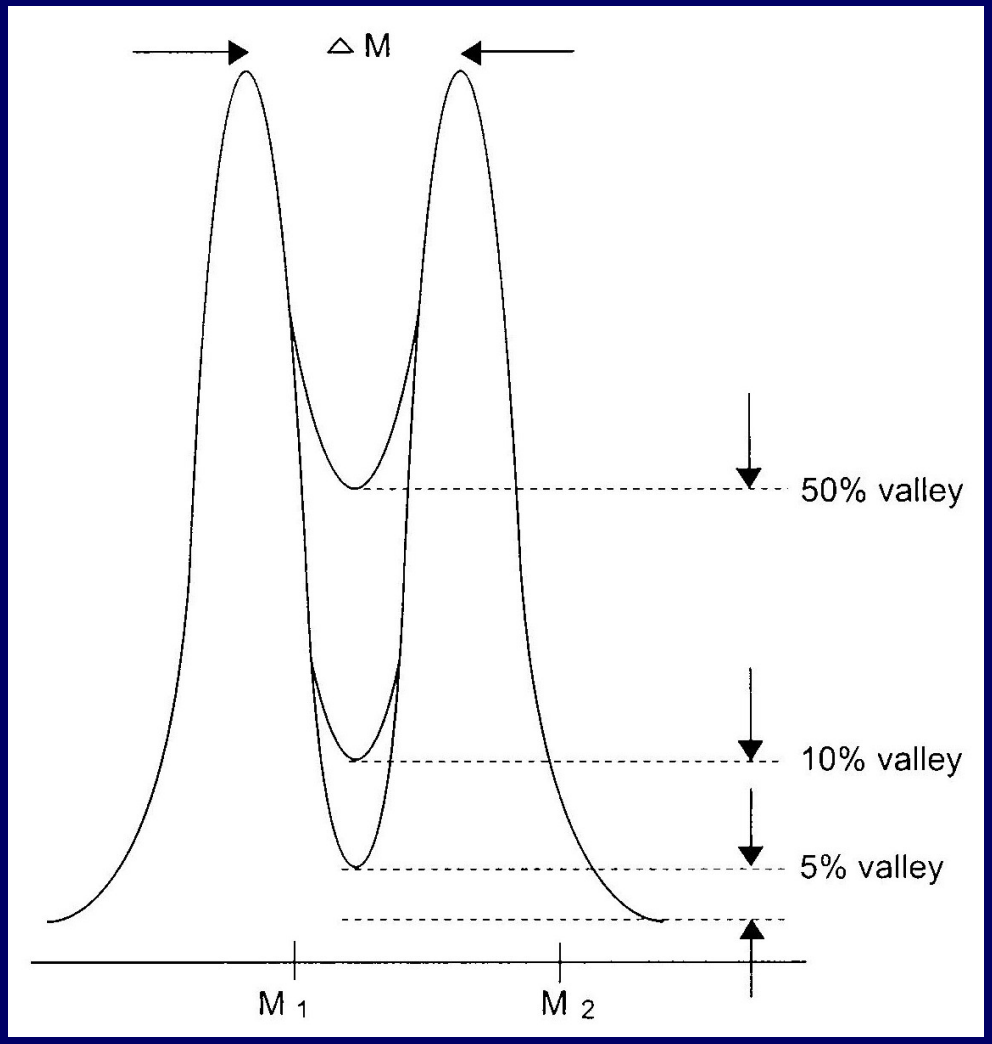
Mass Analysers

- Aim to separate (“resolve”) ions of different m/z ratios
- Sector instruments
  - Magnetic fields (magnetic sectors)
  - Electric and magnetic sectors (double focussing instruments)
- Quadrupoles
  - Quadrupole mass filters
  - Quadrupole ion traps
- Time-of-flight

Resolution 1

- Two peaks are resolved if the valley between them is 10% of the smaller peak intensity
  - Sometimes 10% is applied to sector instruments and 50% to quadrupoles
- Commonest definition
  - Resolution = $m_1/(m_2 - m_1)$
  - m_1 is mass of lighter peak; m_2 mass of heavier peak

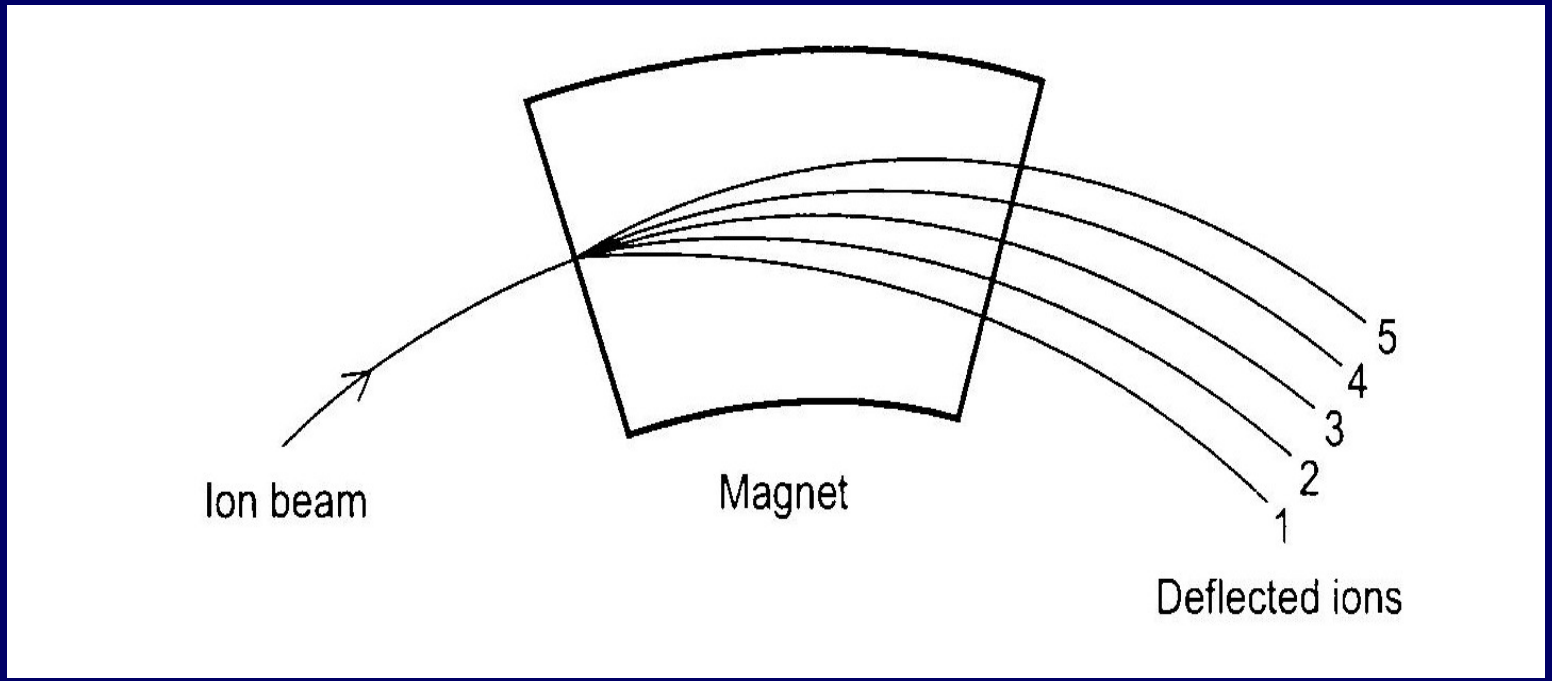
Resolution 2



Magnetic Sectors 1

- Ions accelerated from source by potential,V
- On entering magnetic field, B, ions follow a circular path of radius, r
- For ions of given m/z ratio
  - $m/z = r^2B^2/2V$
- If r is fixed (using flight tube)
  - Only one m/z will exit magnetic sector for given B
    - Varying B allows all ions to be transmitted sequentially
- If r is not fixed
  - All m/z will exit but at different points
    - All ions focussed onto plane, e.g. photographic plate ("dispersive instruments")

Magnetic Sectors 2



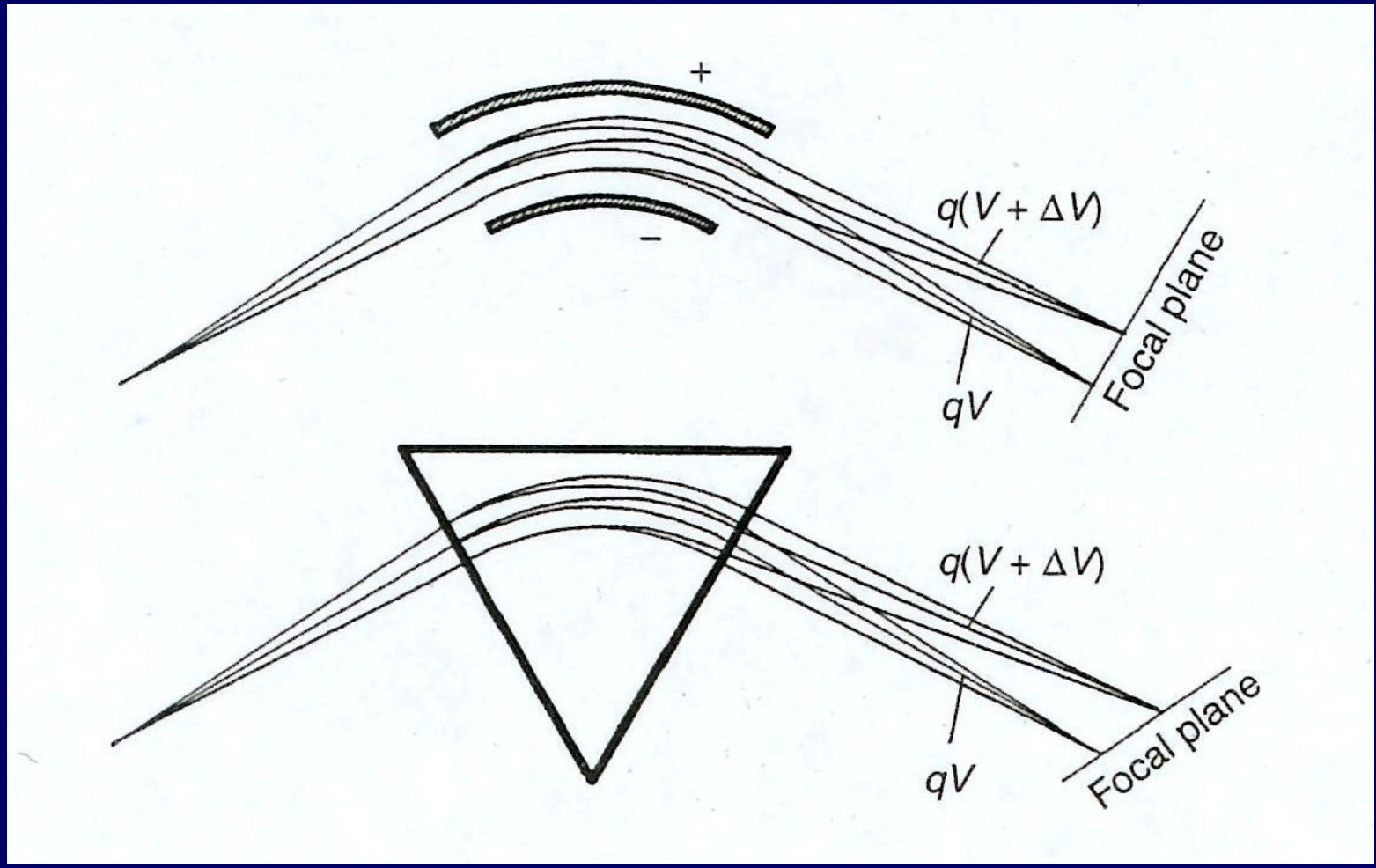
Magnetic Sectors 3

- Problem
  - For a given m/z ratio there is a spread of kinetic energies
    - Thermal effects and collisions in the source ensure that there is a spread of velocities
    - Different k.e. for same m/z lead to “energy dispersion”
    - This dispersion may be exacerbated by the journey through the field – “angular dispersion”
    - Inaccuracies in determined m/z ratio may result with loss of resolution
- Solution
  - Include electric field (sector)

Double Focussing Analysers 1

- In a radial electric field of intensity, E,
  - the ions move in a trajectory where centrifugal electrostatic forces balance
    - $zE = mv^2/r$ or
    - $r = mv^2/zE$
  - Trajectory depends on k.e. rather than just mass
  - Electric sector is a k.e. analyser
- Electric sectors also suffer dispersion

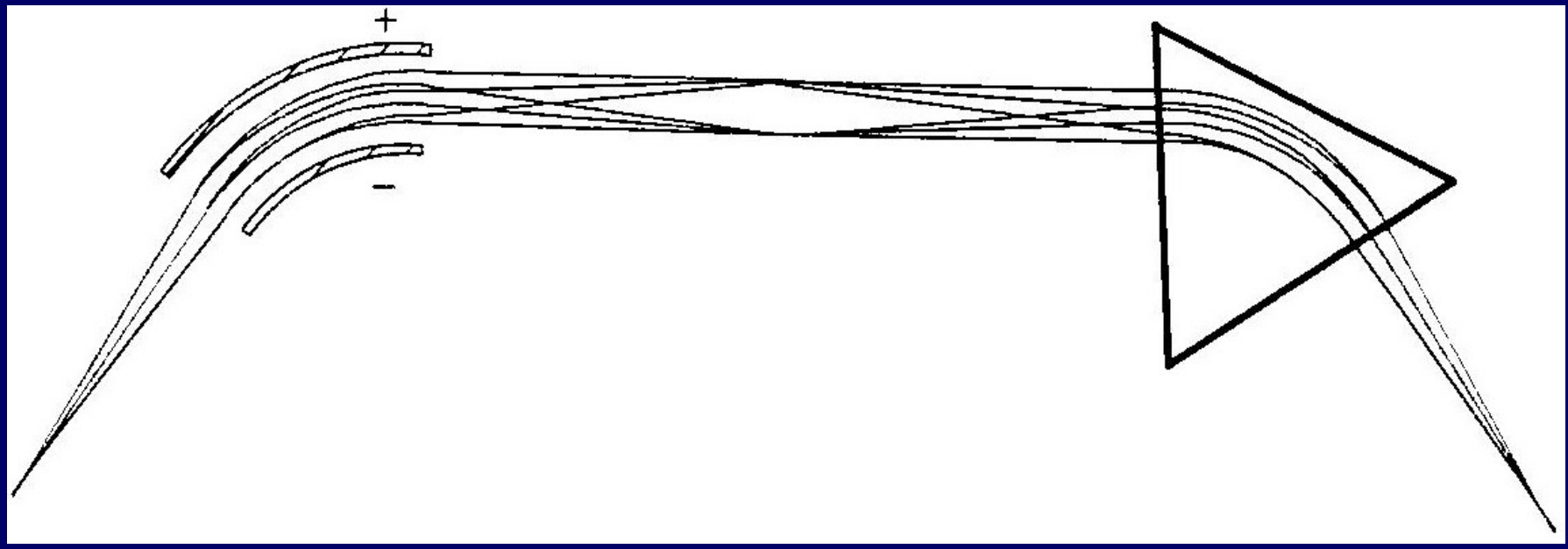
Double Focussing Analysers 2



Double Focussing Analysers 3

- If electric (E) and magnetic (B) sectors with same dispersion are combined the dispersions cancel out
- Order of sectors defines “geometry”
  - EB Nier-Johnson (forward) geometry
  - BE reverse Nier-Johnson (reverse) geometry

Double Focussing Analysers 4



Double Focussing Analysers 5

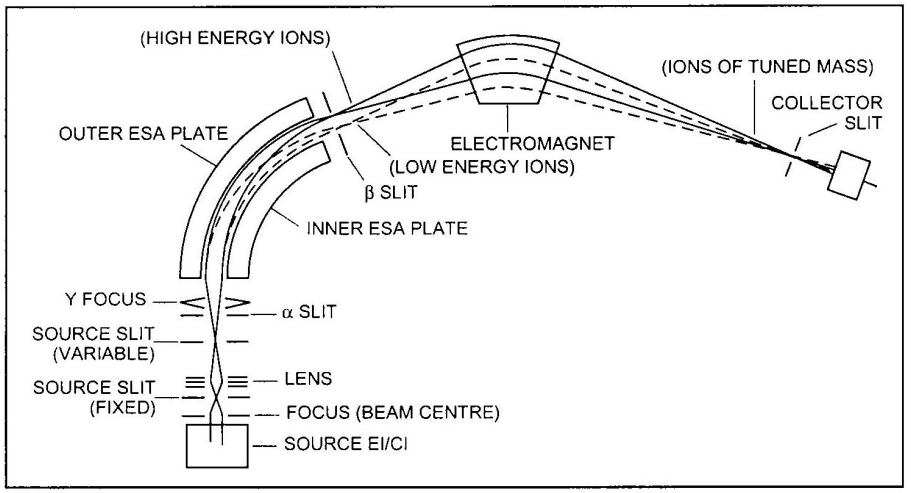


Figure 5 Double focusing ion optics (forward geometry).

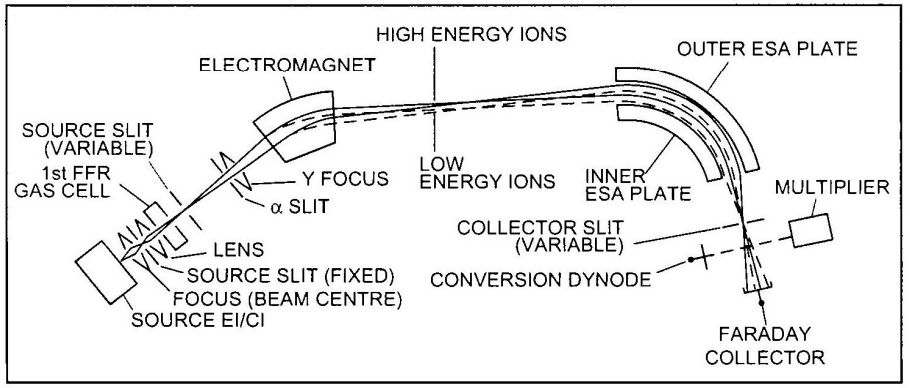
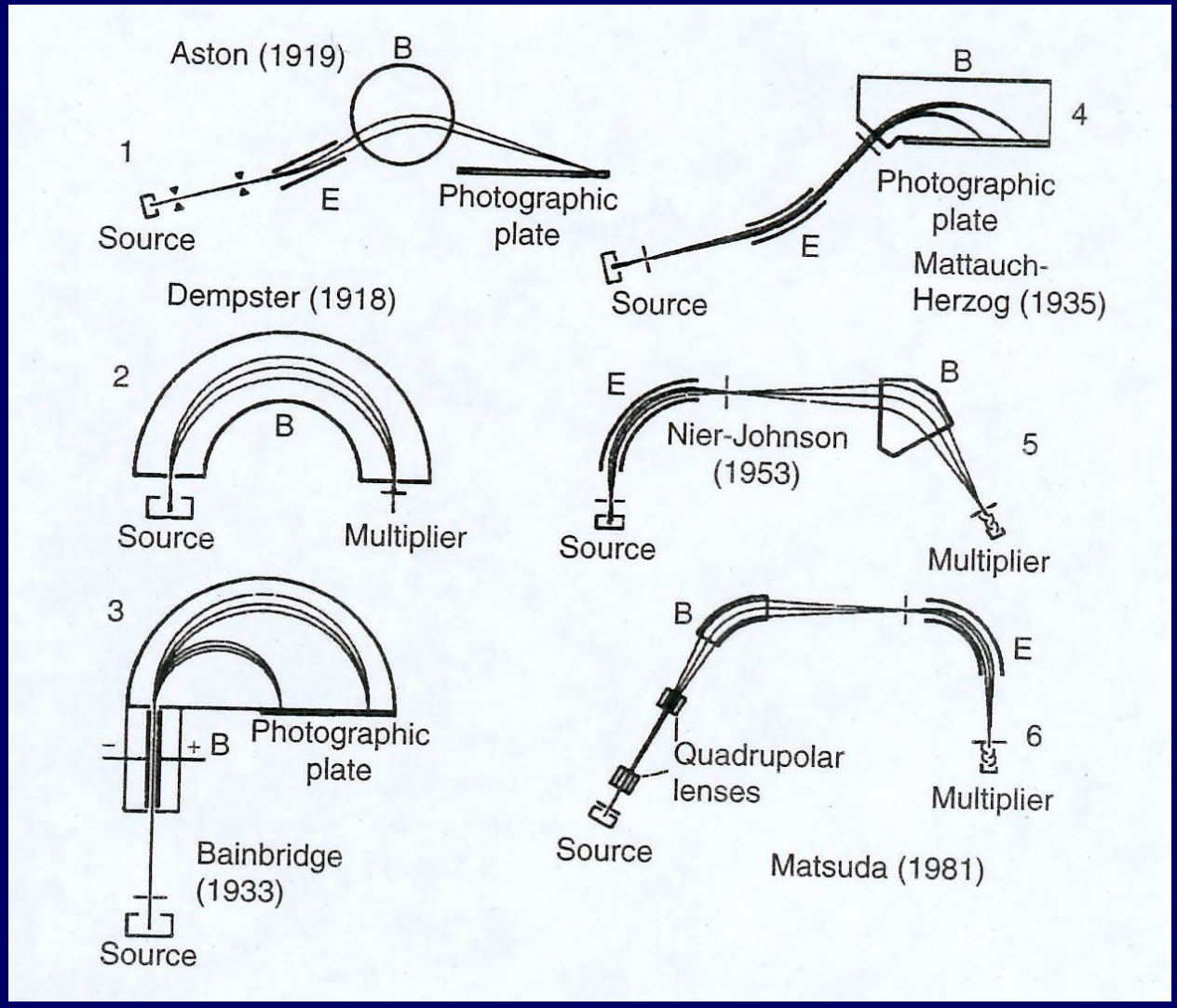


Figure 6 Double focusing ion optics (reverse geometry).

