# GCMS in the Metabolic Laboratory

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**MetBioNet BMS Training Group Workshop :**
**Tackling all techniques** **April 2015**

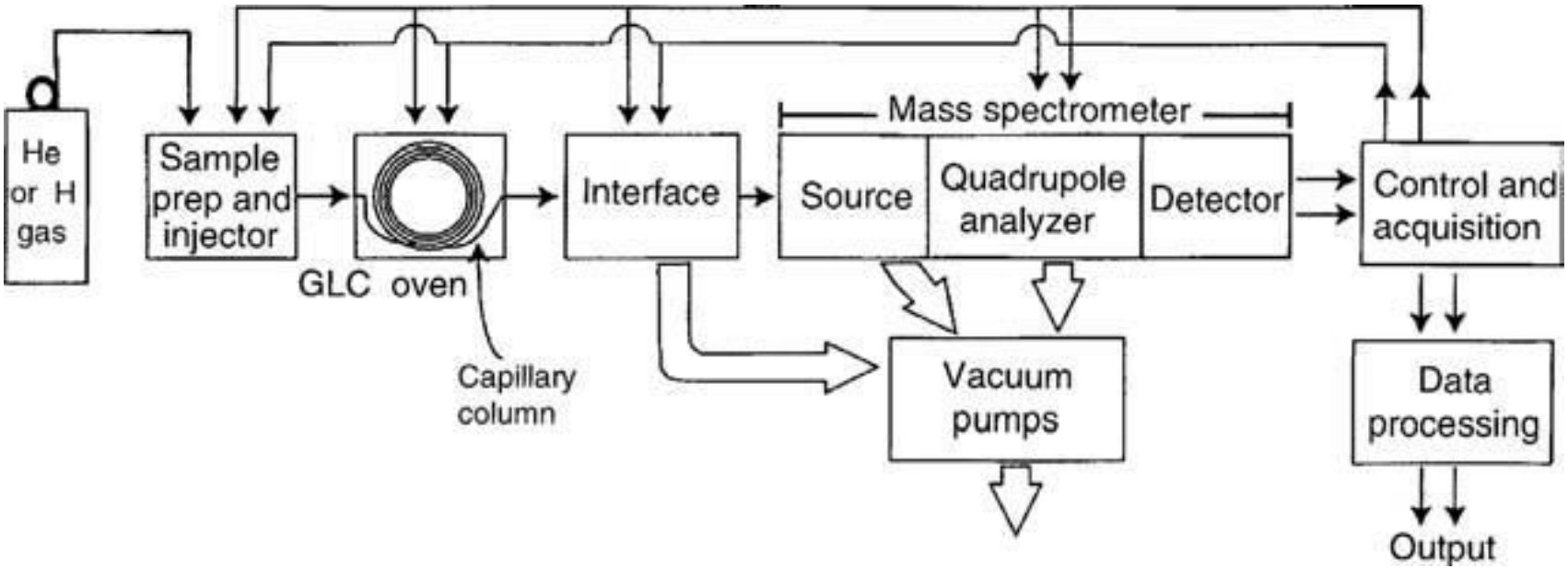
# Fundamentals of chromatography

- Fundamentals: lab technique for the separation of a mixture of compounds
- Mixture of analytes dissolved in a fluid called “the  mobile phase” travel through a structure holding a material called “the stationary phase”
- Separation is based on differential partitioning between the mobile and stationary phases – various constituents travelling at different speeds- varying “retention times”

# Topics for discussion

- Principles of GC-MS ( EI )
- Maintenance/Troubleshooting
- Applications of GC-MS in Clinical Biochemistry
- Cases