Double Focussing Analysers 6



Double Focussing Analysers 7

- Advantage
  - Correction of energy dispersion focuses ion beam
    - Increased resolution
    - Useful for isotope ratio work
- Disadvantage
  - Size
  - Cost

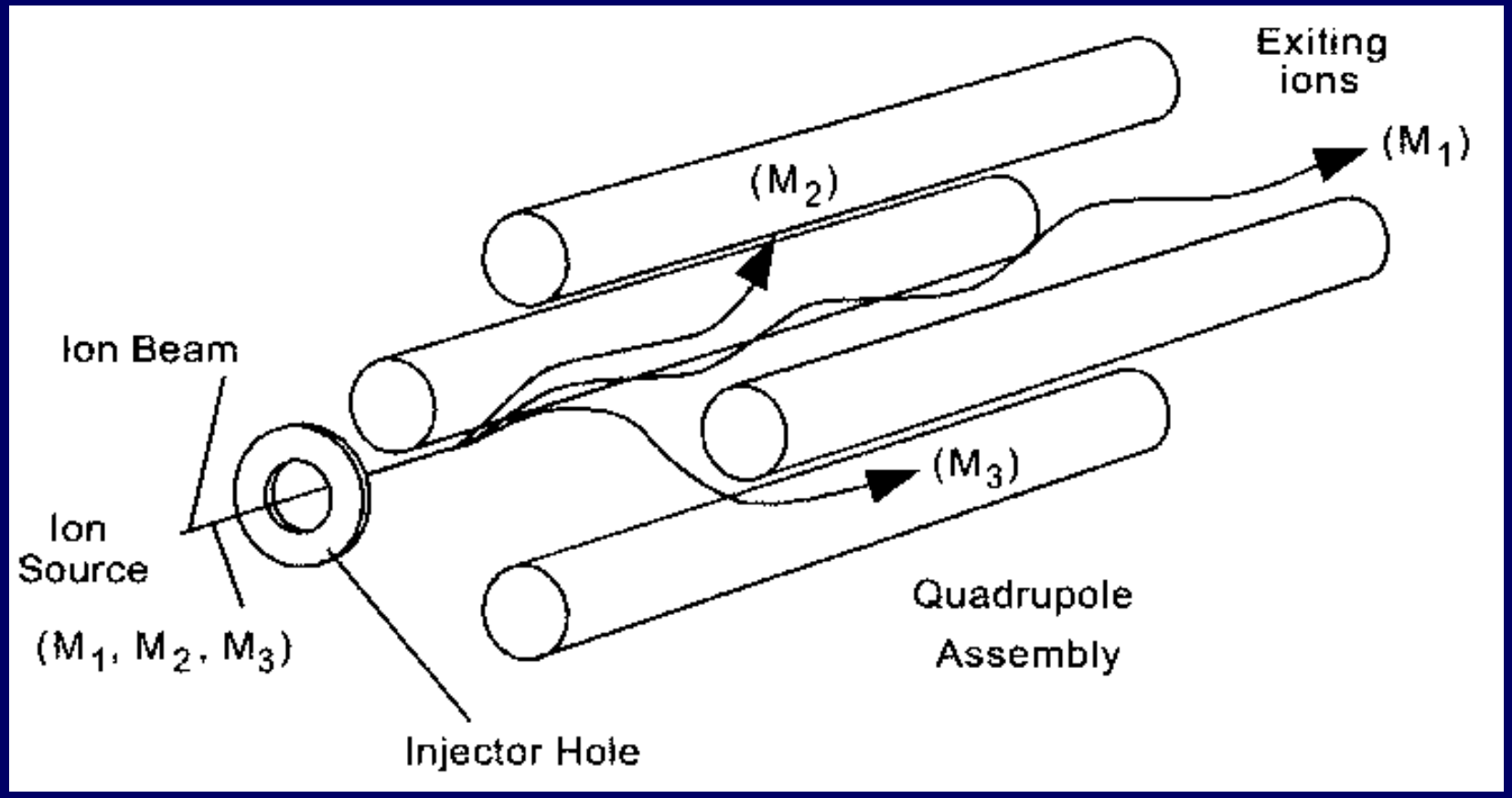
Quadrupole Analysers 1

- Quadrupole mass filters
  - Four rods arranged precisely with DC and RF alternating voltages applied to pairs
- Quadrupole ion traps
  - Effectively the circular equivalent of above
- Resolution typically 1000

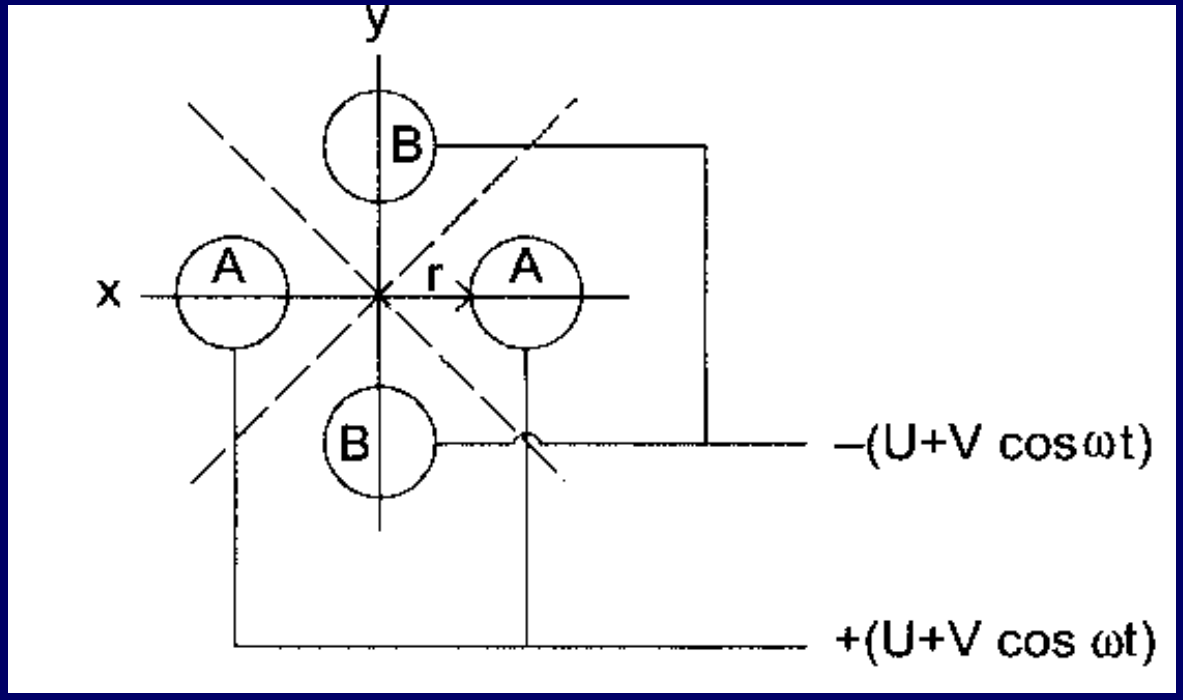
Quadrupole Mass Filters 1

- Ions enter quadrupole region
  - Because of RF voltage and DC offset the polarity of each pair of rods continually changes
  - Ion in quadrupole is alternately repelled and attracted to given rod
  - Ion follows helical path through quadrupole
  - For given RF and DC voltage settings only certain m/z ions have stable trajectory to detector – the rest collide with rods
  - By changing values of the voltages different m/z ions can be focussed onto the detector
- Quadrupole mass filter transmits one m/z ratio at once

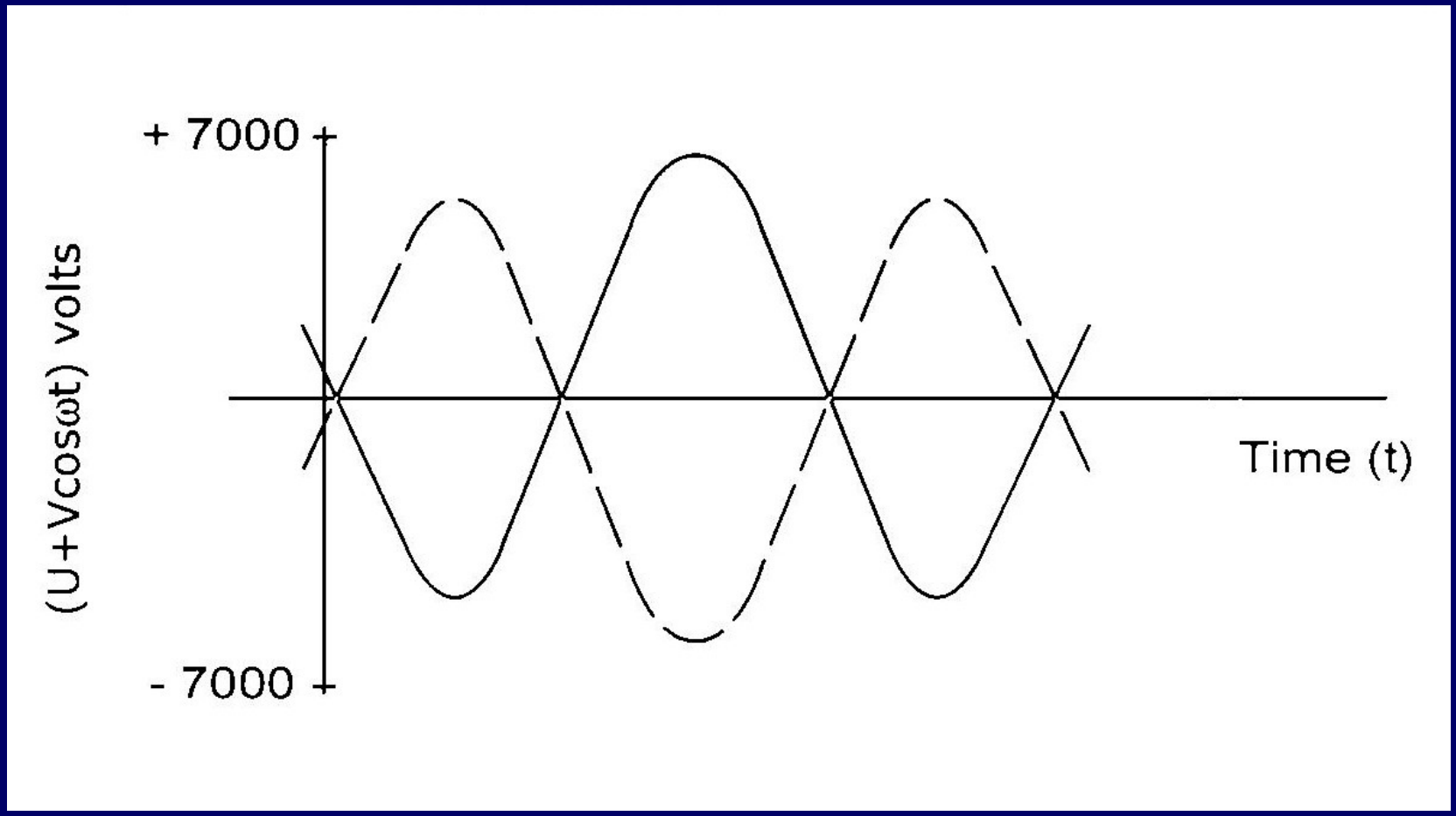
Quadrupole Mass Filters 2



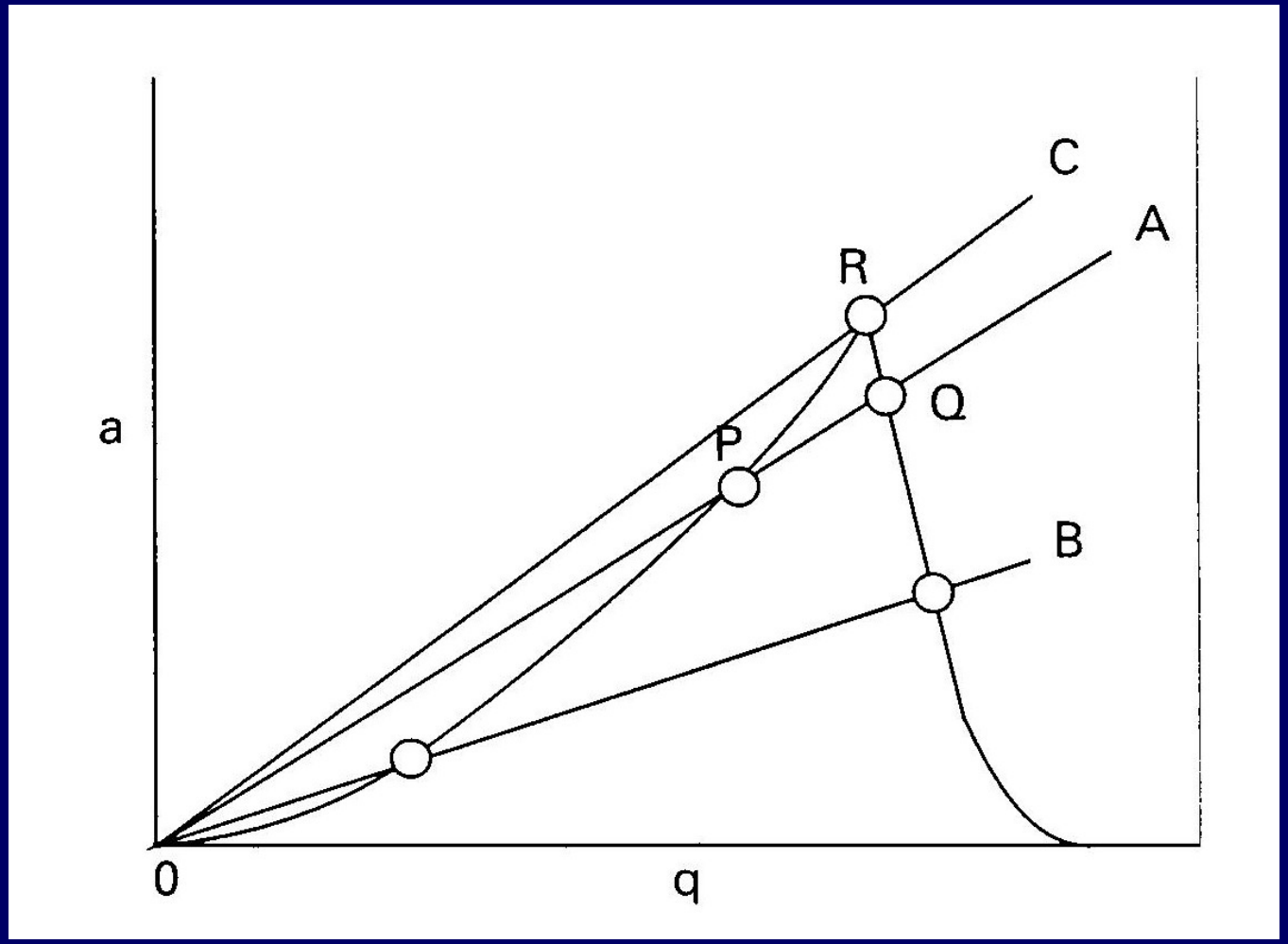
Quadrupole Mass Filters 3



Quadrupole Mass Filters 4



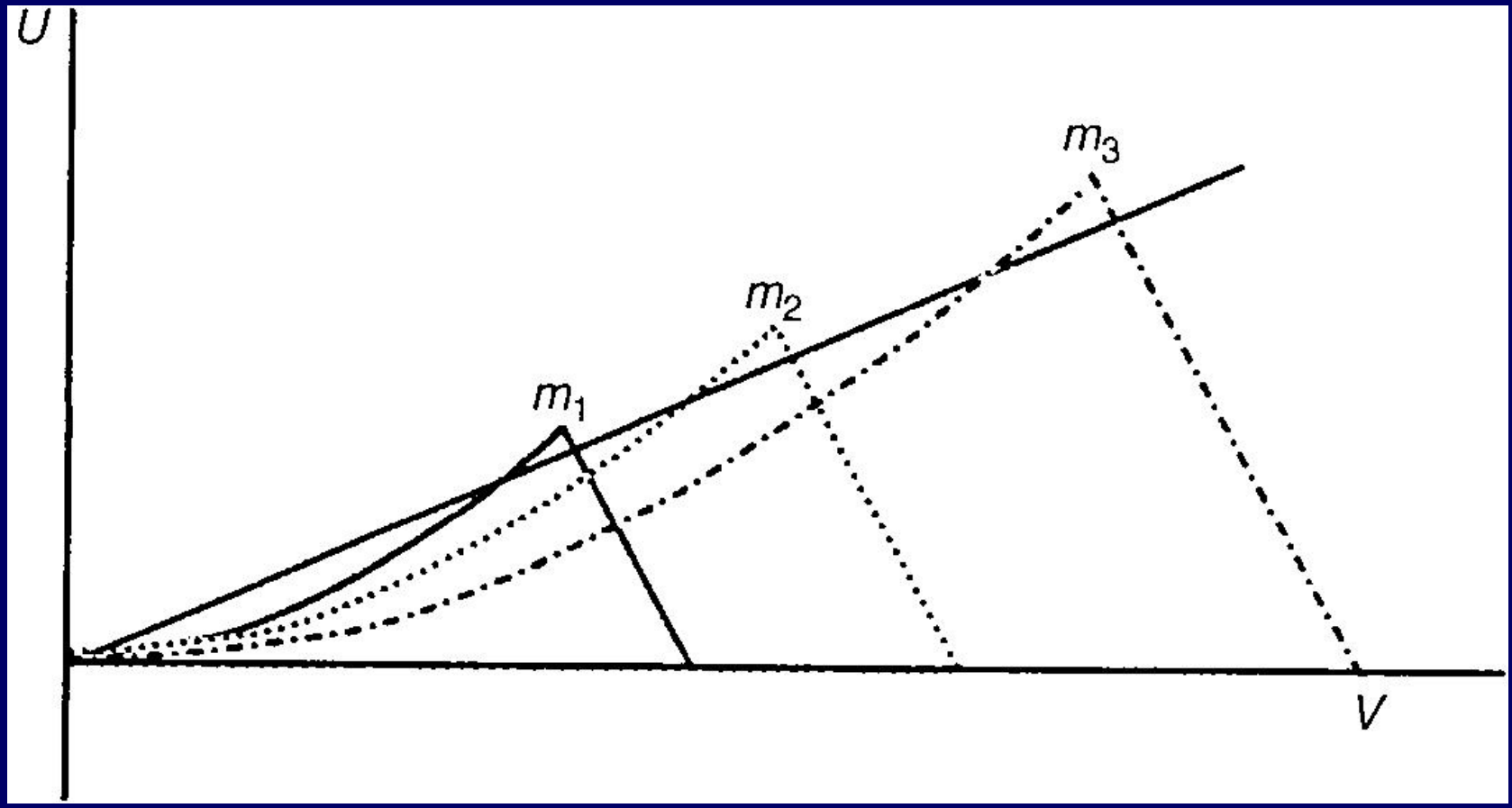
Quadrupole Mass Filters 5



Quadrupole Mass Filters 6

- Resolution
  - Discrimination between ions of similar m/z ratio
  - Variation of RF and DC voltage with fixed ratio allows mass range to be scanned
  - Variation of ratio alters resolution
    - Running the voltages closer to apices of regions of stability leads to higher resolution
    - Ion counts fall with increasing ratio
      - Better resolution ∴ fewer extraneous ions transmitted
      - Ions have more energy ∴ more for given m/z ratio lost to collisions with rods

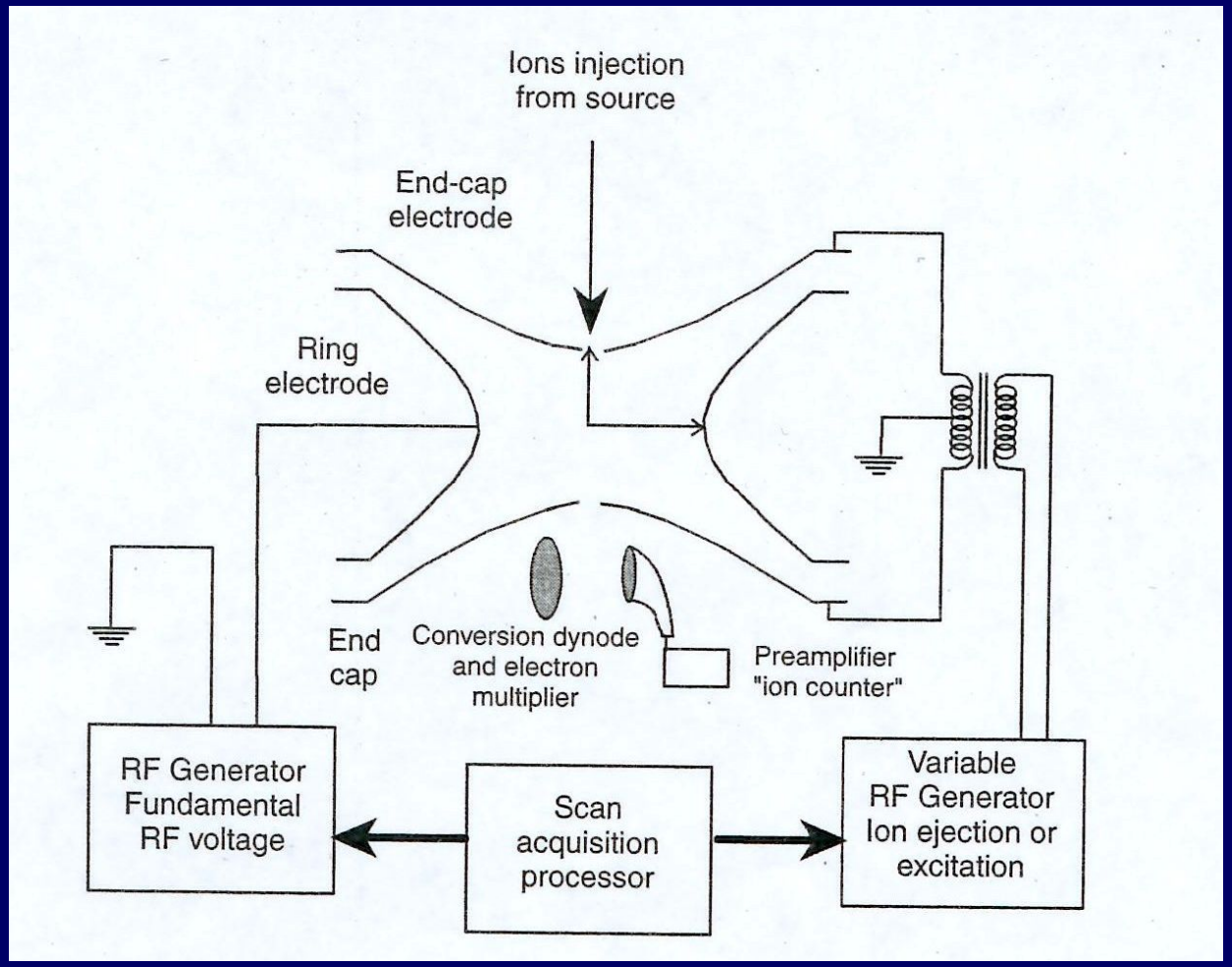
Quadrupole Mass Filters 7



Quadrupole Ion Traps 1

- Two end caps and central toroidal ring electrode
- Unlike quadrupole mass filter QIT can be used to store a range of m/z ratios and then eject them sequentially

Quadrupole Ion Traps 2



Quadrupole Ion Traps 3

- RF voltage is applied to trap.
- DC voltage may or may not be applied to end caps
  - How voltages are applied determines how trap works
    - Keep all ions
    - Keep one m/z ratio
- Ions adopt figure of 8 trajectory
  - By altering RF (+/- DC) voltages ion trajectory become unstable and ions leave trap by end plate hole
    - Variation of voltages allows sequential exit of different m/z ratio ions

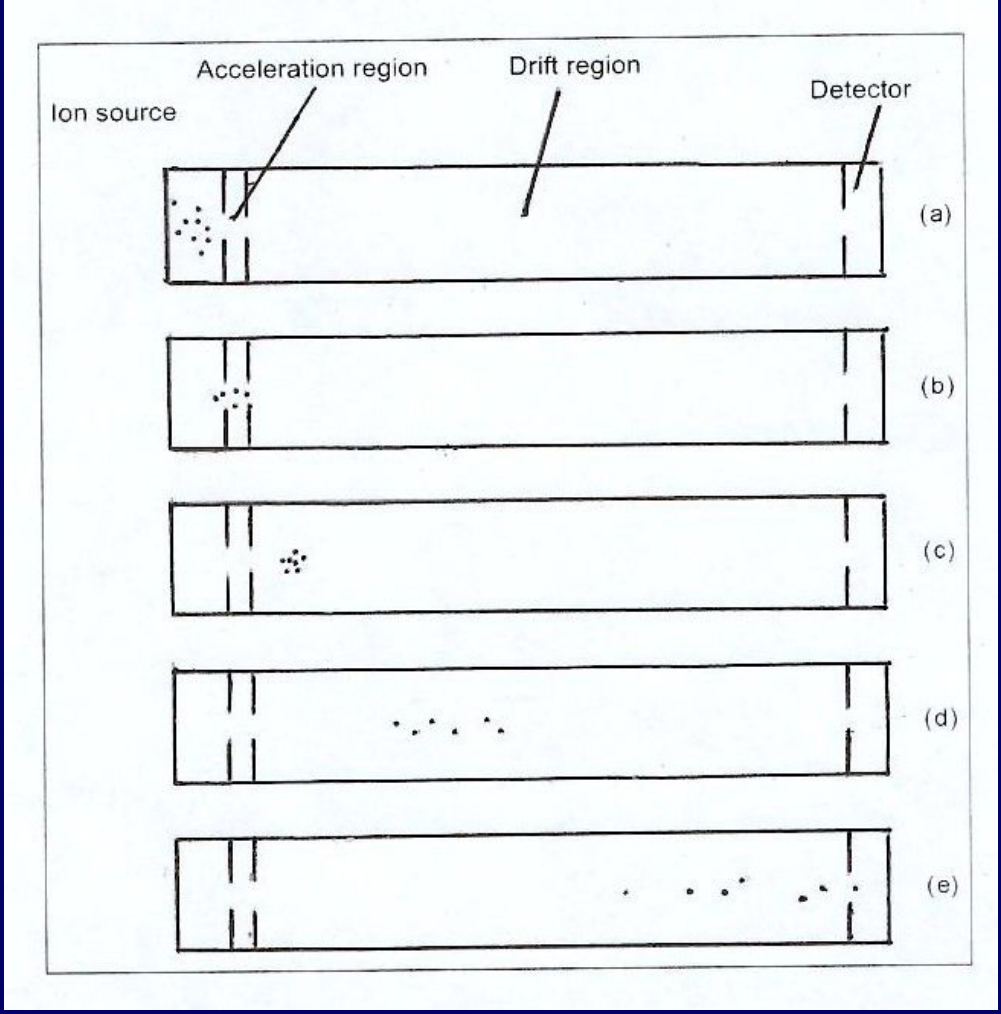
Quadrupole Analysers 2

- Advantages
  - Compact (esp. QIT)
  - Cheap
  - Robust
- Disadvantages
  - Poorer resolution than sector instruments

Time-of-Flight Analysers 1

- All ions are given same k.e. by accelerating potential (ca. 3 keV)
- Ions drift freely down “drift tube” to reach detector 1- 2 m away
- Different ions have different masses and different *velocities*
  - K.e. = $mv^2/2$
- Ions of different masses separated by taking different times to reach detector
  - From knowing start time, m/z can be determined from time to reach detector

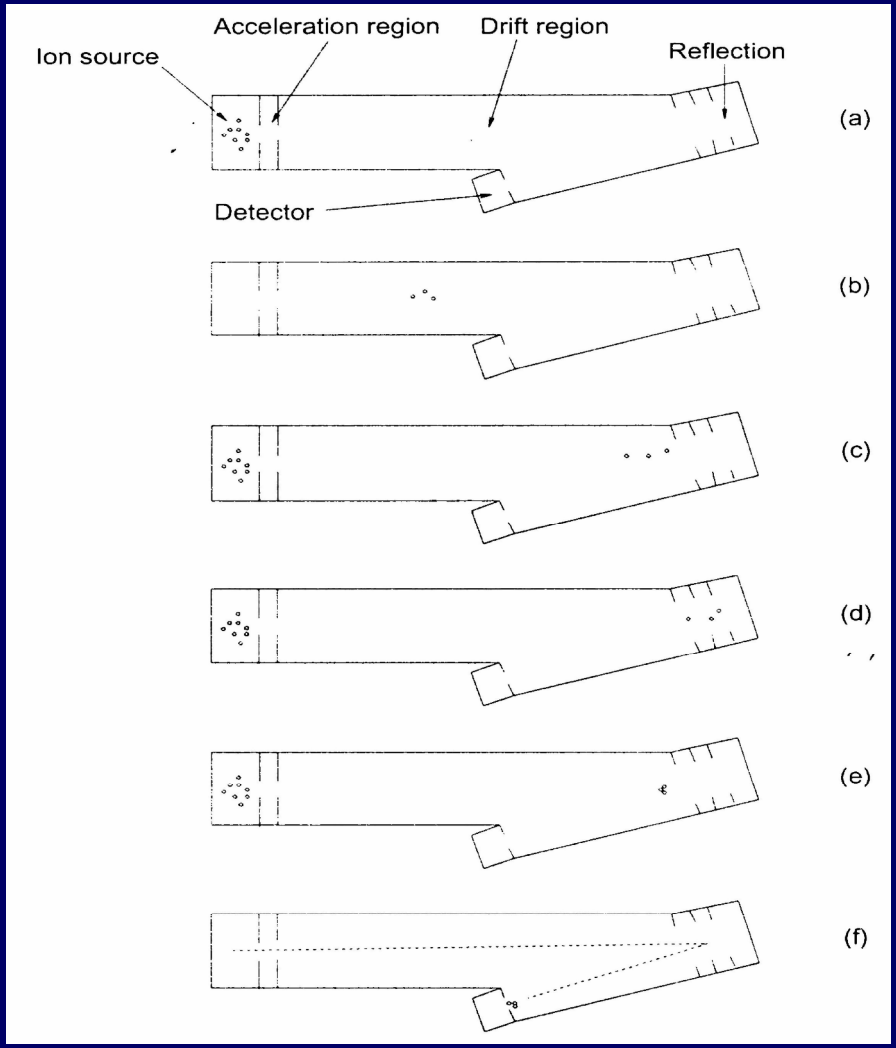
Time-of-Flight Analysers 2



Time-of-Flight Analysers 3

- Some issues with ions of same m/z having slightly different k.e.
- Performance of TOF improved by including “reflectron” (“ion mirror”)
  - Device for reflecting ions
  - Faster moving ions with same m/z penetrate reflectron more than slower ones so that they are re-focused on reflection
  - Additionally by reflecting ions back to drift again drift path and resolution increased

Time-of-Flight Analysers 4



Detectors

- Photographic plate
- Faraday cage
- Electron multiplier
- Photomultiplier
- Charge collectors

Photographic Plate

- Original detector
- Ions of same m/z hit plate at same point
- Intensity of spot relates to relative abundance of ions
- Little used now except for Mattauch-Herzog geometry (plane-focussing instrument)

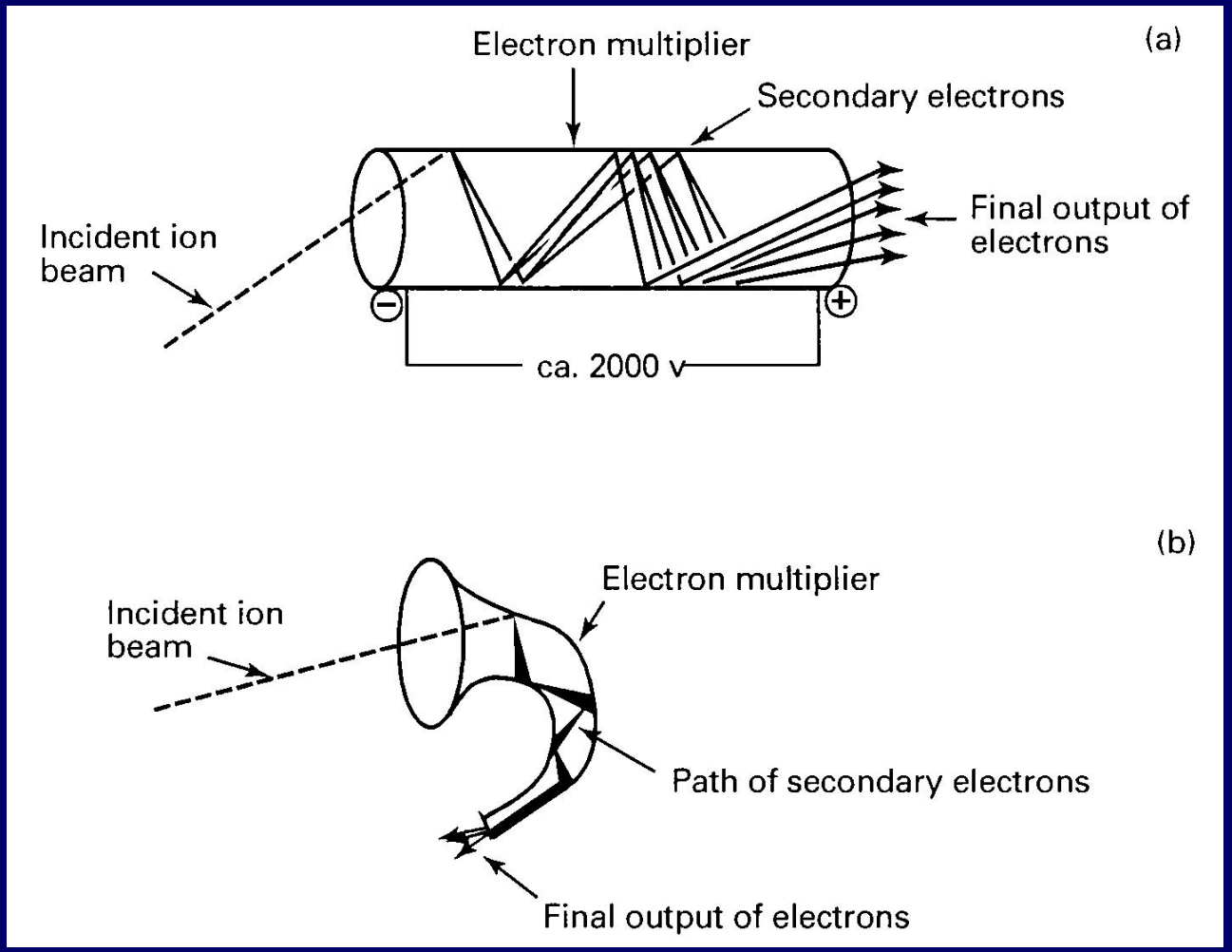
Faraday Cage

- Collection cylinder for ions
- Ions enter cylinder and discharge generating current
- Current amplified and measured
  - Current proportional to ion abundance
- Limited sensitivity

Electron Multiplier 1

- Useful for positive or negative ions
- Ions strike high voltage conversion dynode generating secondary particles of opposite charge
  - Positive ions liberate electrons; negative ions positive ions
- Secondary particles collide with walls of electron multiplier cascading out more electrons which cascade out even more electrons in further collisions
- Amplified current measured as related to ion count
- Sensitive
- Allows rapid scanning

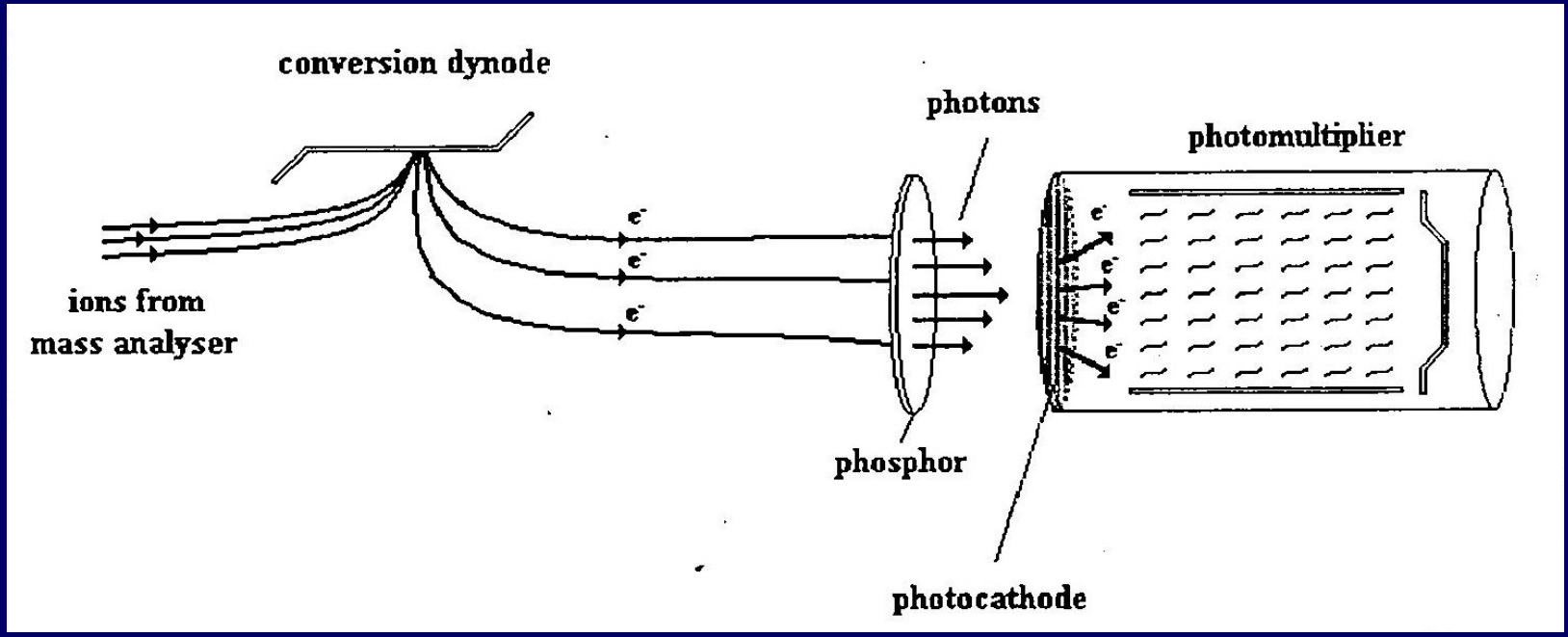
Electron Multiplier 2



Photomultiplier 1

- Detects positive or negative ions
- Two conversion dynodes (+ve and –ve), phosphor screen and photomultiplier
  - Negative ions hit positive conversion dynode
  - Positive ions hit negative dynode
- Secondary particles from conversion dynode hit phosphor ejecting photons
- Photons detected by photomultiplier
- Current proportional to ion count

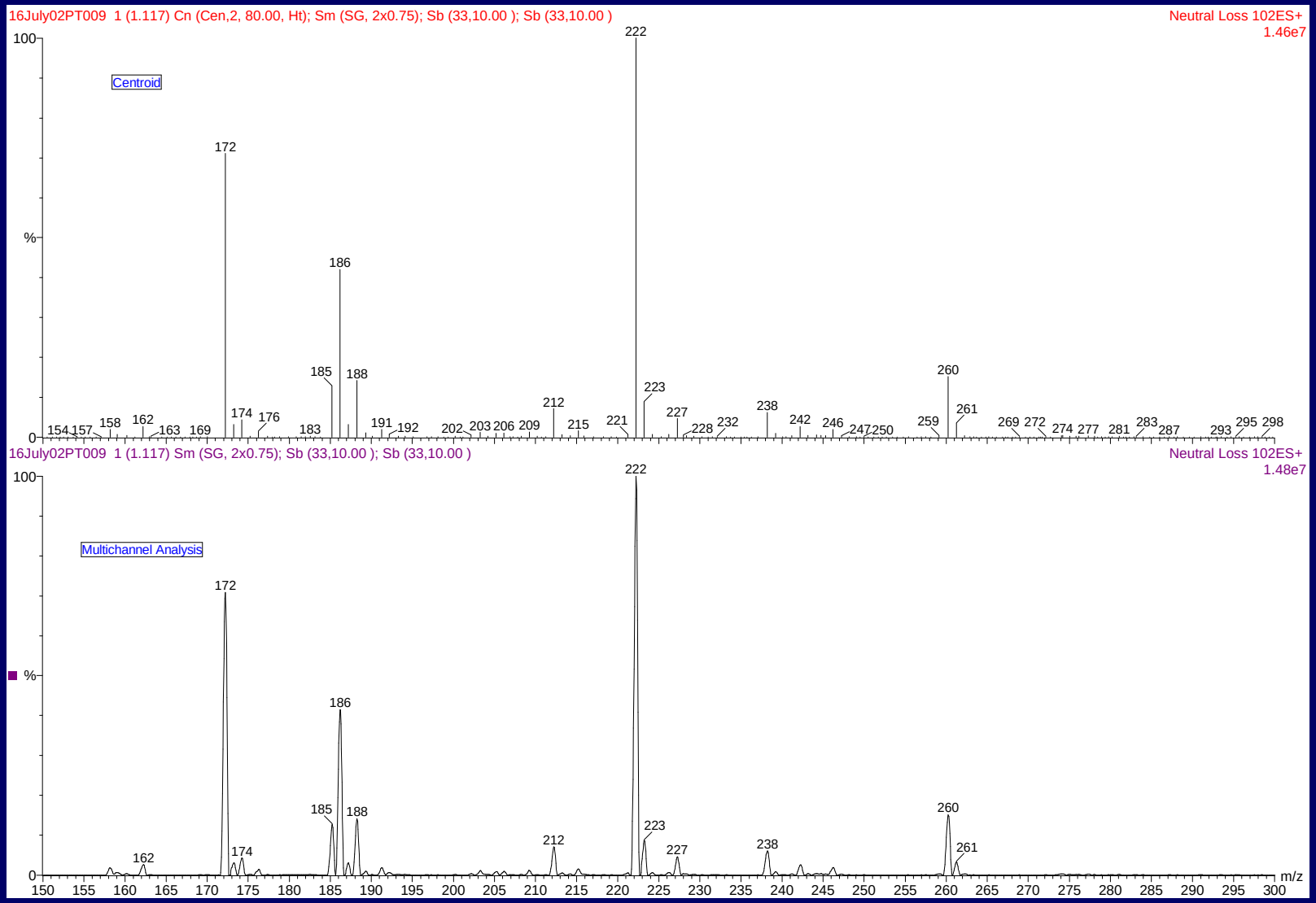
Photomultiplier 2



Mass Spectrum Acquisition

- Three basic types of scanning
  - Full scan
    - Detect all ions in given m/z range
      - Continuum, multichannel analysis (MCA) and centroid variations
  - Selected Ion Monitoring (SIM)
    - Set to detect only one m/z
  - Selected Reaction Monitoring (SRM)
    - Tandem MS experiment – see later
    - Detect specific fragment ion from specific precursor ion
    - Can cycle between a number pairs of ions – Multiple Reaction Monitoring (MRM)