# Schematic of a GCMS



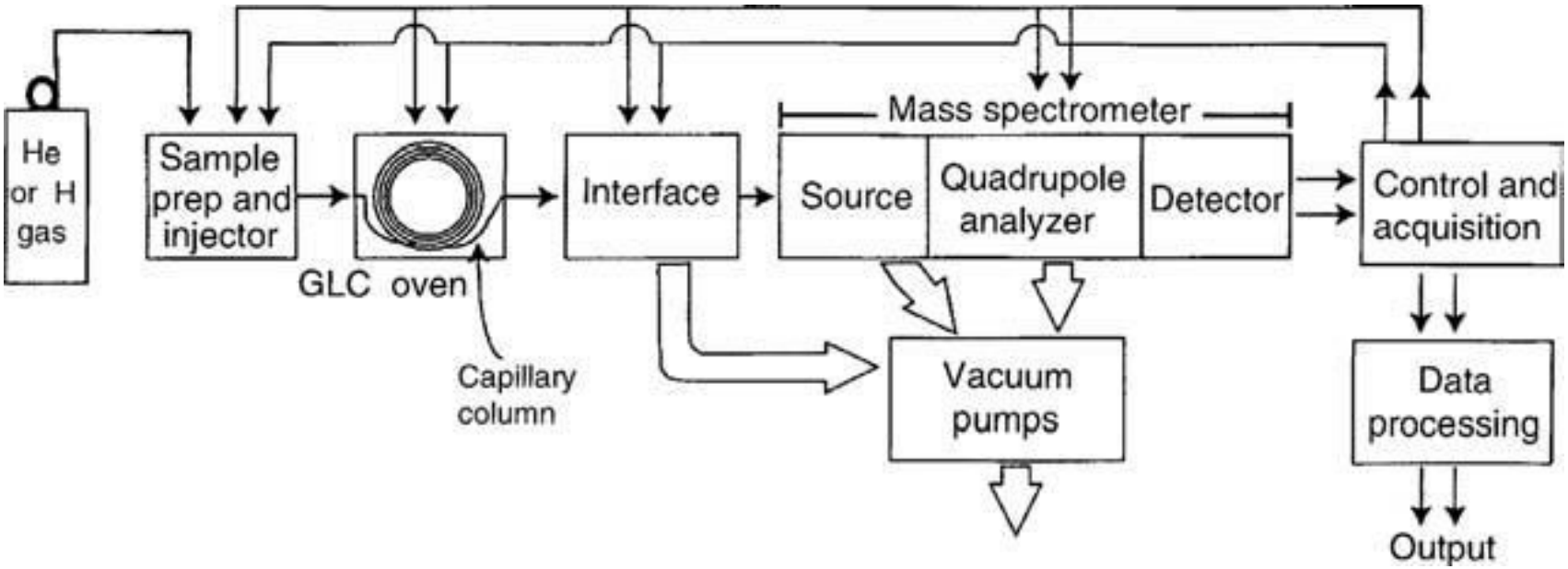
# GC

- Insulated oven with  programmable temperature control
- Autosampler
- Heater inlet for split/ splitless injection
- Column: fused silica capillary with chemically bonded phase eg HP5
- Transfer line to detector: MS

# MS

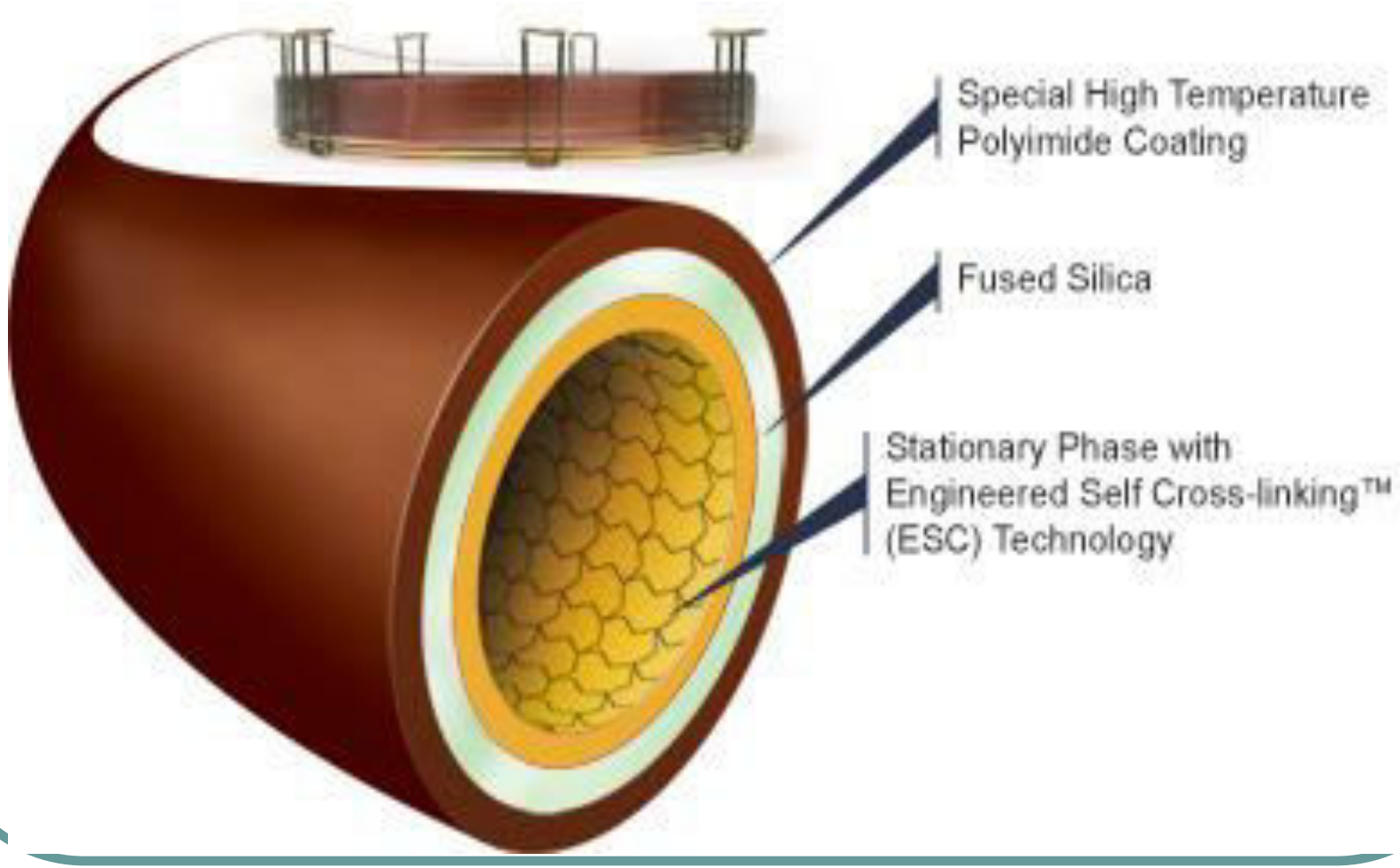
- A MS consists of: a source, an analyser and a detector
- A mass spectrometer produces charged particles ( ions) ; the MS then uses electric and magnetic fields to measure the mass “weight” of the charged particles
- MS is used in environmental work, analysis of petroleum products, forensic medicine, toxicology and analysis of biological materials and now proteomics.