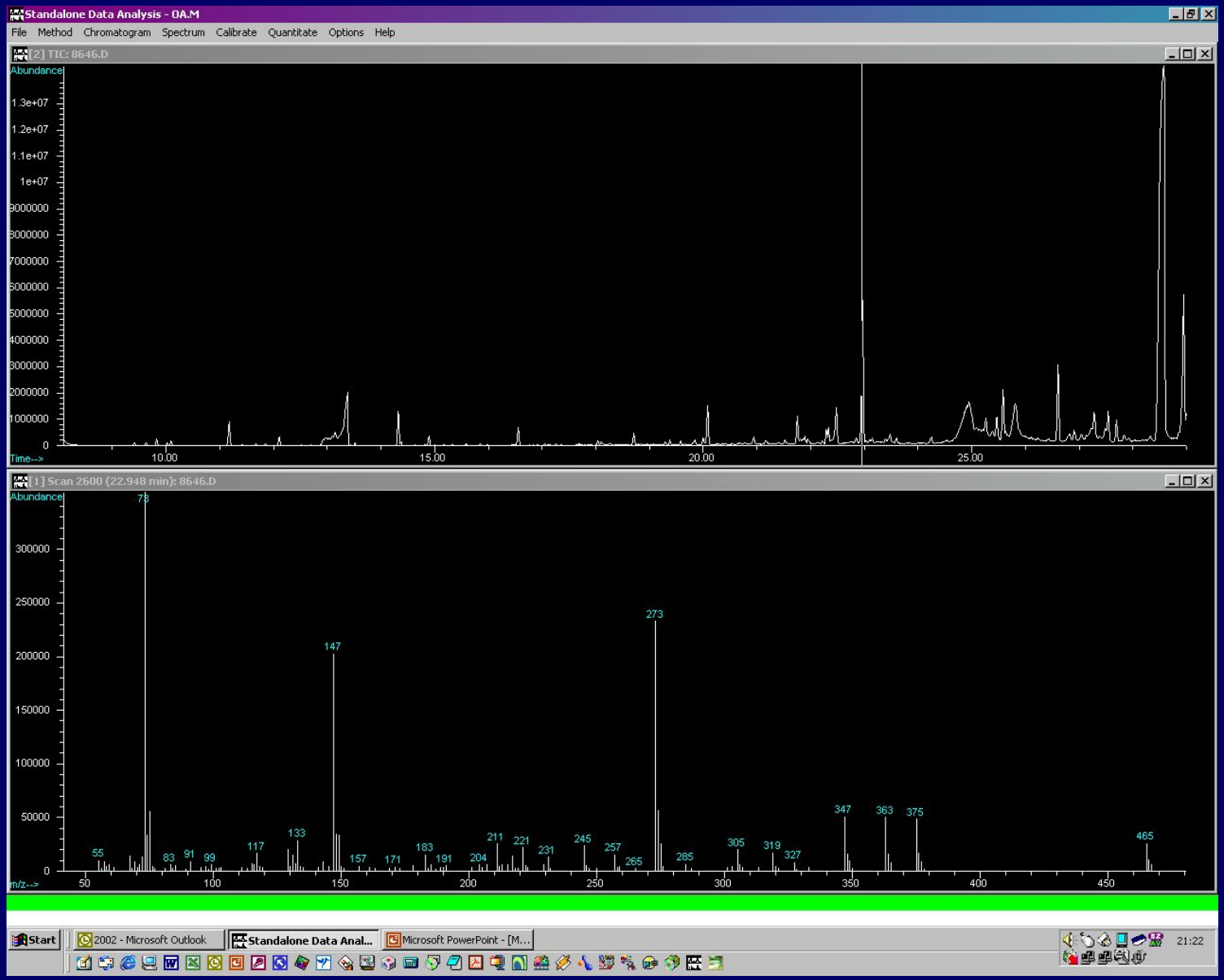
MCA vs Centroid Data



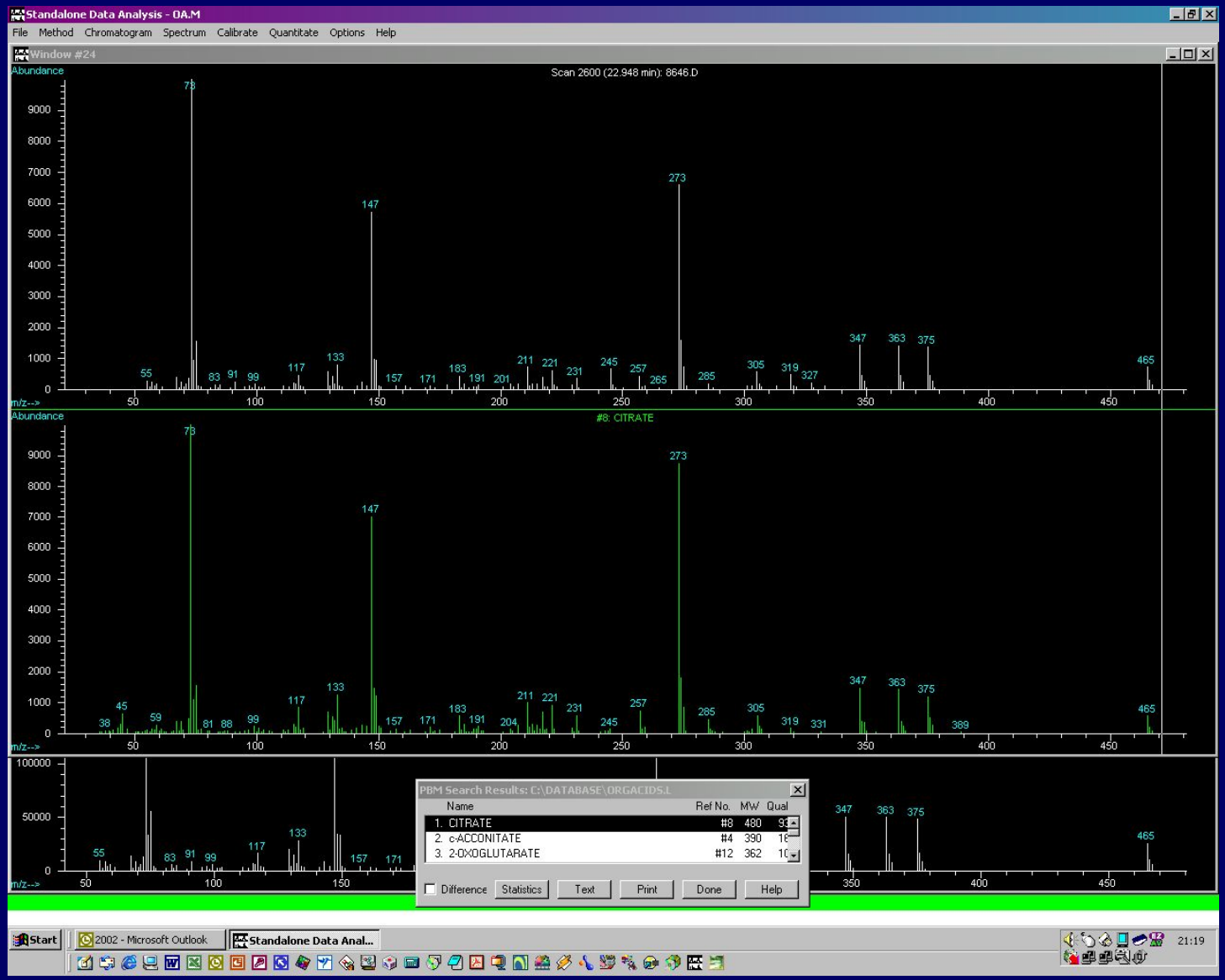
Example of EI Use

- GC/MS for organic acids
  - Organic acids converted to trimethylsilyl esters
  - Esters separated by capillary GC column
  - As compounds elute they enter EI source, are ionised, fragmented and the fragments detected to give mass spectrum
  - Current in detector is plotted vs. time to create chromatogram (“total ion chromatogram”)
  - Mass spectrum for each chromatogram peak can be inspected and likely compounds identified by library matching

Library Matching 1



Library Matching 2



Tandem Mass Spectrometry 1

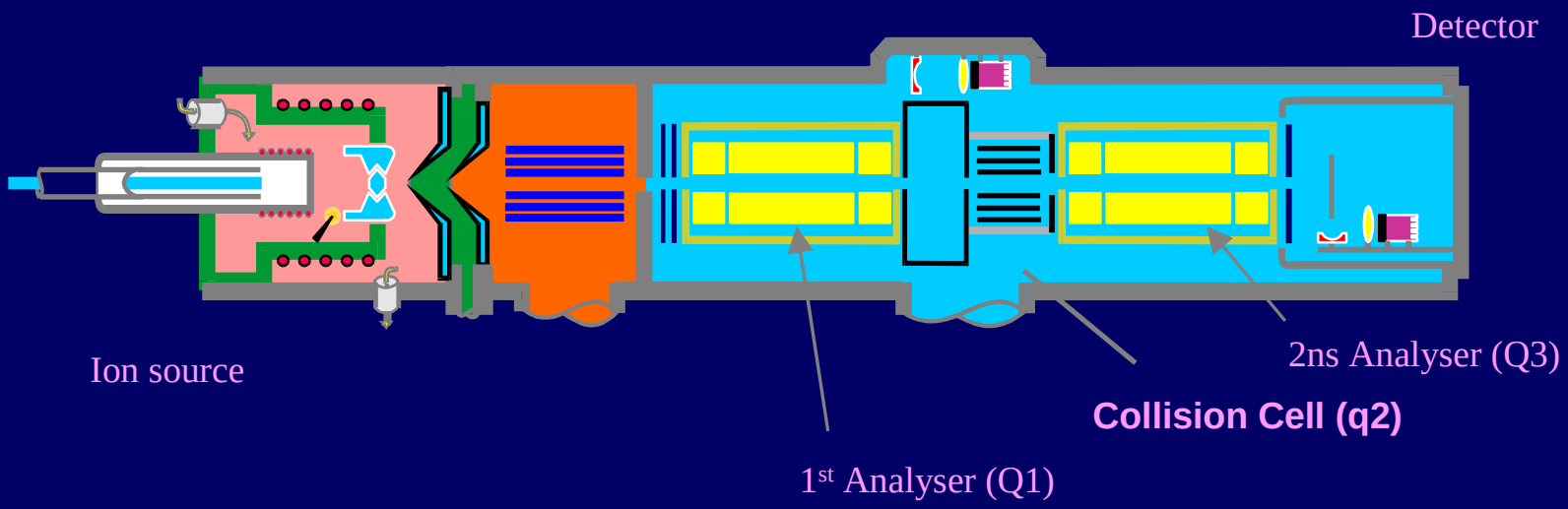
- Abbreviated MS/MS or MS^2
  - TMS should not be used!!!
- Essentially two mass analyser in series (tandem)
- Placed between analysers is a collision cell
  - Ions from first analyser collide with Ar in cell and fragment (collision-induced dissociation)
  - Fragments then analysed by second analyser
- Allow greater range of investigations

Tandem Mass Spectrometry 2

- Ion source usually of soft type to form ions with little fragmentation
- Commonly ESI and ACPI sources used
- Commonest instruments are “triple quadrupoles”
  - Generic name for class of instruments
  - 1st and 3rd quadrupoles (Q1, Q3) have RF and DC voltages to effect ion separation
  - Middle quadrupole (q2)is RF only
    - In absence of DC voltage time averaged voltage experienced by ions is zero
    - RF only focuses ions for transmission to next stage

Tandem Mass Spectrometry 3

- Generic triple quadruple MS/MS



Tandem Mass Spectrometry 4

Tandem Mass Spectrometry 5

- Types of MS/MS experiments
  - Simple MS scan using one quadrupole
  - Neutral Loss
    - Also (rarely) Neutral Gain
  - Precursor ion scan (“parents of”)
  - Product ion scan (“daughters of”)

Neutral Loss Experiment

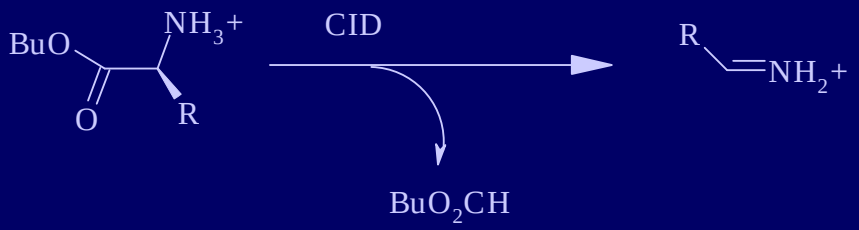
- Ion transmitted by Q1
- Collision induced dissociation in collision cell causes ion to lose a neutral molecule and form a smaller ion
- Smaller ion transmitted by Q3
- By scanning Q1 and Q3 at the same time with an offset equal to the neutral fragment mass only ions that lose the neutral fragment are detected
  - Increased sensitivity as background signal reduced

Precursor Ion Experiment

- Aim to find all precursor ions that generate a given product ion
- Q1 scanned to transmit ions to collision cell
- Ions fragment and fragments transmitted to Q3
- Q3 static for specific m/z of product ion of interest
- Only ions generating specific product ion are detected
  - Increased sensitivity

Amino Acids 1

- Amino acids measured as butyl esters
  - butyl esters lose butyl formate in collision cell (mass 102 Da)



  - Scanning MS1 and MS2 together but with MS2 lagging 102 behind MS1 only those species losing 102 Da fragments are detected

Amino Acids 2

- Phenylalanine butyl ester (m/z 222)
- ions ---> MS1 ---> Col cell ---> MS2 --> detector

  scan 120 - 300 scan 18 - 198

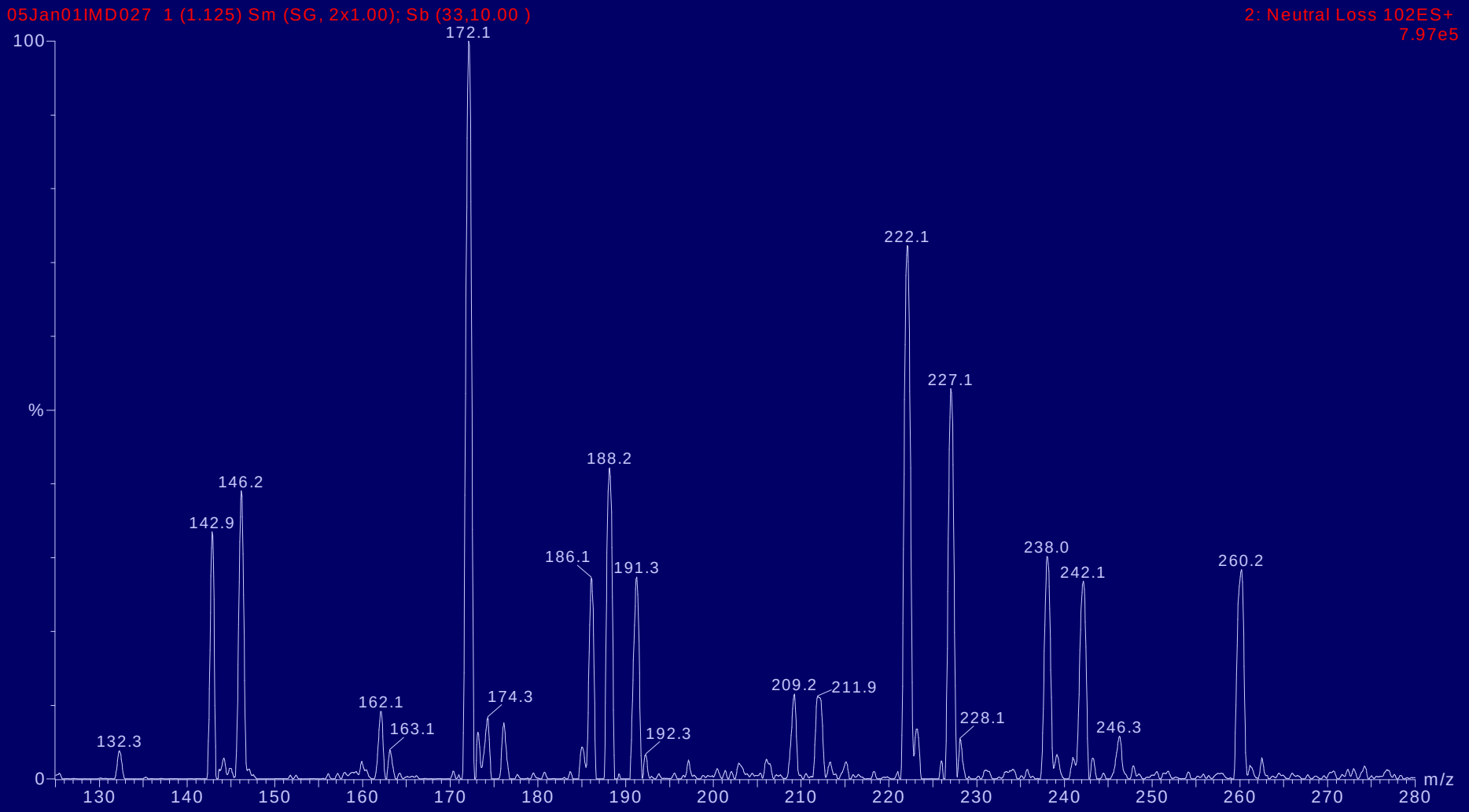
  222 -----> -102 ------> 120 --->

  – when MS1 transmits m/z 222 MS2 is set to transmit m/z 120
  – only ions of m/z 222 losing 102 Da fragment detected

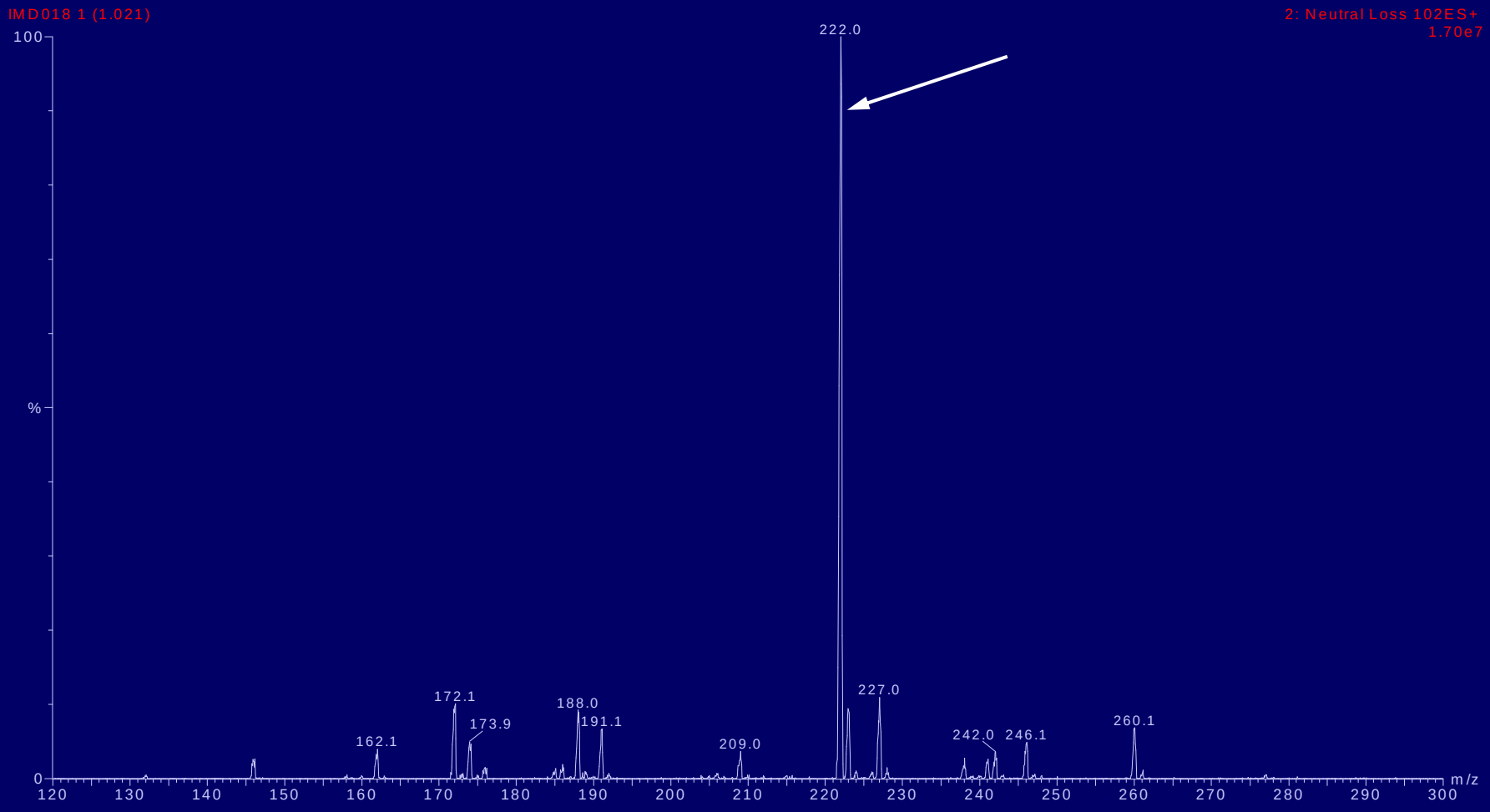
Amino Acids 3

- NL 102 generic experiment
  - Some amino acids are better detected by other scans
    - Basic amino acids NL 119 (butyl formate + ammonia)
    - Glycine NL 56
    - Arginine NL 161

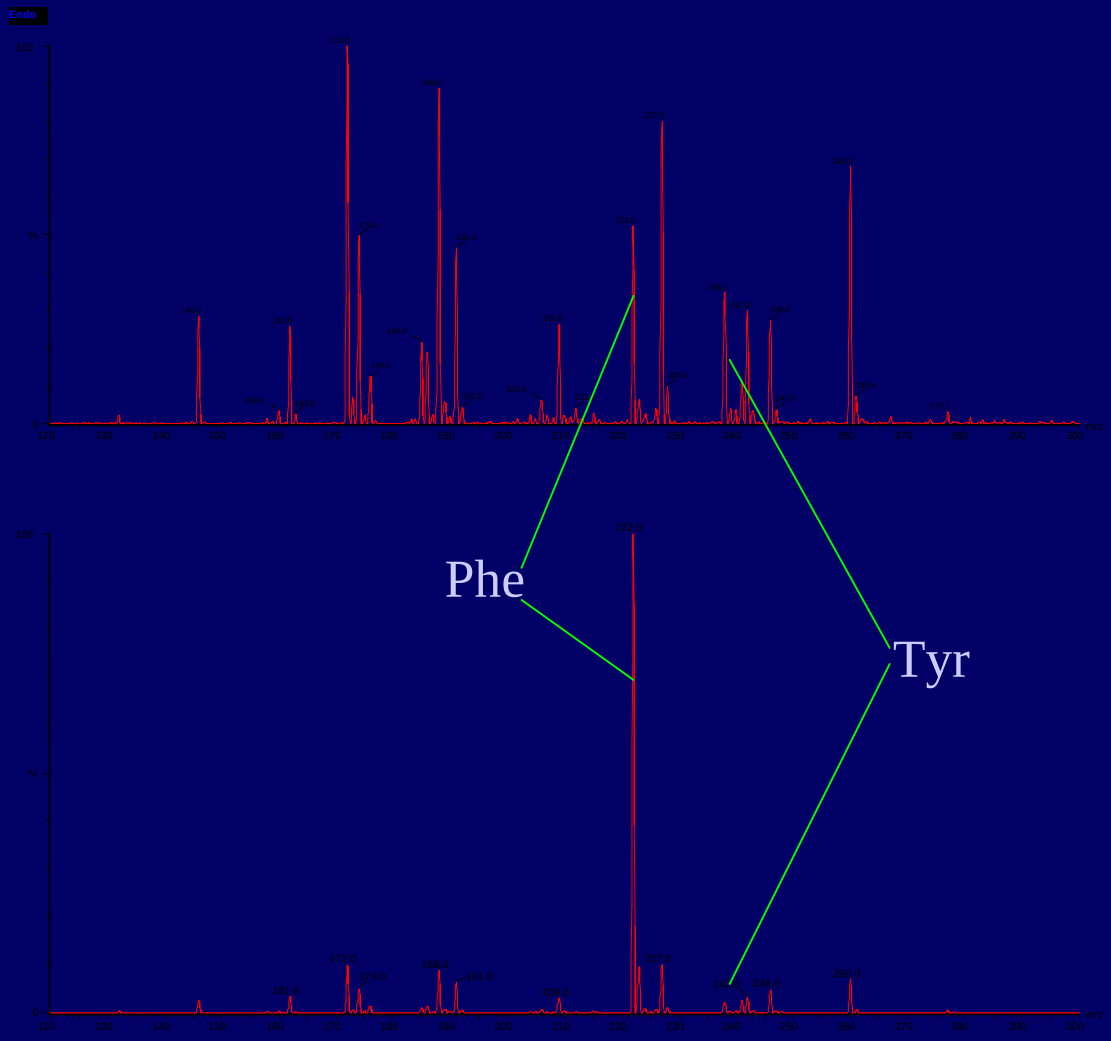
Normal Amino Acid Spectrum



Phenylketonuria



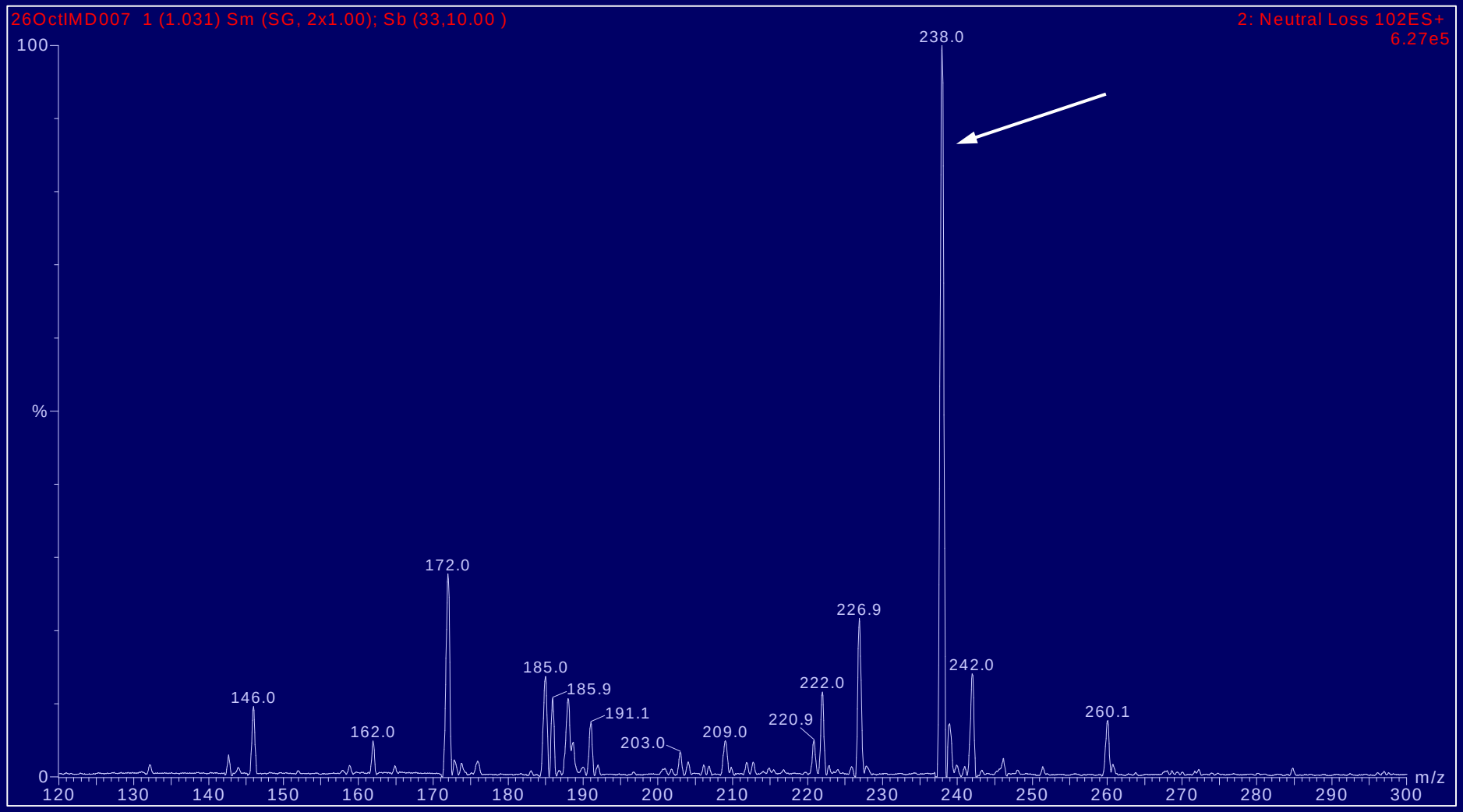
MS/MS Spectra For Normal vs. PKU



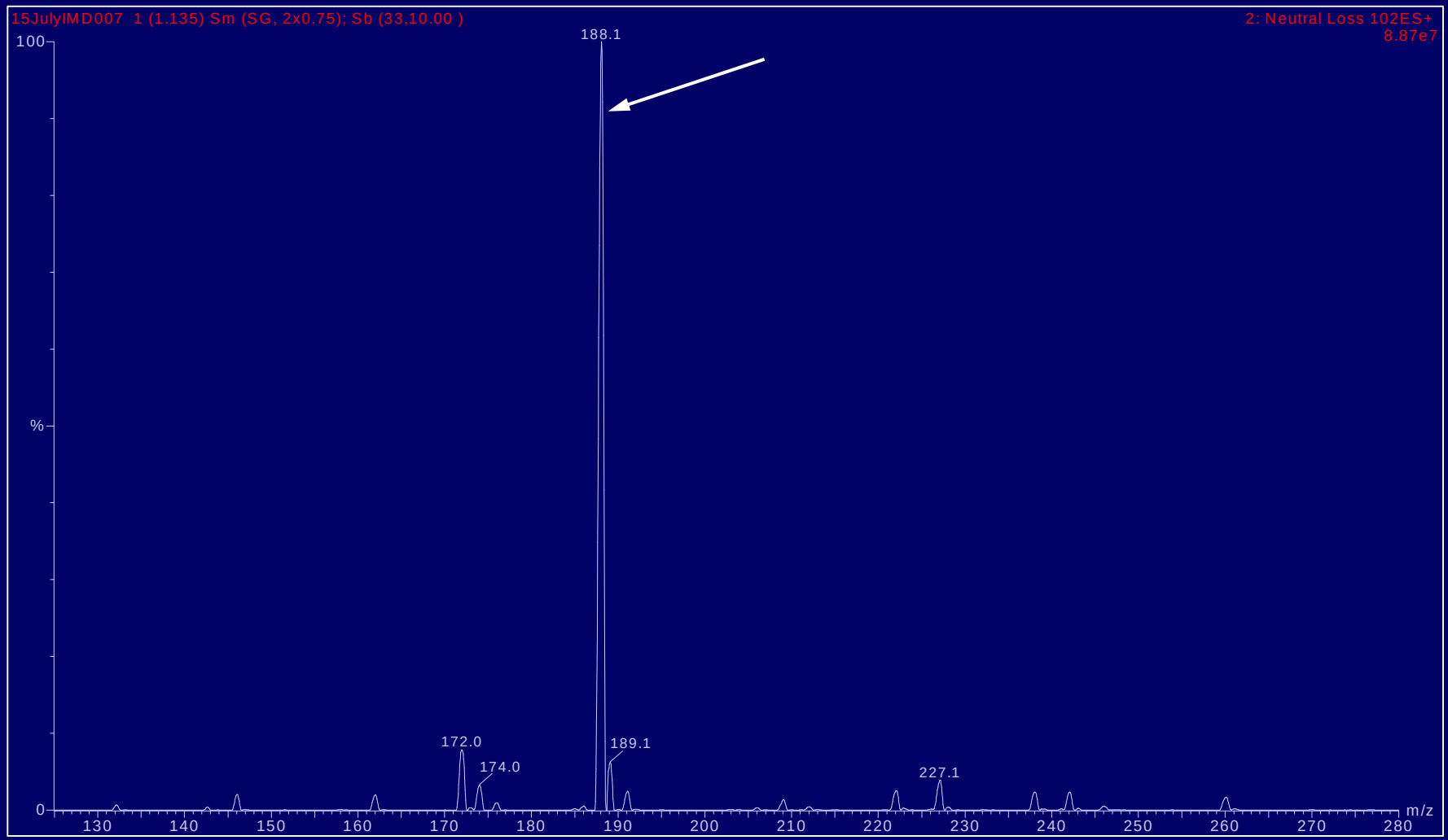
Normal Subject

PKU Patient

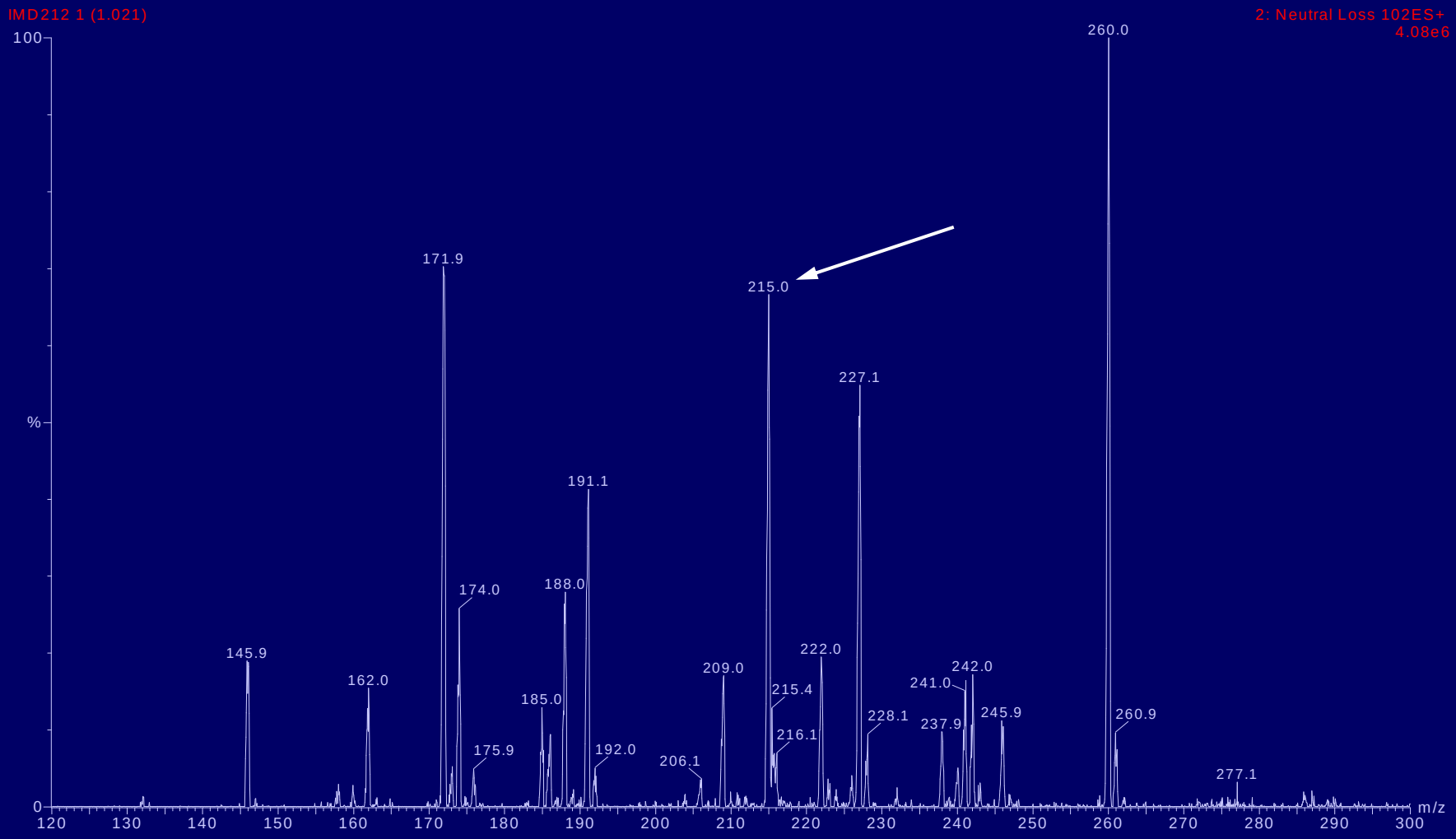
Tyrosinaemia



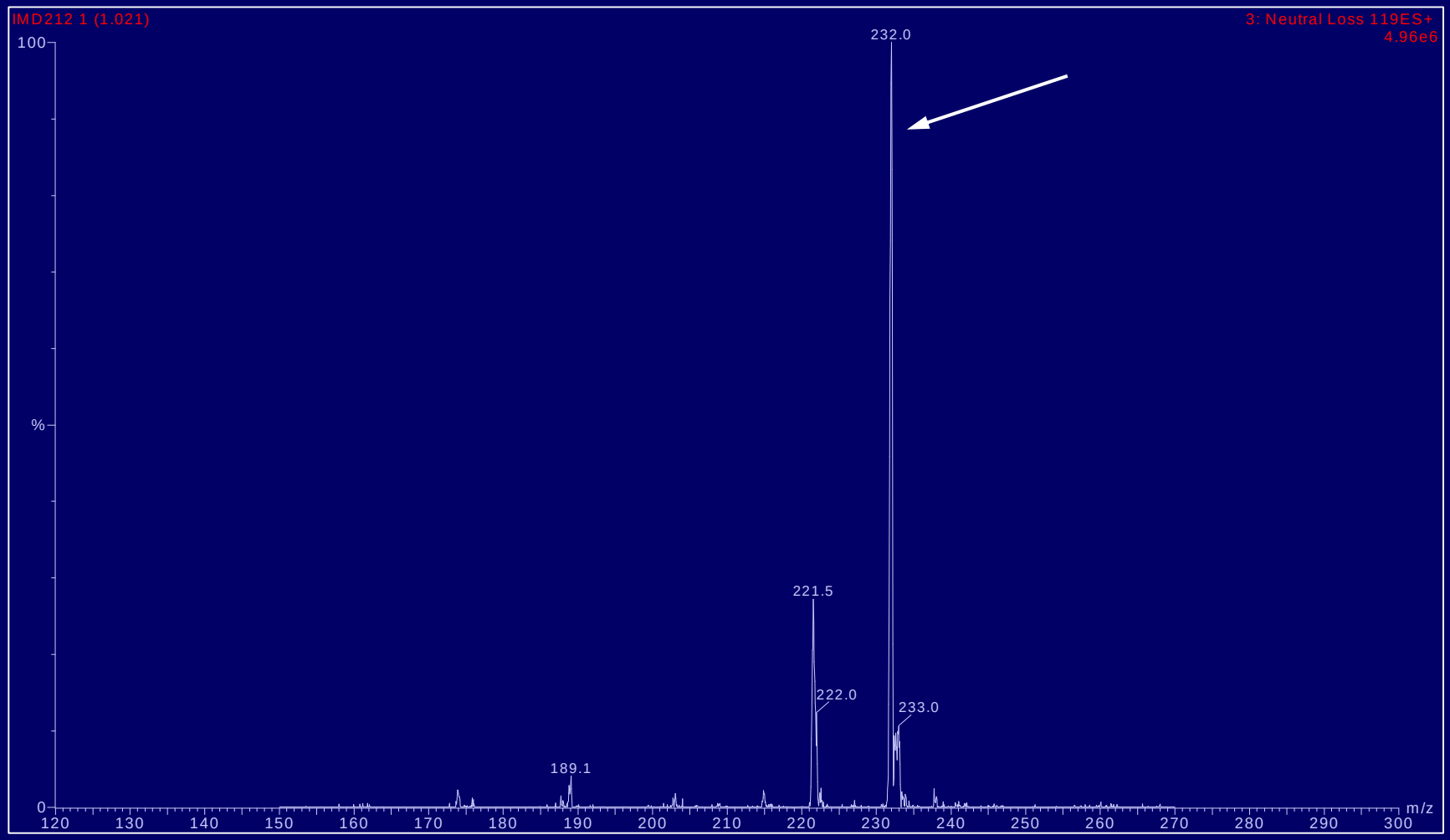
Maple Syrup Urine Disease



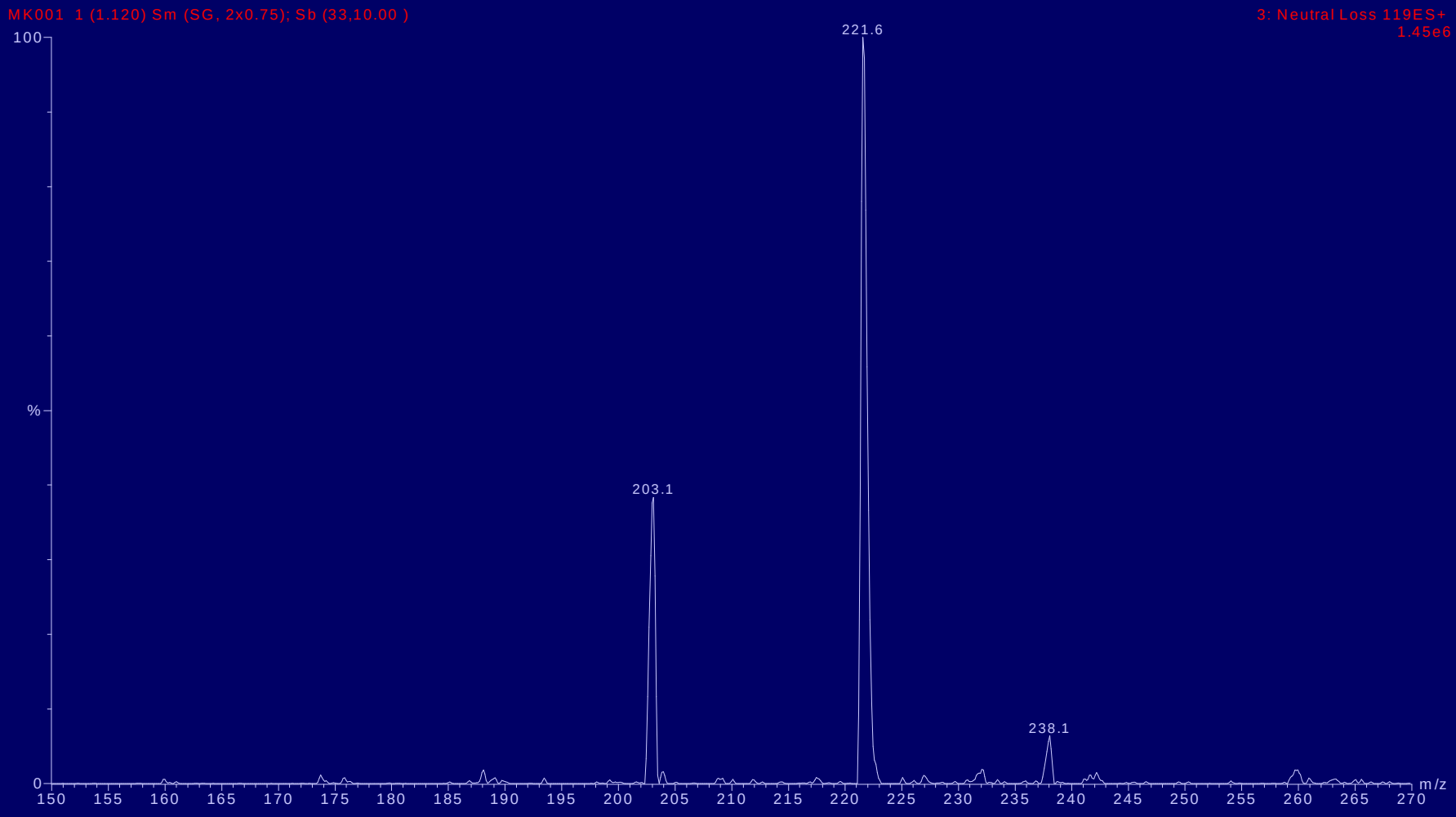
Citrullinaemia NL 102



Citrullinaemia NL 119

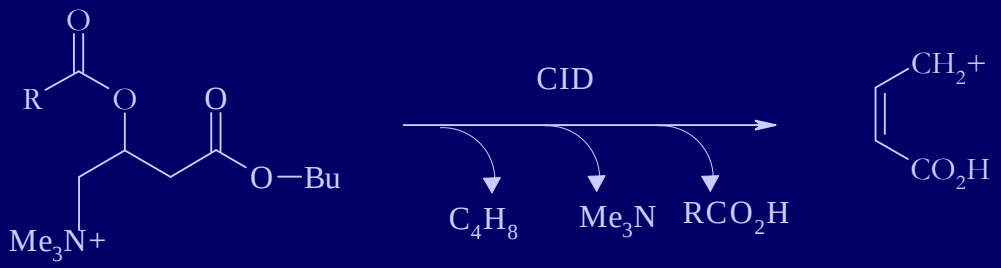


Normal Blood Spot NL 119



Acyl carnitines 1

- Butyl esters of acylcarnitines
  - prepared in same way as amino acids
  - esters all fragment to form an ion of m/z 85



  - by fixing MS2 to transmit m/z 85 but scanning MS1 only ions forming a m/z 85 fragment will be detected.

Acyl carnitines 2

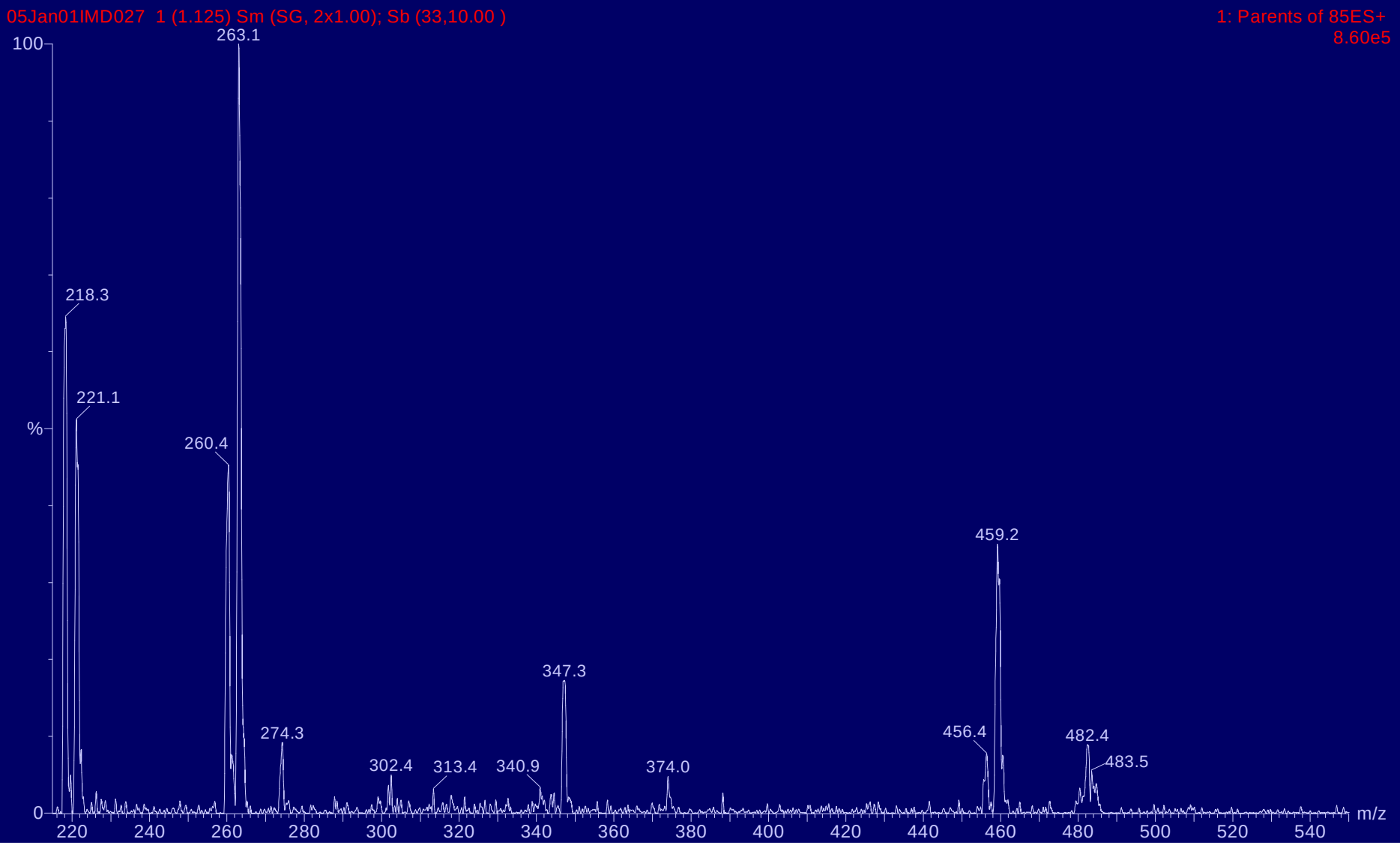
- Butyl esters of free carnitine (m/z 218) and acetylcarnitine (m/z 260)
- ions ---> MS1 ---> Col cell ---> MS2 --> detector