# Schematic of a GCMS

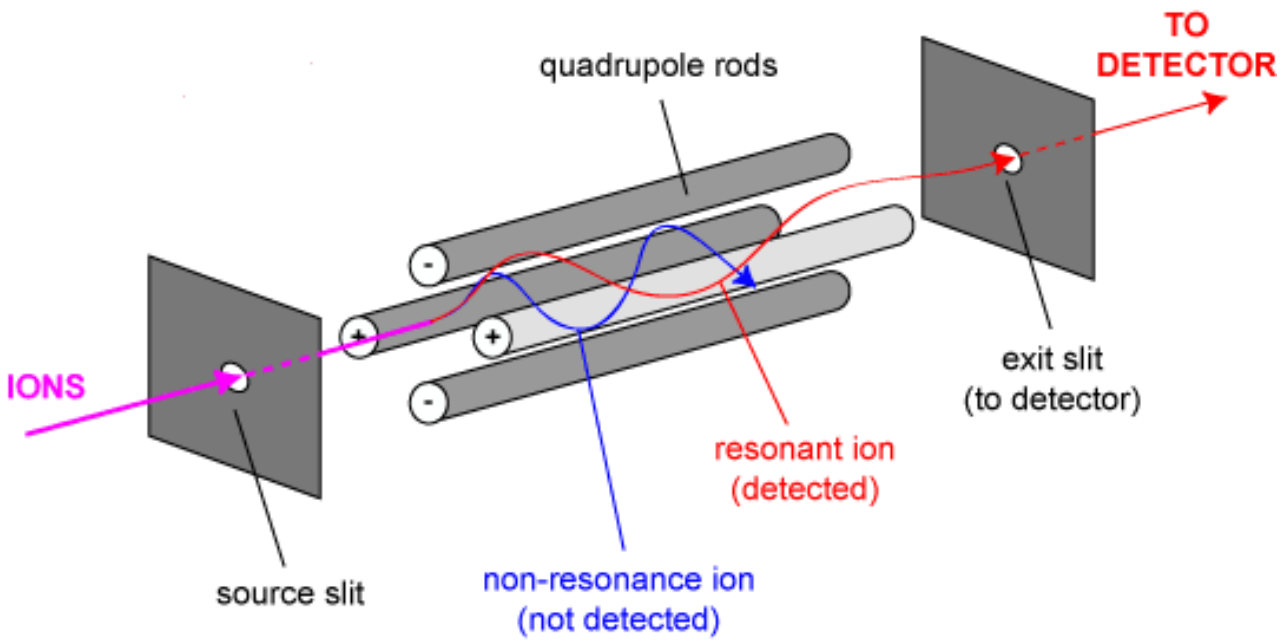


# Capillary columns

# Schematic of a capillary column



# Quadropole


TO
DETECTOR
quadrupole rods
IONS
exit slit
(to detector)
resonant ion
(detected)
source slit
non-resonance ion
(not detected)

# What does the mass tell us?

| | | |
|---|---|---|
| H20 | 2 x H | ( 2 x 1 amu) |
| | 1 x O | (1 x 16 amu) |
| H2O | = | 18 u |

Water vapour is introduced into the ion source of the MS ( under vacuum).

Shoot a beam of electrons through the water vapour, some of the electrons will hit water molecules and knock off an electron. Loss of a negatively charged electron, the water will be left with a net positive charge.

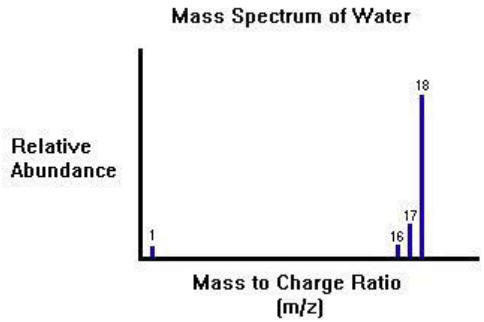
H20 + 1 (fast) electron → [H20}+ + 2electrons

# Mass spectra

For water, the only possible fragments will be [OH]+,

O+

and H+




The mass spectrum will show peaks at the masses of 1,16,17 and 18.

# Modes of operation

- Full scan – considers all peaks in a sample and their individual spectrums
- Selective Ion Monitoring ( SIM) - only monitors selected ions associated with a specific substance

# Software

- Software that can control temperature gradient programmed methods with “ retention time locking”, collect full scan large data files, store them, allow retrospective analysis
- Acquisition of MS data, processing of MS data.
- Full scan: across mass range 50-500. Scan speeds increasing over time Fragmentation pattern ; structure and “fingerprint”
- SIM Selective ion monitoring – screening out background concentrating on a particular ion for quantification and a second conffirmatory ion
- Computer programs, such as those that search libraries of mass spectra for the best match can be used to interpret a mass spectrum “ Library Search” eg NIST, AMDIS etc
- Automatic labelling of peaks: deconvolution software. Shimadzu, Agilent ( Masshunter)

# Modes of operation

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# Analysis of Physiological fluids

- Liquid/liquid extraction ( extraction proportional to creatinine)
- Addition of internal standards
- SPE
- Preparation of volatile derivatives for GC
- Metabolic profiling

# Preparation of derivatives

- High polarity, low volatility, and thermal instability of most organic compounds makes it essential to convert them into stable volatile derivatives
- Nature of the derivative selected obviously affects the mol wt and mass spectra
- TMS (trimethylsilyl derivatives) - commercial preparations widely available eg BSTFA with 1% TMCS and with some pyridine
- Methyl esters ( esterification of fatty acids)

# TMS derivatives

- TMS derivatives widely used in organic acid analysis
- Easy to prepare: low polarity/high volatility
- High reactivity to water: dry extracts required for preparation and stable storage
- In TMS spectra, the base peak is almost invariably m/z 73, in compounds with two or more TMS groups the ion m/z 147
- Molecular ions invariably small, with an ion at M-15 ( loss of CH3). Indicator of molecular weight.