scan 215 - 550 transmit 85

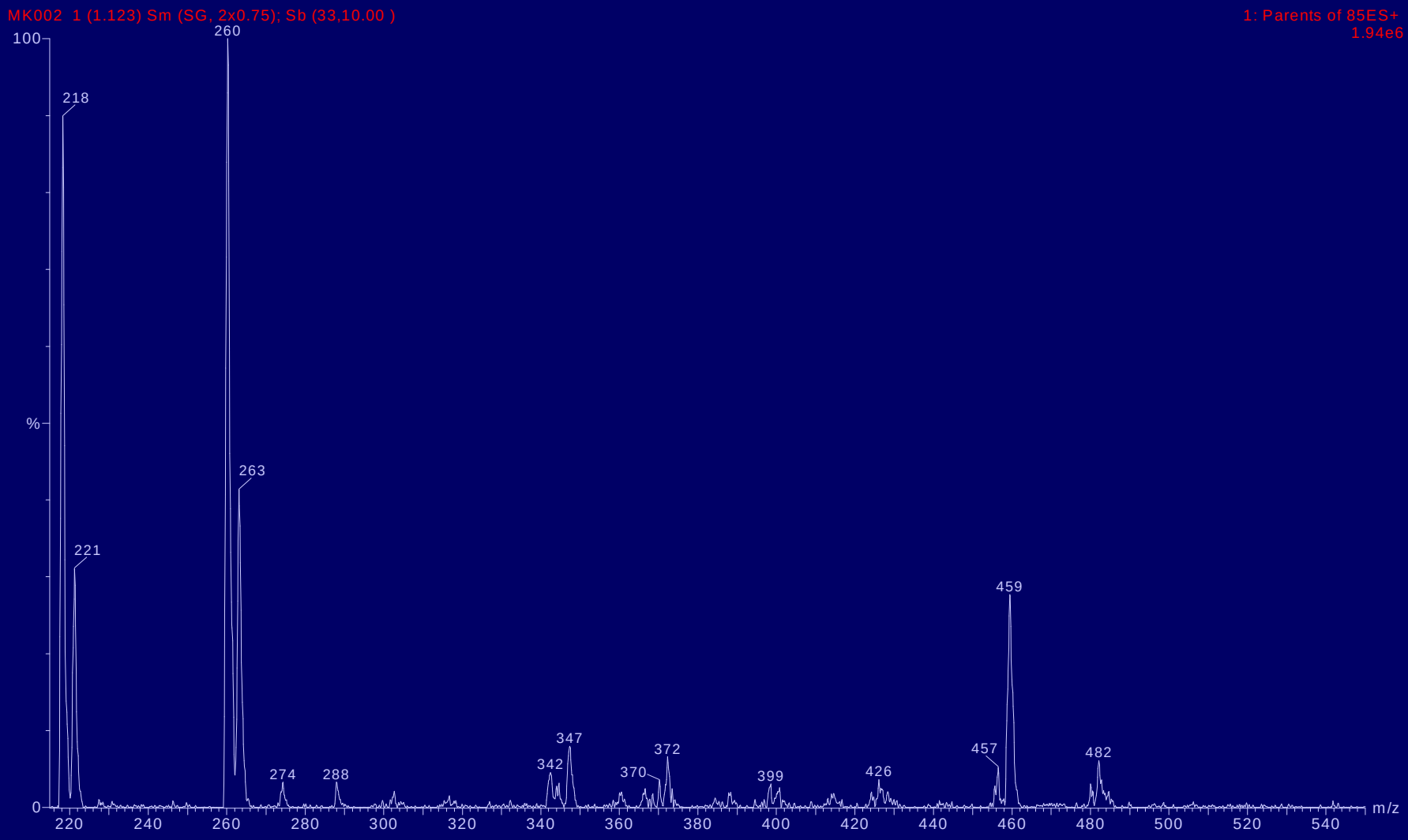
218 -----> -133 ------> 85 --->

260 -----> -175 ----> 85 --->

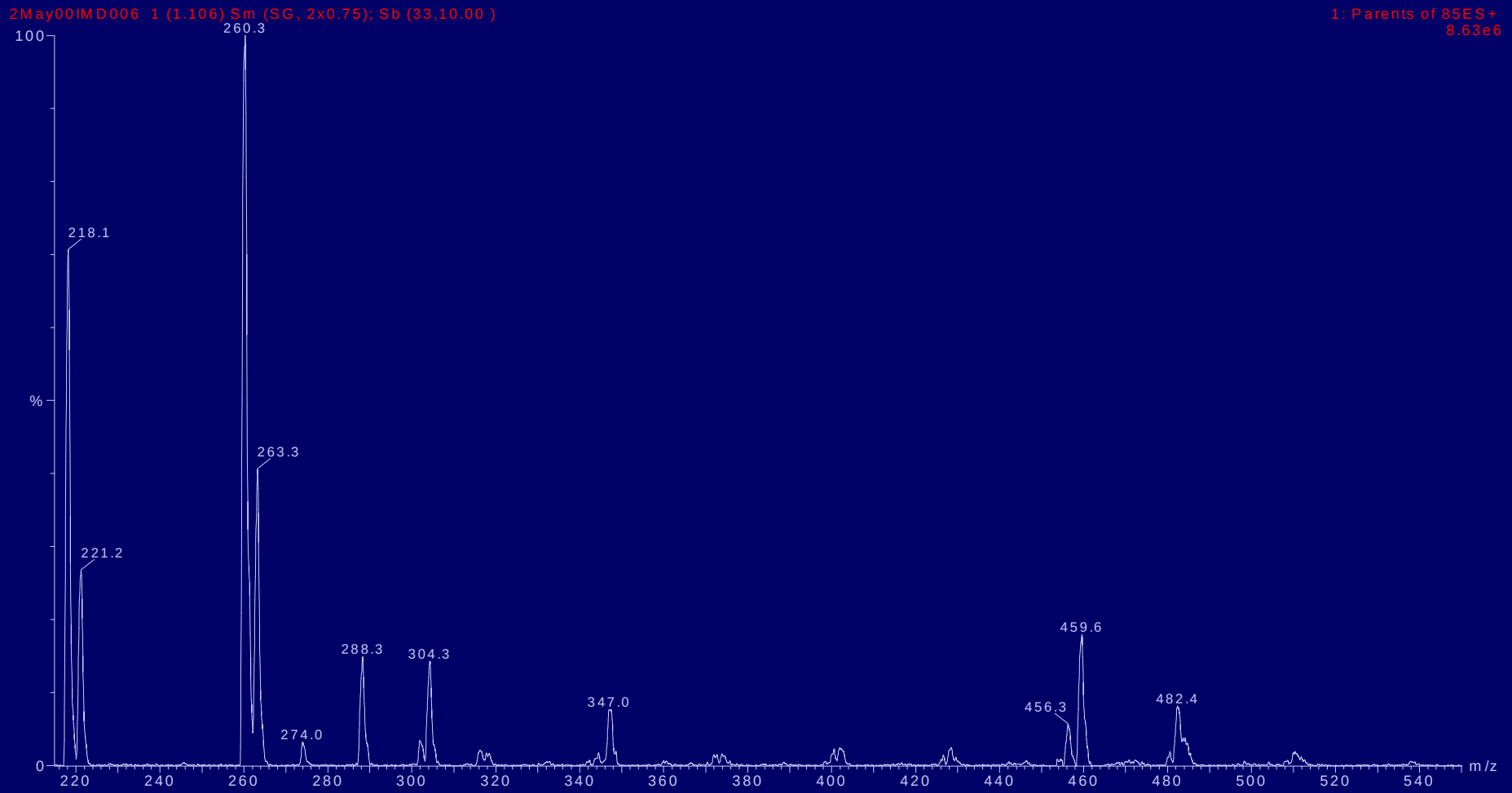
Normal Bloodspot Acyl Carnitine Spectrum



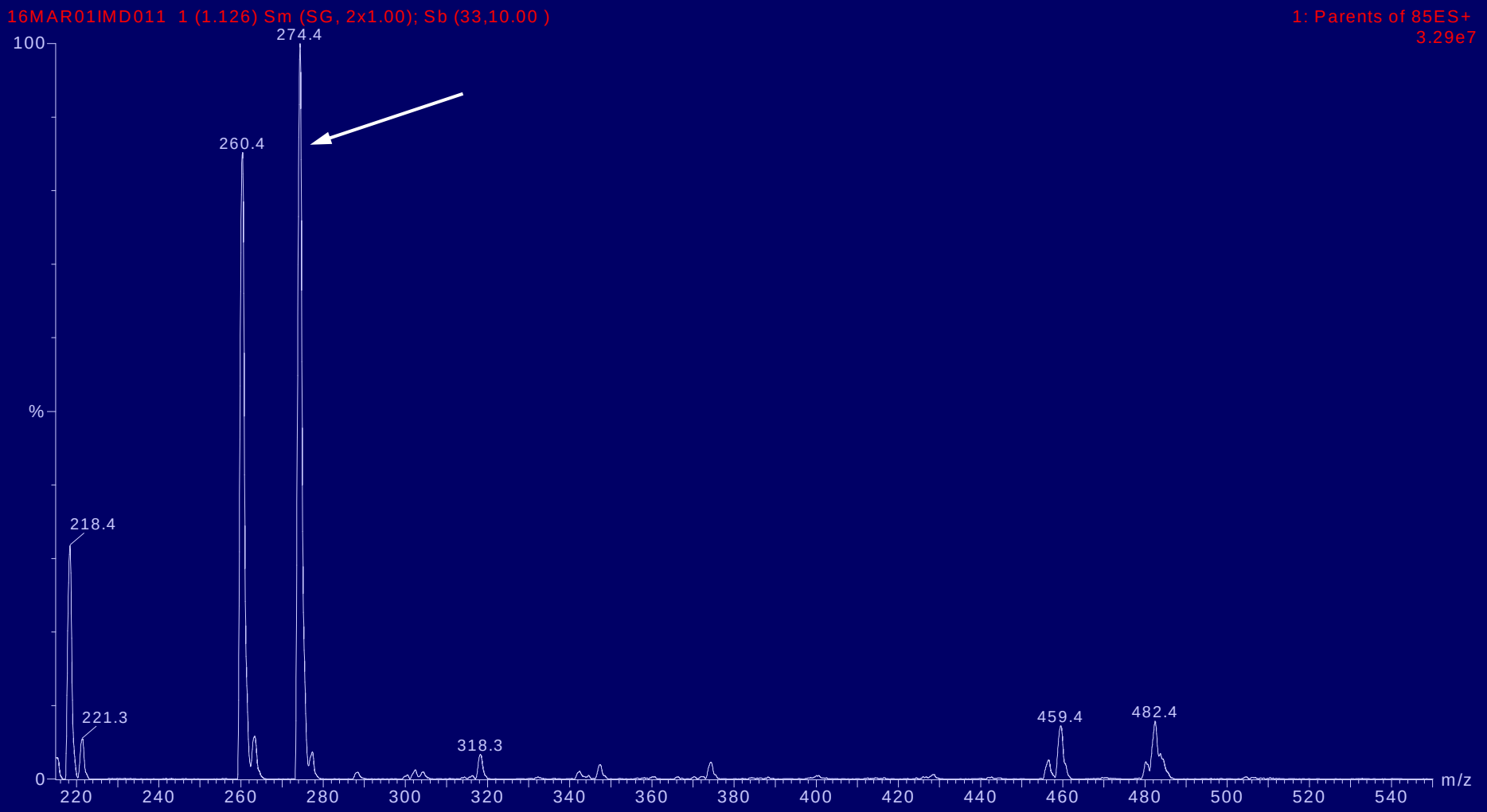
Normal Plasma Acyl Carnitine Spectrum



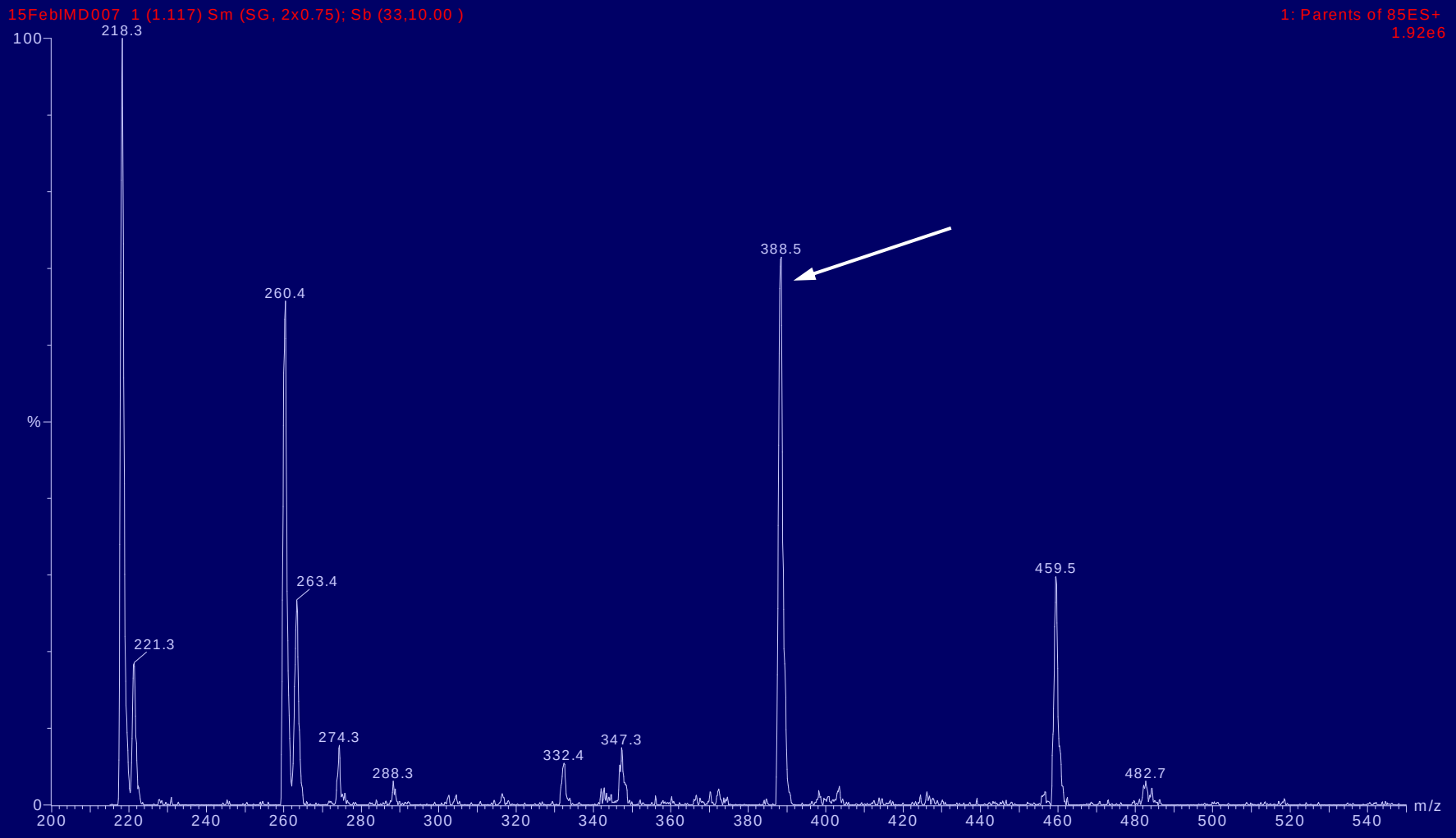
Post-mortem Specimen



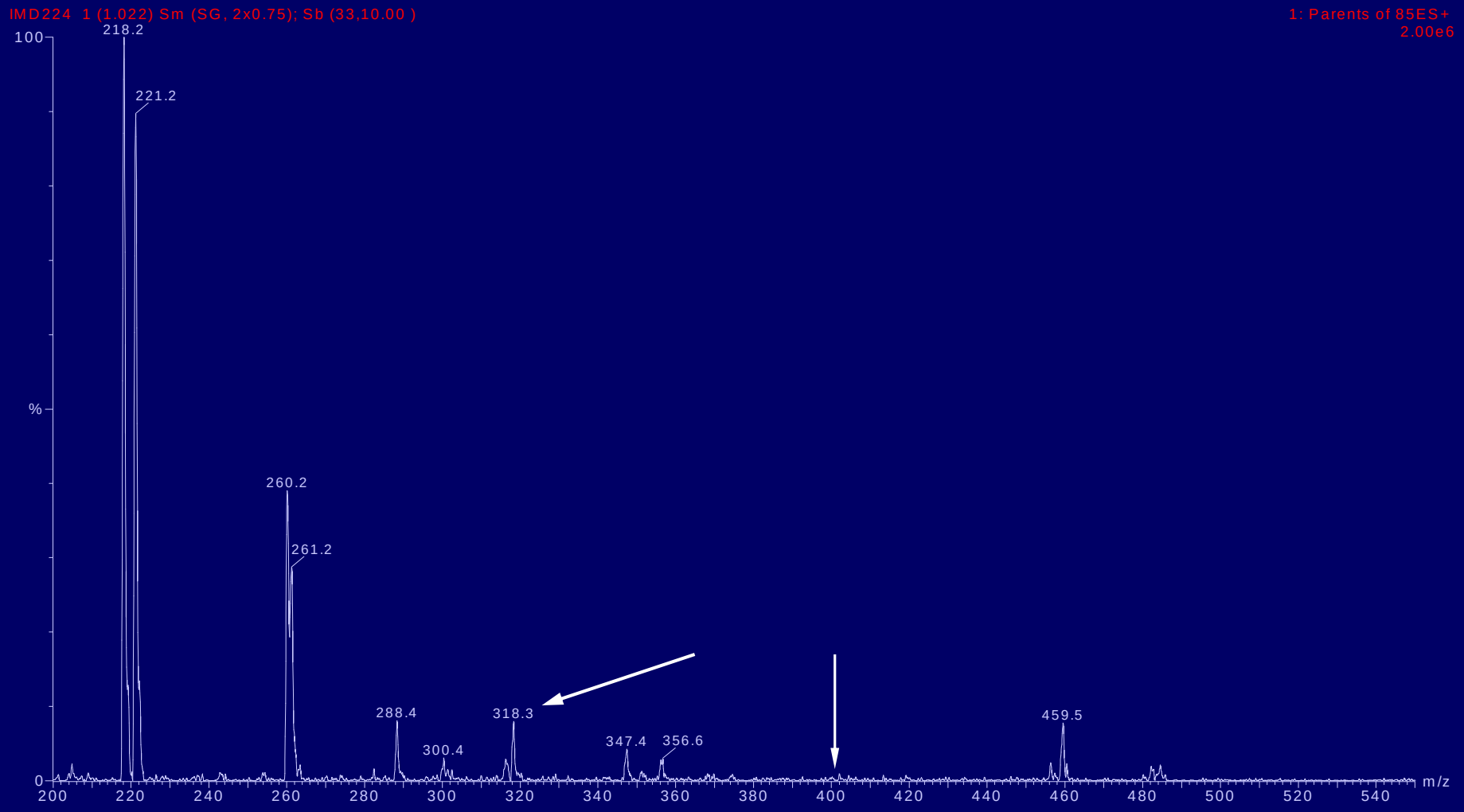
Propionic Acidaemia



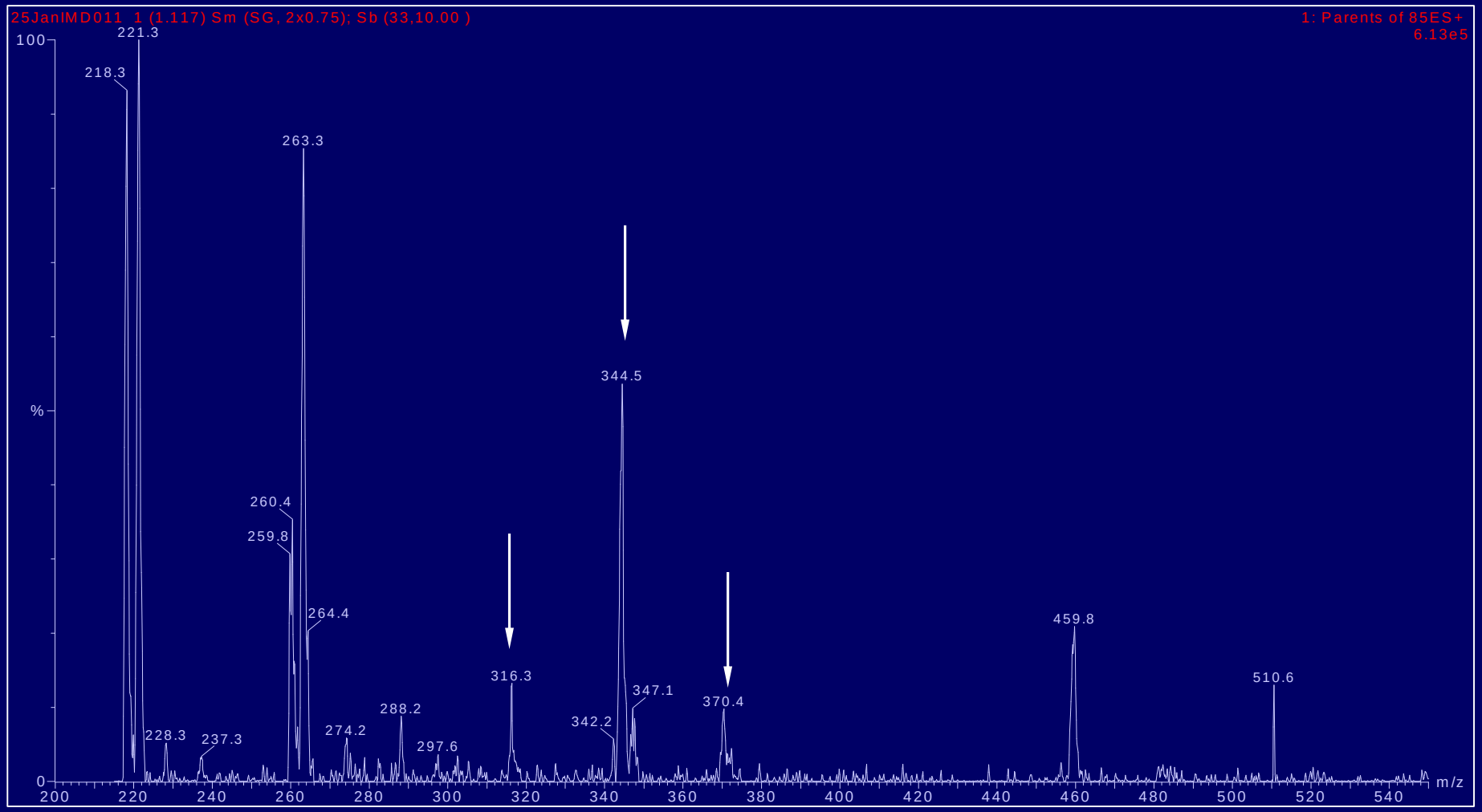
Glutaric Aciduria Type 1



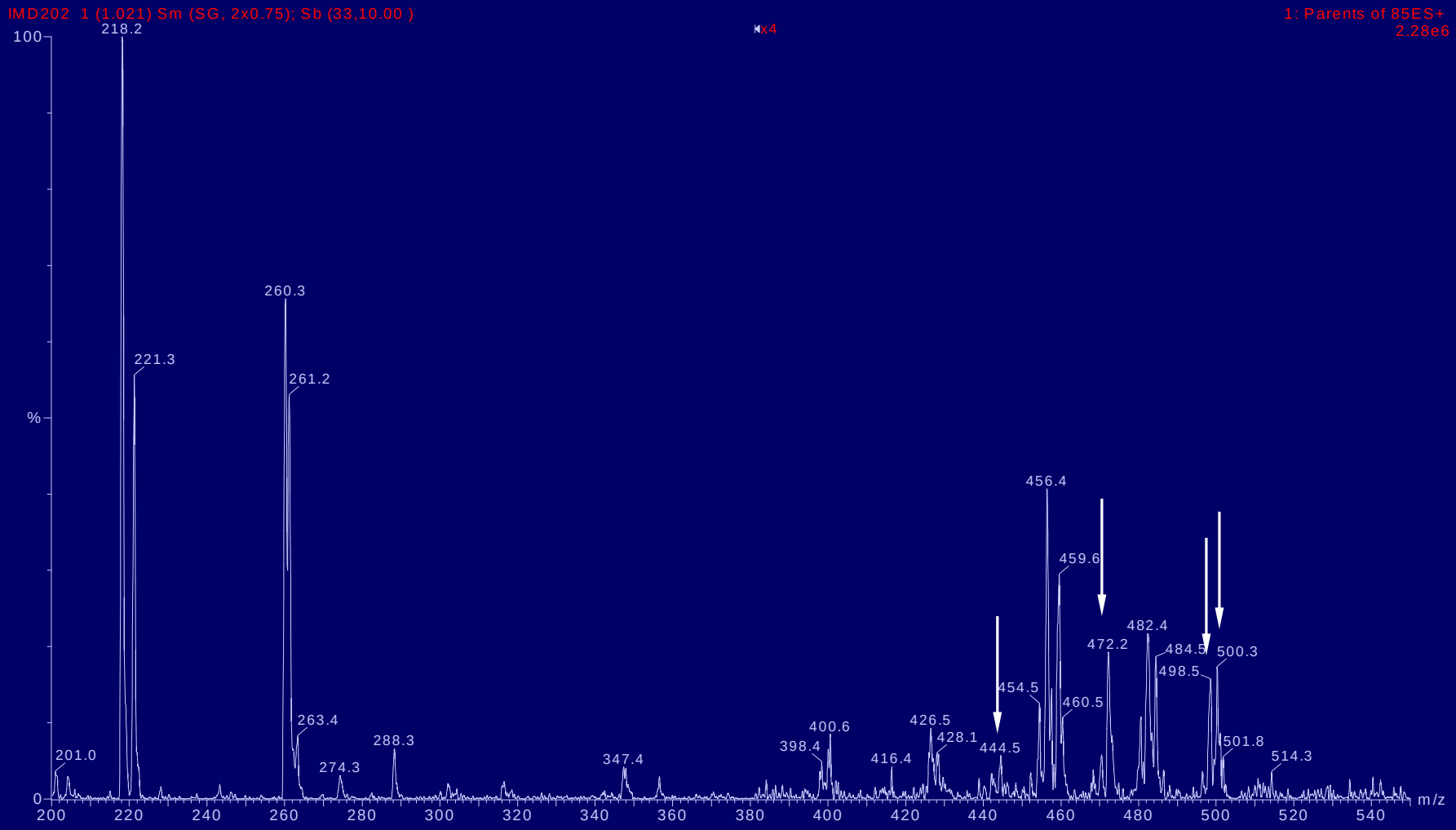
HMG CoA Lyase Deficiency



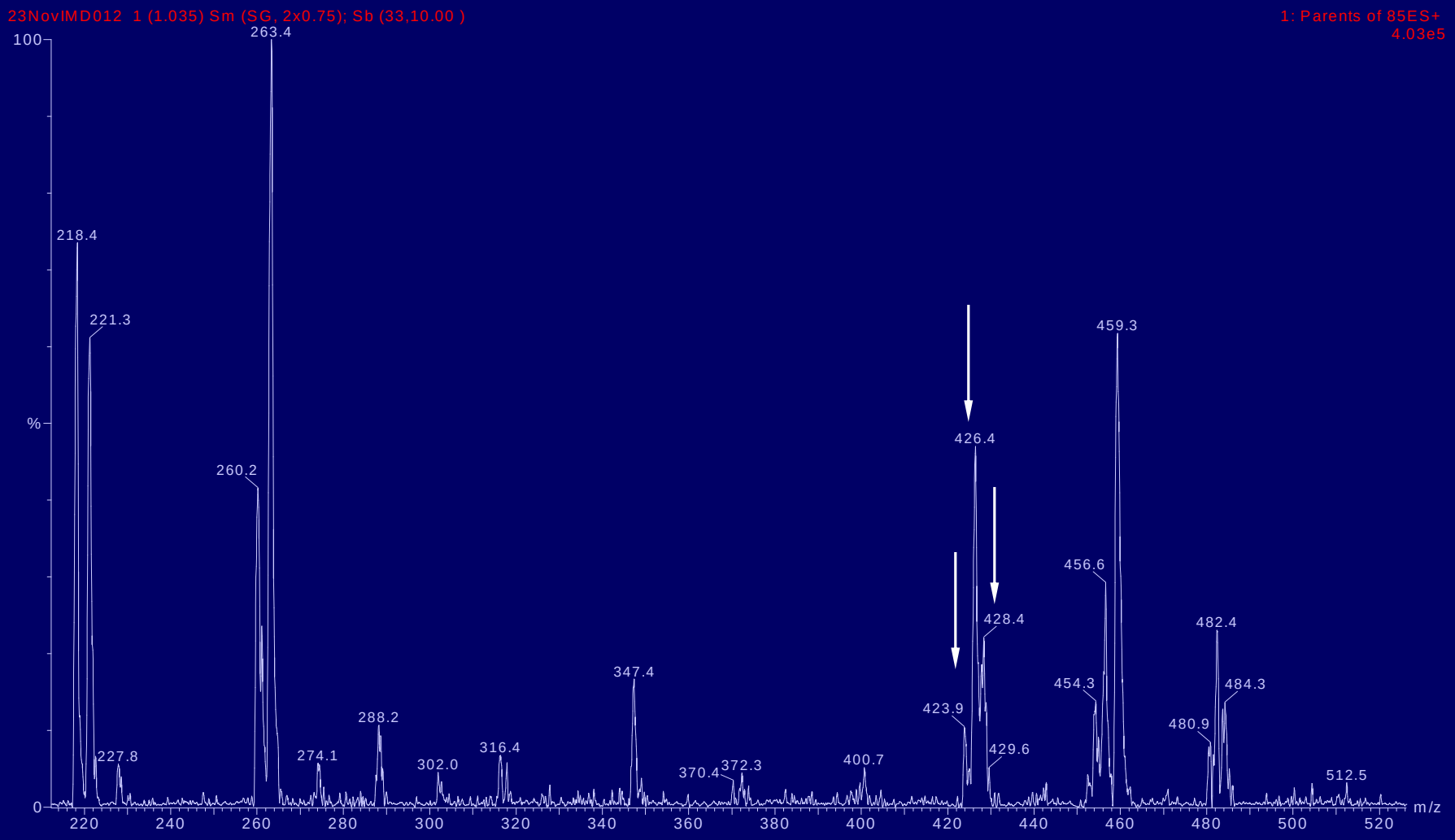
MCADD


25JanIMD011 1 (1.117) Sm (SG, 2x0.75); Sb (33,10.00)
1: Parents of 85ES+
6.13e5
100
%
0
221.3
218.3
263.3
344.5
260.4
259.8
264.4
316.3
459.8
510.6
347.1
370.4
288.2
342.2
274.2
228.3
237.3
297.6
200
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
520
540
m/z

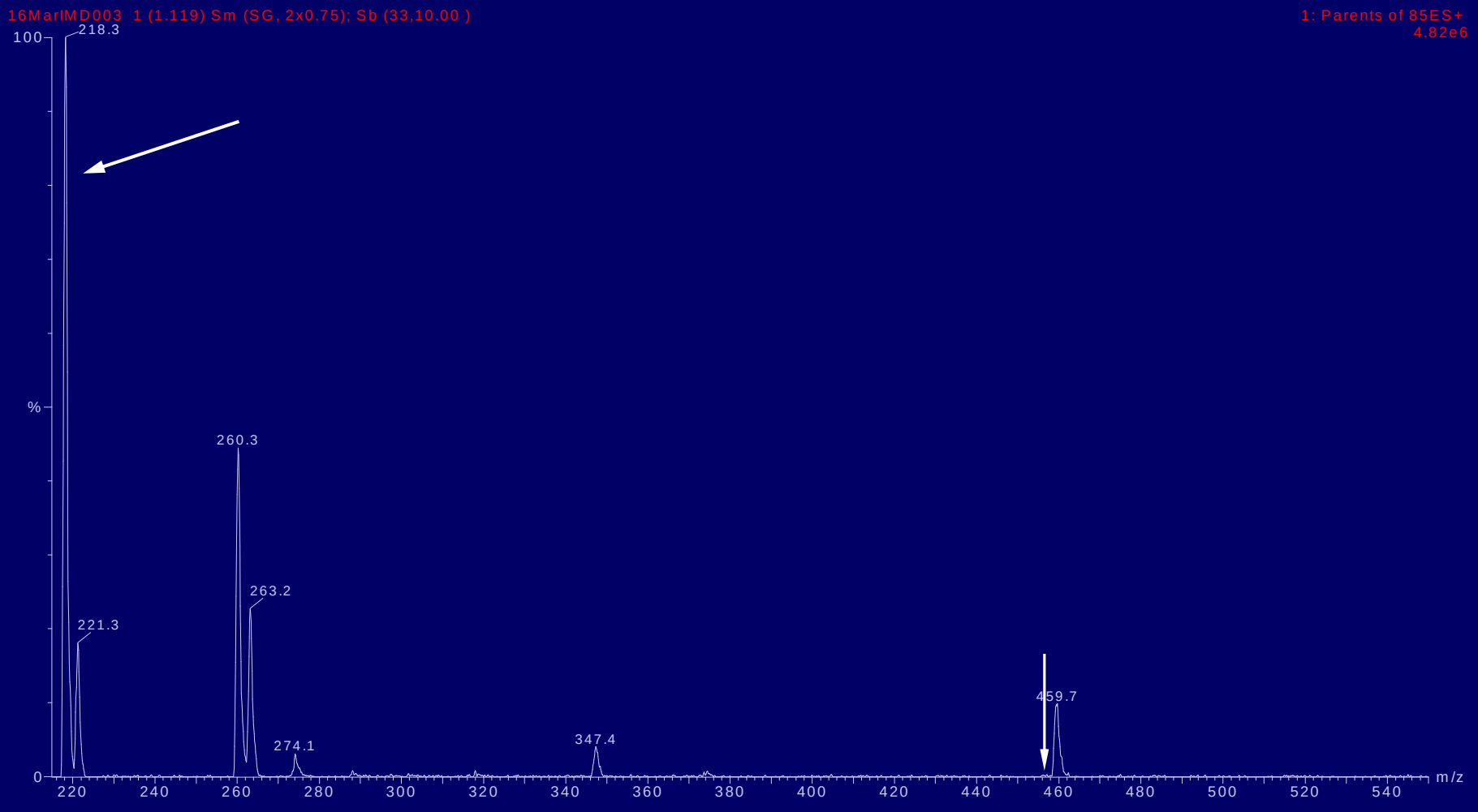
LCHADD


IMD202 1 (1.021) Sm (SG, 2x0.75); Sb (33,10.00)
x4
1: Parents of 85ES+
2.28e6
100
%
0
218.2
260.3
221.3
261.2
263.4
288.3
201.0
274.3
347.4
400.6
398.4
416.4
426.5
428.1
444.5
454.5
456.4
459.6
460.5
472.2
482.4
484.5
498.5
500.3
501.8
514.3
200
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
520
540
m/z

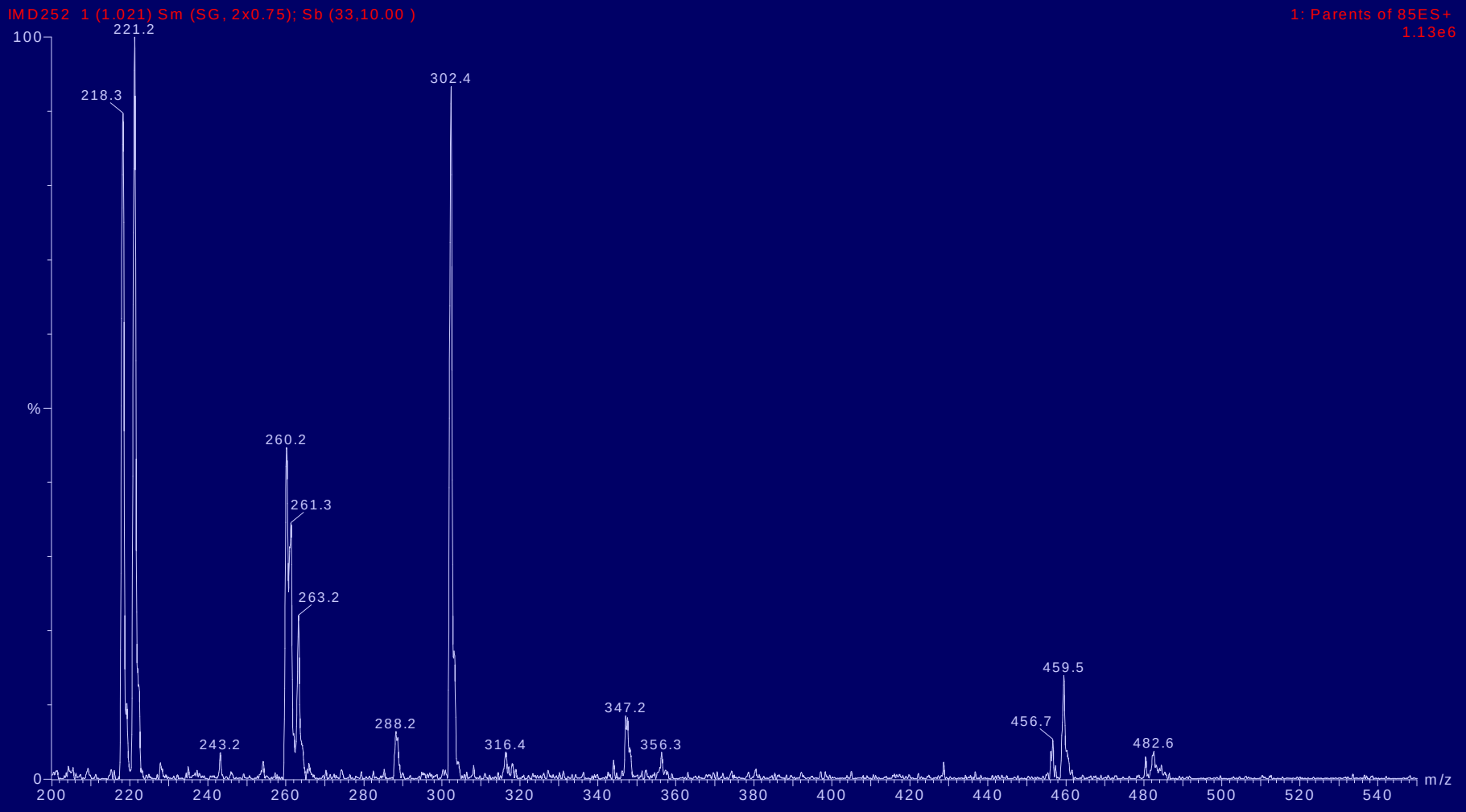
VLCADD


23NovIMD012 1 (1.035) Sm (SG, 2x0.75); Sb (33,10.00)
1: Parents of 85ES+
4.03e5
100
%
0
263.4
218.4
221.3
260.2
227.8
274.1
288.2
302.0
316.4
347.4
370.4
372.3
400.7
423.9
426.4
428.4
429.6
454.3
456.6
459.3
480.9
482.4
484.3
512.5
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
520
m/z

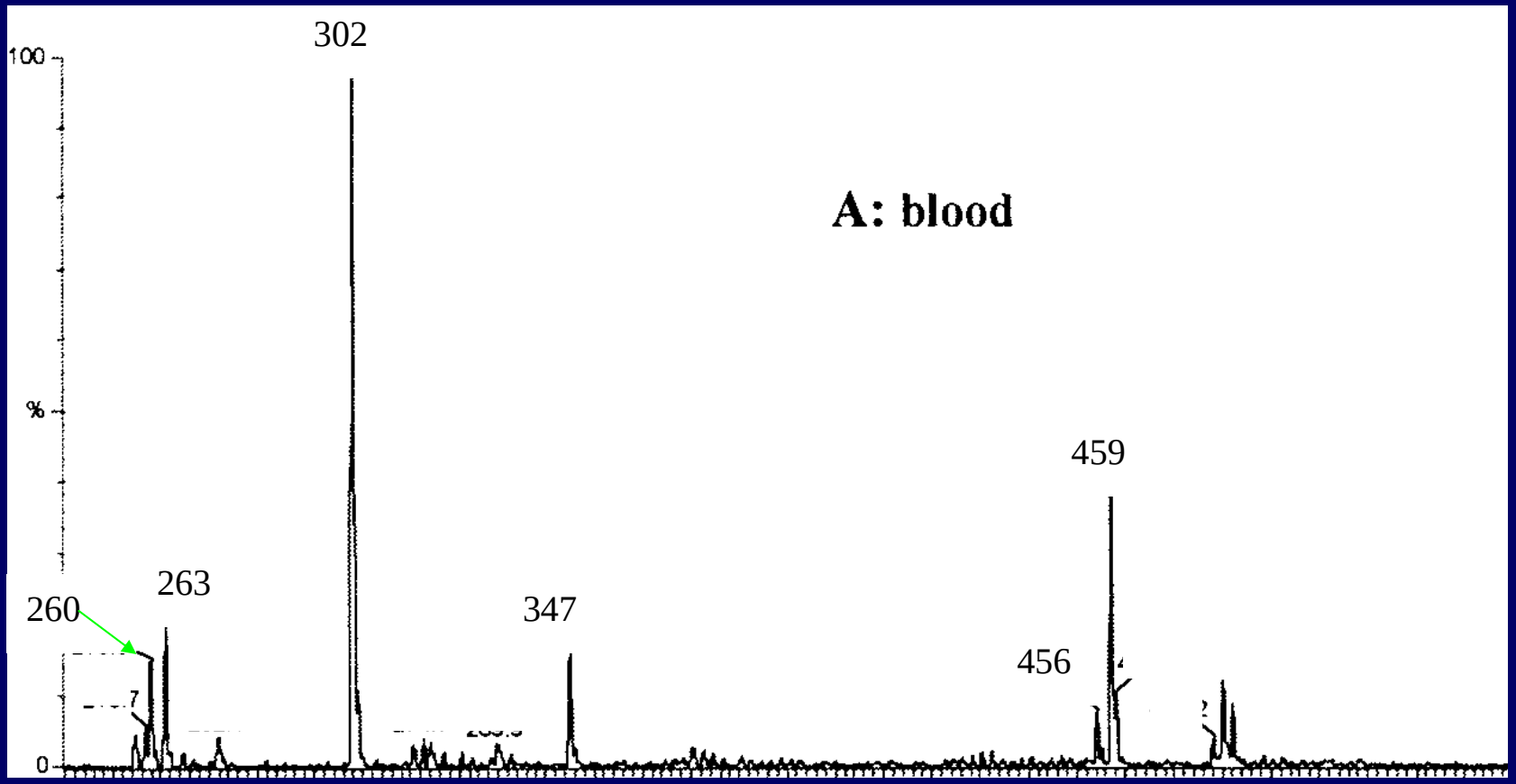
CPT-1 Deficiency



Isovaleric Acidaemia



What is the diagnosis?



Wrong!

J. Inher. Metab. Dis. 21 (1998) 624–630

Diagnosis of isovaleric acidaemia by tandem mass spectrometry: False positive result due to pivaloylcarnitine in a newborn screening programme

J. E. ABDENUR*, N. A. CHAMOLES, A. E. GUINLE, A. B. SCHENONE and A. N. J. FUERTES

Fundación para el Estudio de las Enfermedades Neurometabólicas (FESEN), Buenos Aires, Argentina

* *Correspondence: Fundación para el Estudio de las Enfermedades Neurometabolicas, Uriarte 2383 (1425), Buenos Aires, Argentina*

MS received 9.9.97 Accepted 14.1.98

Why?