# Maintenance

- A preventative maintenance contract covering the GC, MS & vacuum system should be in place in order to ensure optimal operation of the instrument.
- Most day-to-day maintenance is directed towards the injector & injection port. Need to ensure inertness & an air-tight seal.
- Maintenance schedule depends on workload & sample quality.
  - Septum replacement after approximately 150 injections.
  - Replace liner – usually silanized to remove active sites in the glass.
  - Clean the autosampler syringe with (DCM/acetone/methanol) regularly & change the on-board wash solvents regularly
- Observe the column manufacturers specifications – column life will be shortened & performance compromised if the oven temperature is too high.

# Maintenance Logbook

- Record of maintenance

Eg inlet: septum , liner , gold seal replacement

- Instrument tuning : see tune reports
- Replacing columns
- Cleaning the ion source, replacing filaments

Operators/ assay sequence lists/

# Gastight system

- Do not over-tighten fittings.  Only ½ turn past finger tight is required, otherwise the ferrule will fail & leak.
- Routine MS maintenance:
    - Perform a leak check before each run. Look for the water (18 amu), nitrogen (28 amu), and oxygen (32 amu) peaks. If present, there probably is a leak around the column-to-source seal.
    - Tune MS before each use.
- Maintain a supply of spare parts and keep a dedicated tool box to hand.
- Be proactive with instrument maintenance – develop & follow a schedule.
- Record all maintenance, scheduled & unscheduled in a log book.

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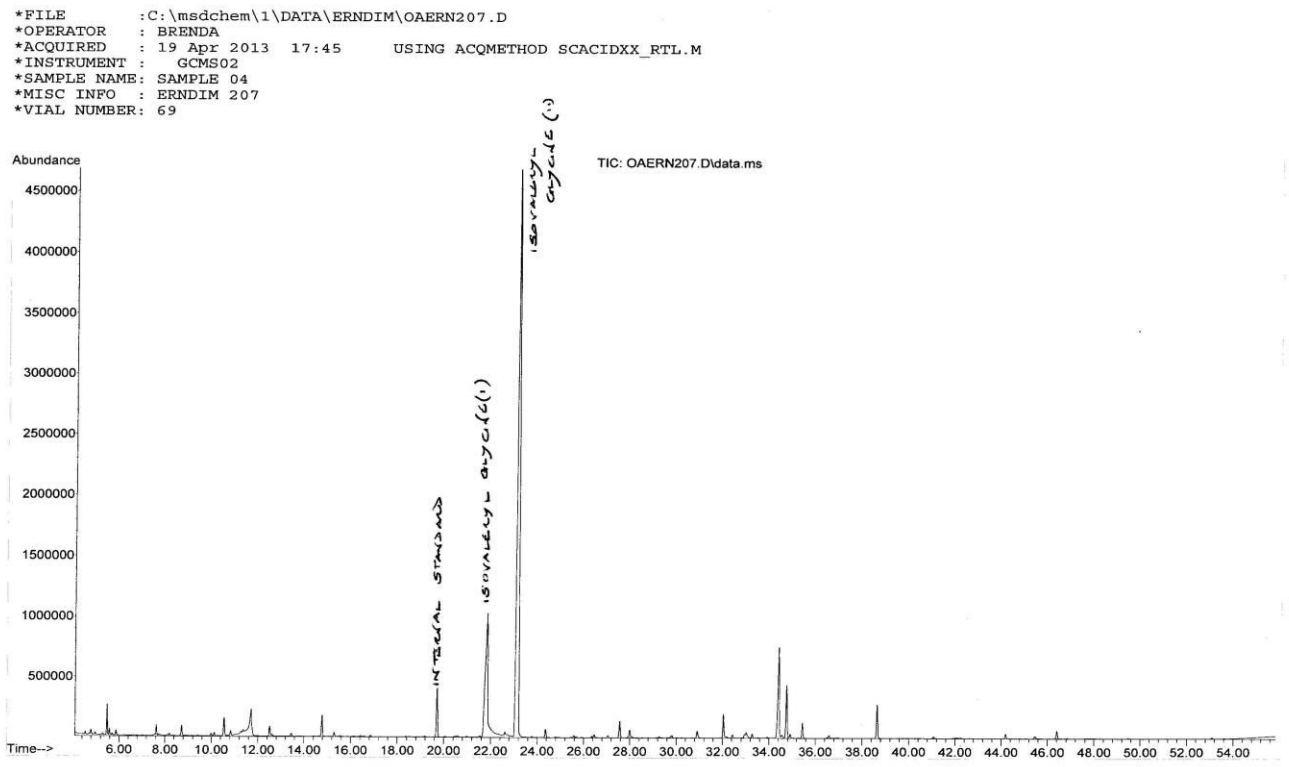
# Applications of GCMS