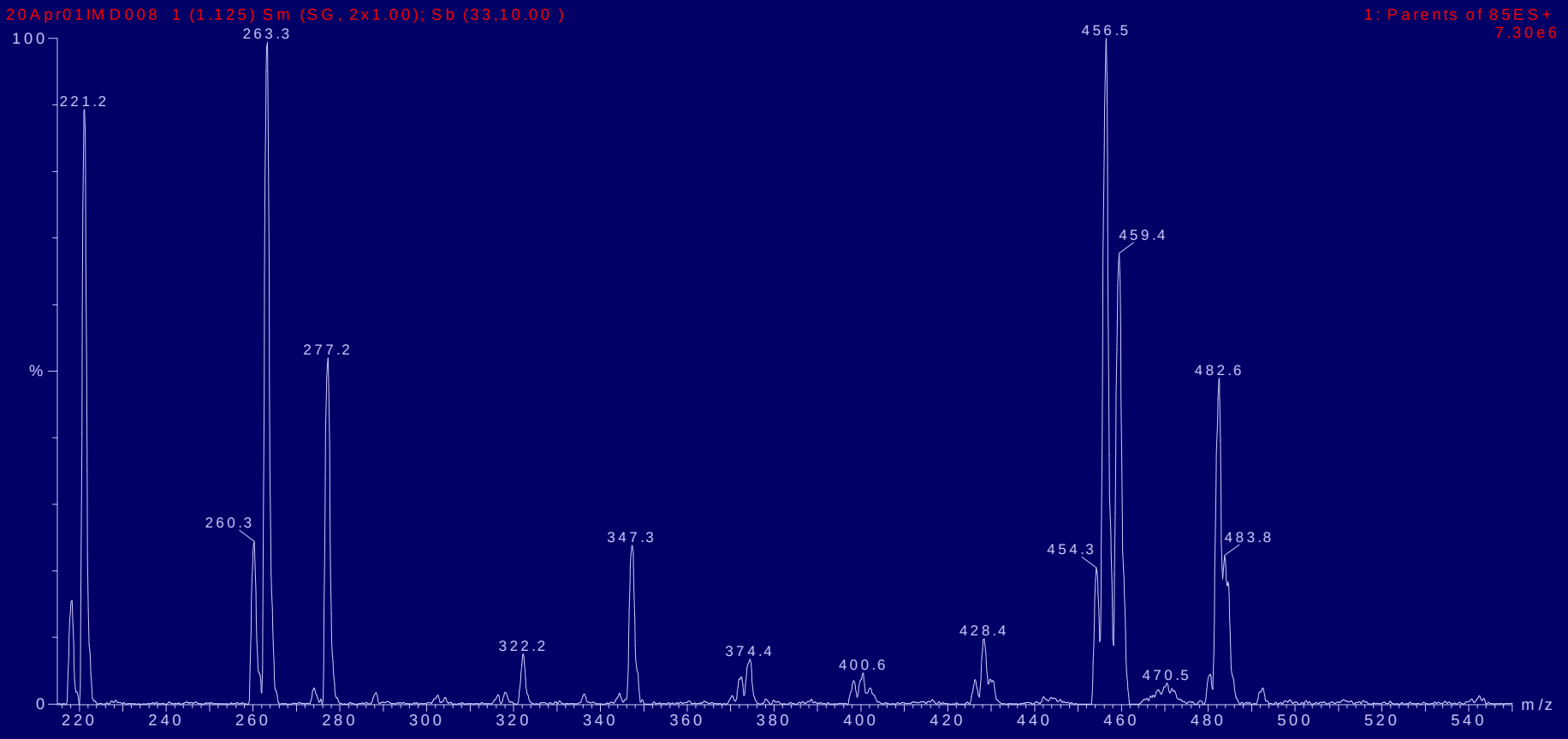
- PAR 85 experiment *not specific* to acylcarnitines
  - acylcarnitines are detected because they form a m/z 85 fragment
  - other species forming m/z 85 fragments will also be detected
  - possible diagnostic problems if other species has same mass as an acylcarnitine

Isobaric Species

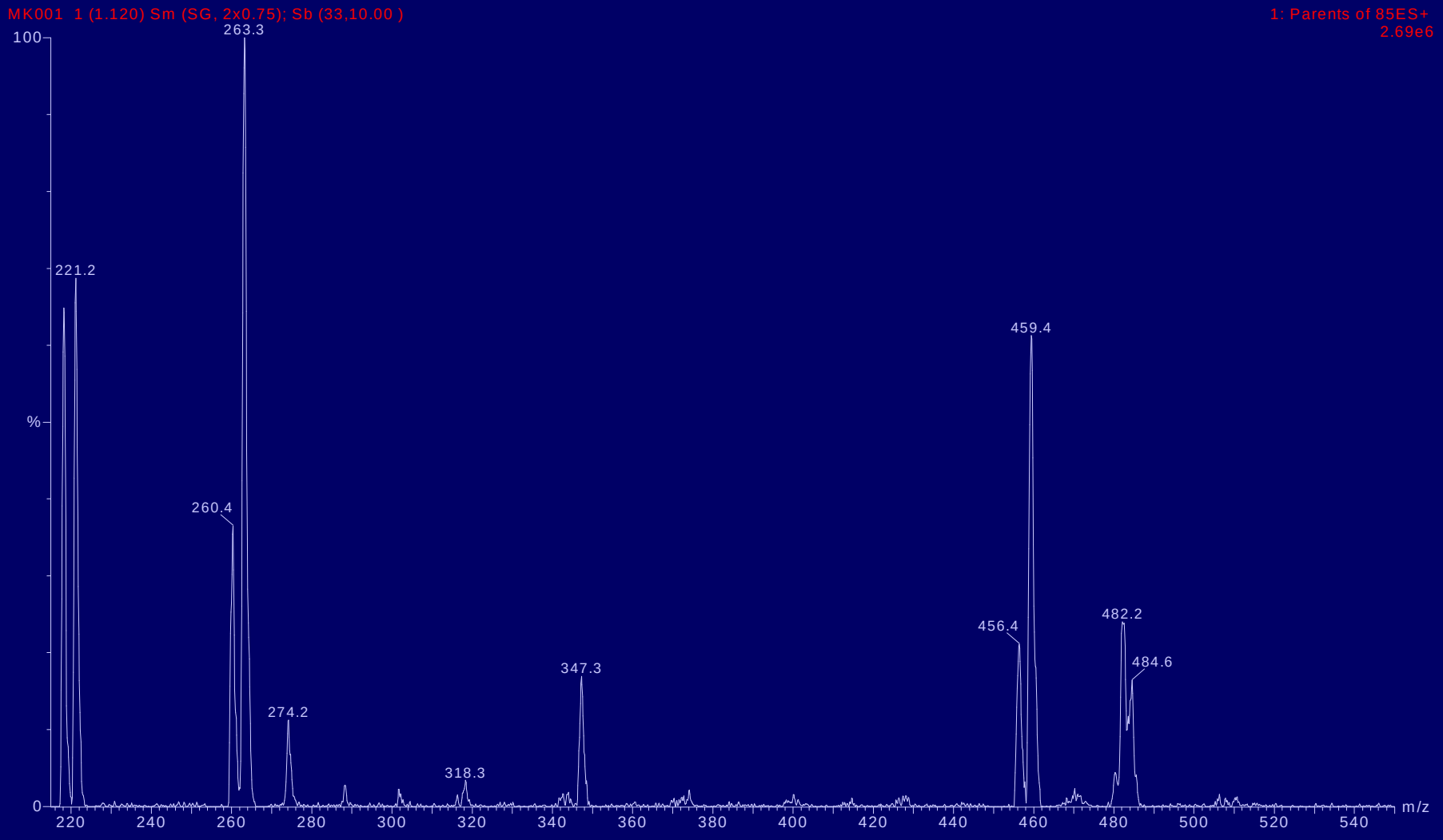
- Pivaloylcarnitine and isovalerylcarnitine
- Valproylcarnitine and octanoylcarnitine

Blood Spots Or Plasma? 1

- Translocase deficiency patient - blood spot

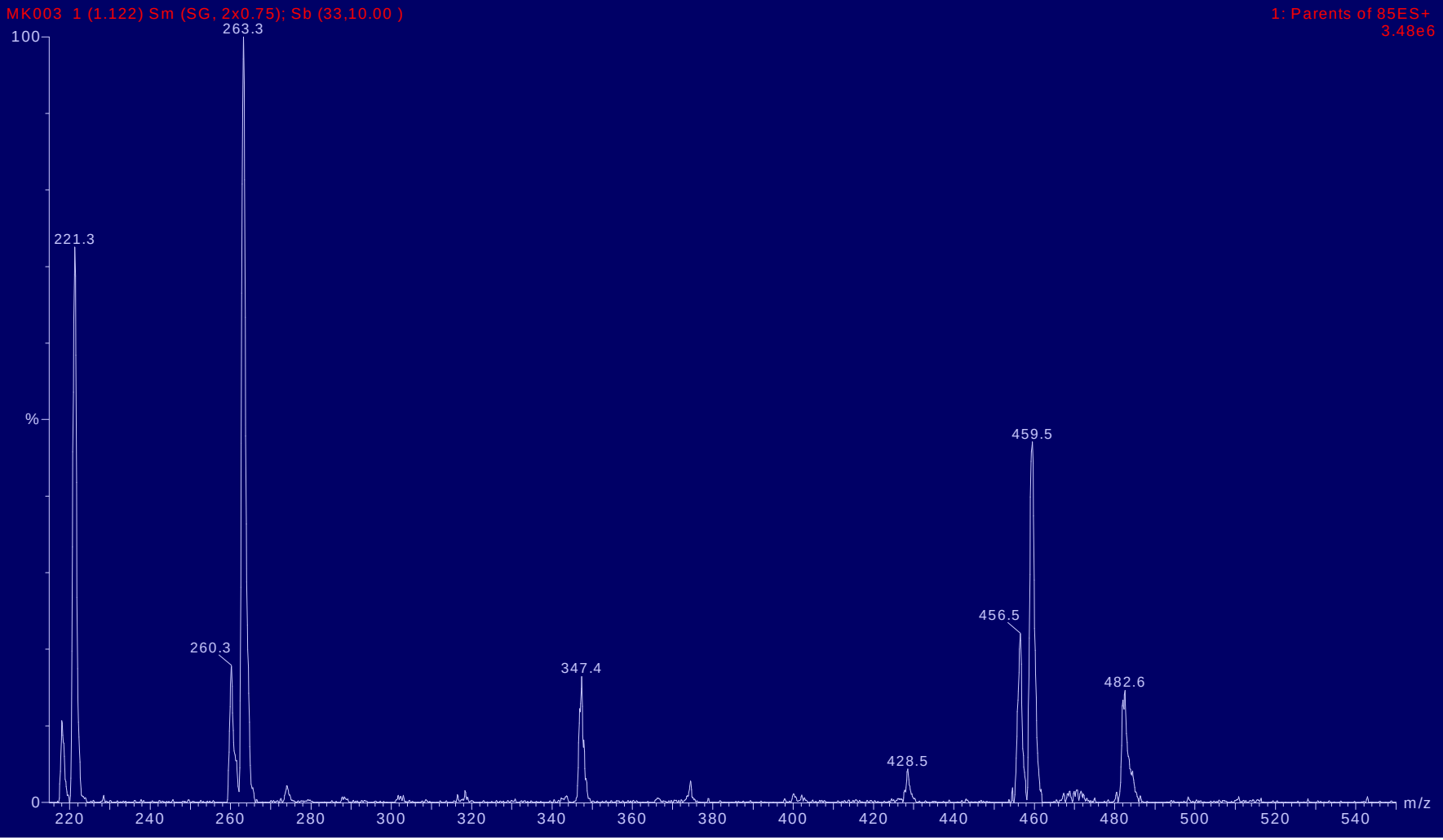


Normal Blood Spot Acyl Carnitine Spectrum



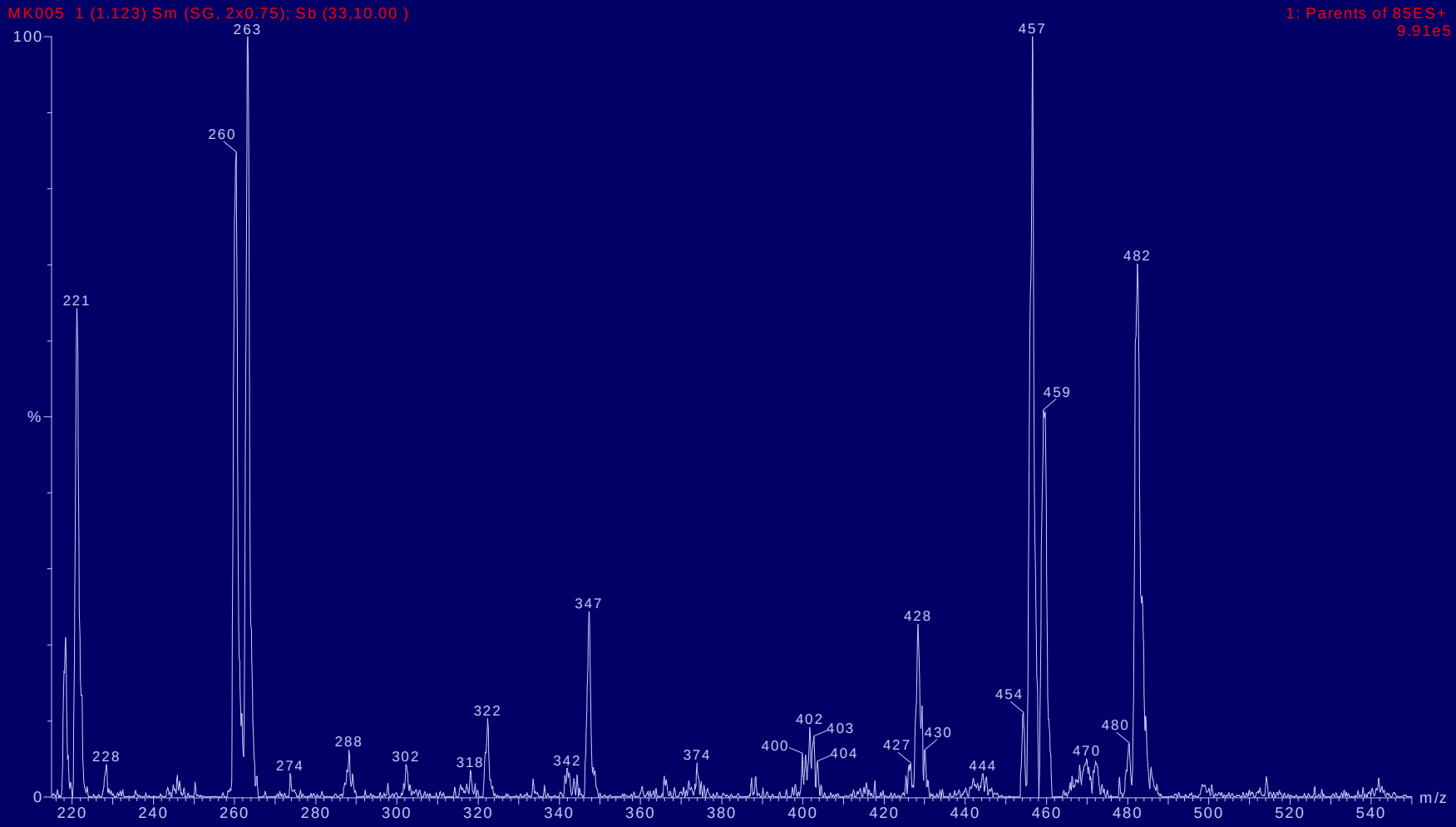
Blood Spots or Plasma? 2

- Normal blood spot?

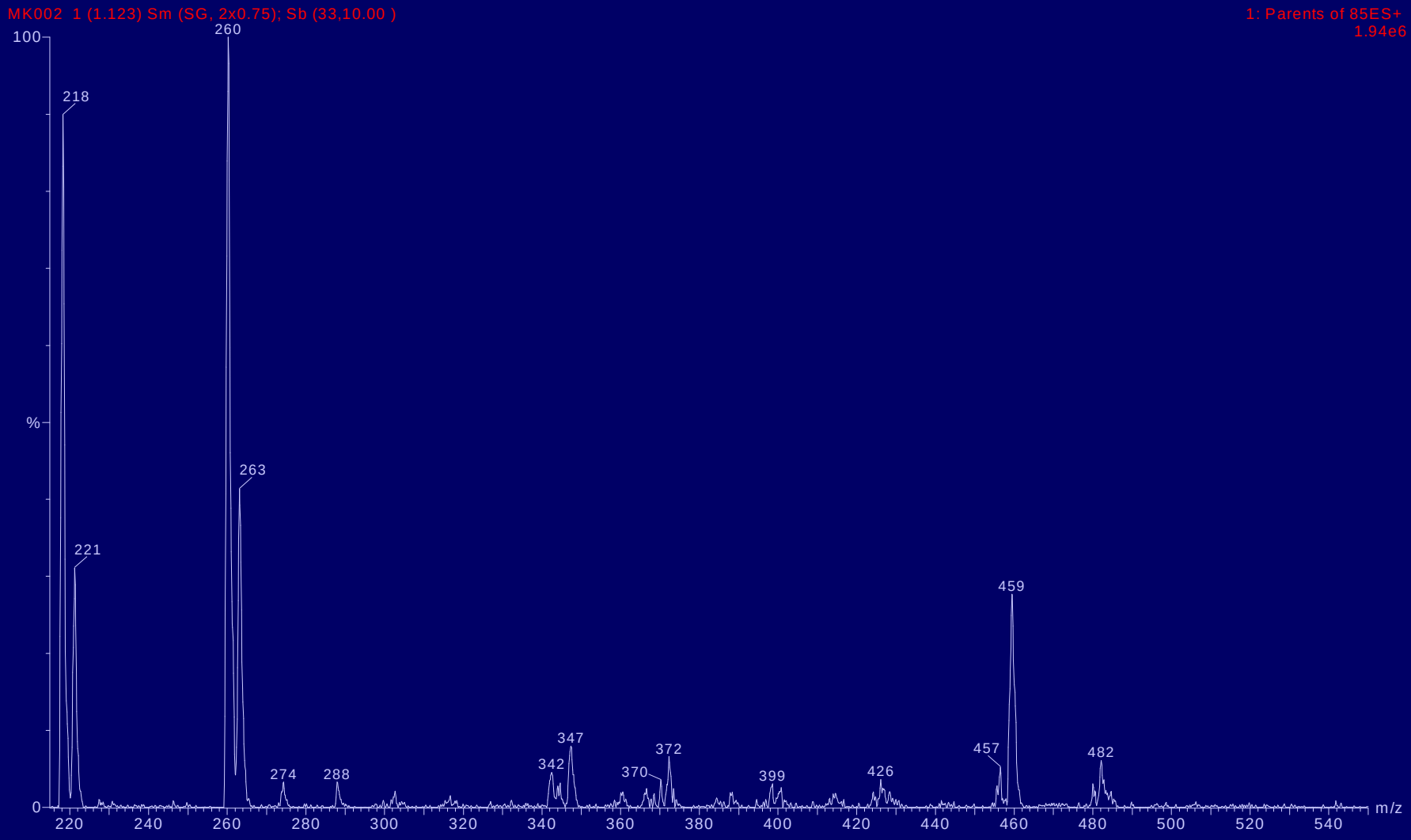


Blood Spots or Plasma? 3

- But abnormal plasma!



Normal Plasma Acylcarnitine Spectra



Blood Spots or Plasma? - 4

- Plasma appears to be a more sensitive medium for detecting acylcarnitine abnormalities in most cases
  - an exception is HMG CoA lyase deficiency where the reverse is true
- Send blood spots and plasma!!!

Amino Acids & Acyl Carnitines - Comment

- MS/MS for acyl carnitines and amino acids is diagnostically powerful
- Results can be obtained rapidly on small samples
- But problems:
  - isomers not differentiated
    - leucine/isoleucine not separately measurable this way
    - interference from drugs etc
  - not specific for MMA/PA or CPT-II/translocase
  - blood spots or plasma can be used but both ideally

MRM For FK506 1

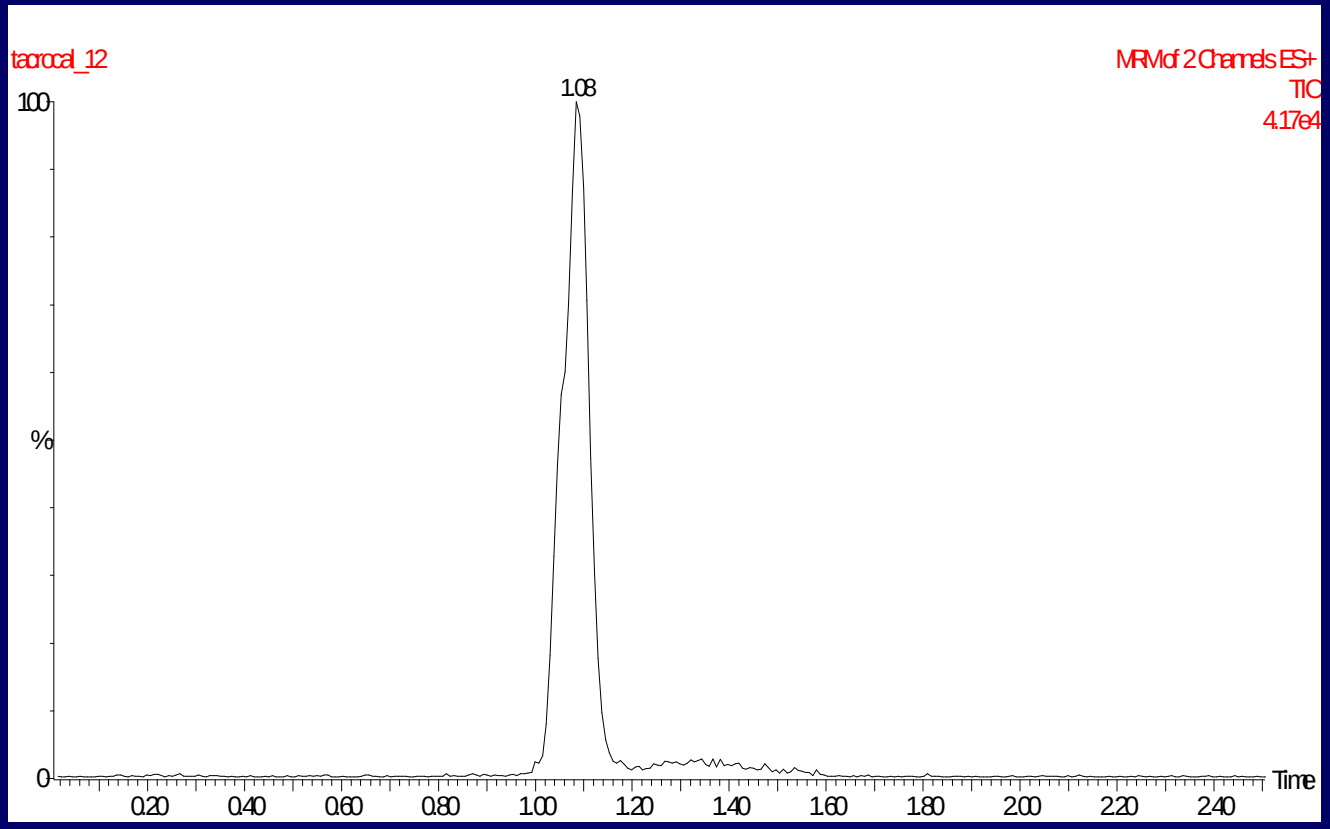
- Amino acid and acyl carnitine determination look for classes of compounds using scans
- For specific compounds it is better to use MRM acquisitions
  - MRM allows specification of precursor and product ion pairs
  - Q1 stays set to transmit precursor ion
  - Q3 stays set to transmit product ion
    - Quadrupoles dwell on set mass for longer ∴ better s/n ratio and sensitivity compared to scan
  - Can do this for one or more compounds
    - If more than one Q1 and Q3 keep switching between settings to transmit the different ion pairs

MRM For FK506 2

- FK506 assayed against ascomycin as internal standard in presence of NH_4^+ ions
  - FK506 and ascomycin form ammoniated ions ($[M+NH_4]^+$)
    - $[FK506+NH_4]^+$ m/z 809.4
    - $[Asco+NH_4]^+$ m/z 821.4
  - Collision induced dissociation causes both to fragment giving product ions of m/z 756.5 and 768.4 respectively
    - MRM monitoring of following transitions gives allows sensitive detection of FK506 and ascomycin
      - FK506 809.4 > 756.5
      - Asco 821.4 > 768.4

MRM For FK506 3

- By looking for specific ion pairs during elution a total ion chromatogram can be formed



MRM For FK506 4

- Because only specific ions are monitored no mass spectrum is formed; only specific precursor ions are recorded