- Urine organic acids
- Plasma/Urine MMA quantitation
- Very long chain fatty acids (peroxisomal disorders)
- Steroids and their metabolites

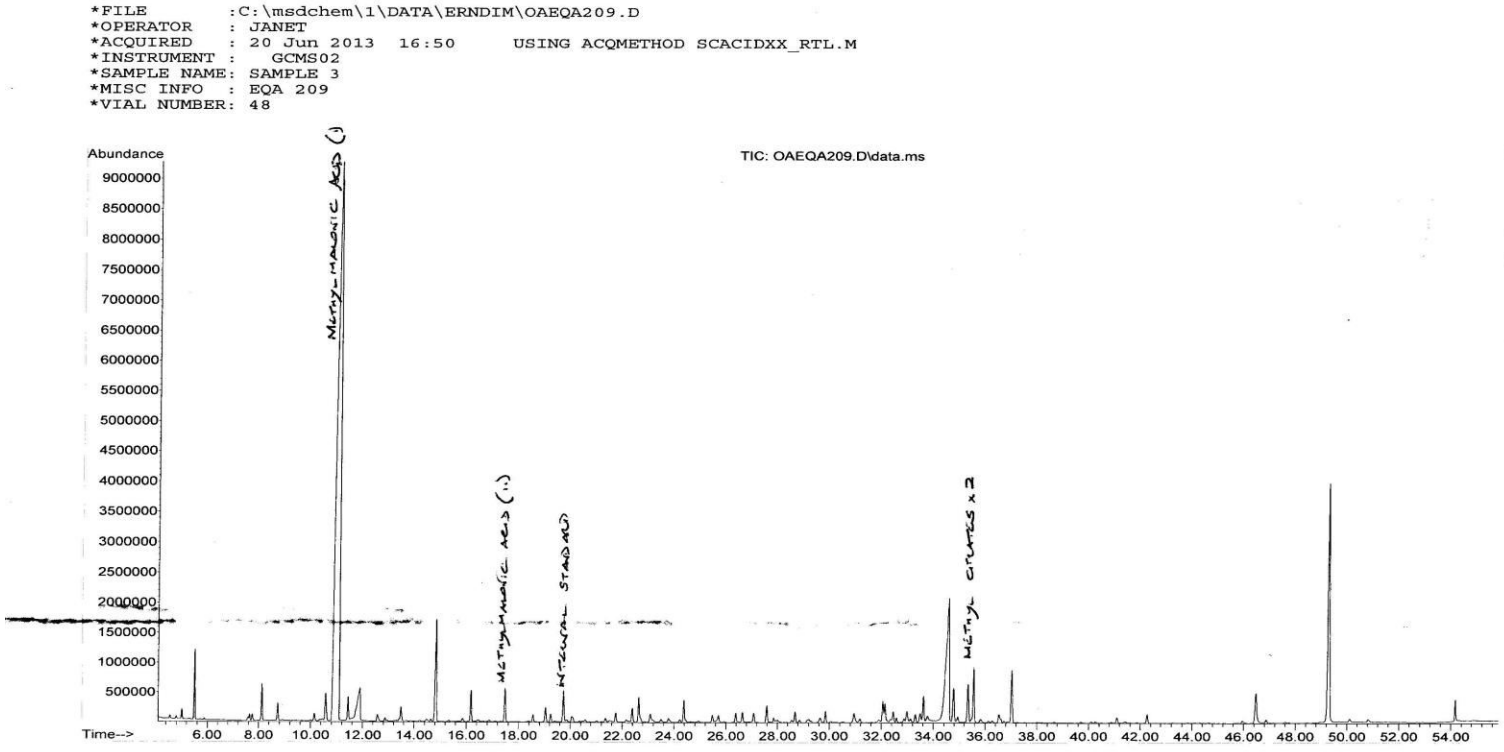
# Cases: organic acid disorders

- Methylmalonic aciduria
- Isovaleric aciduria
- MCAD
- Beta ketothiolase deficiency
- MSUD

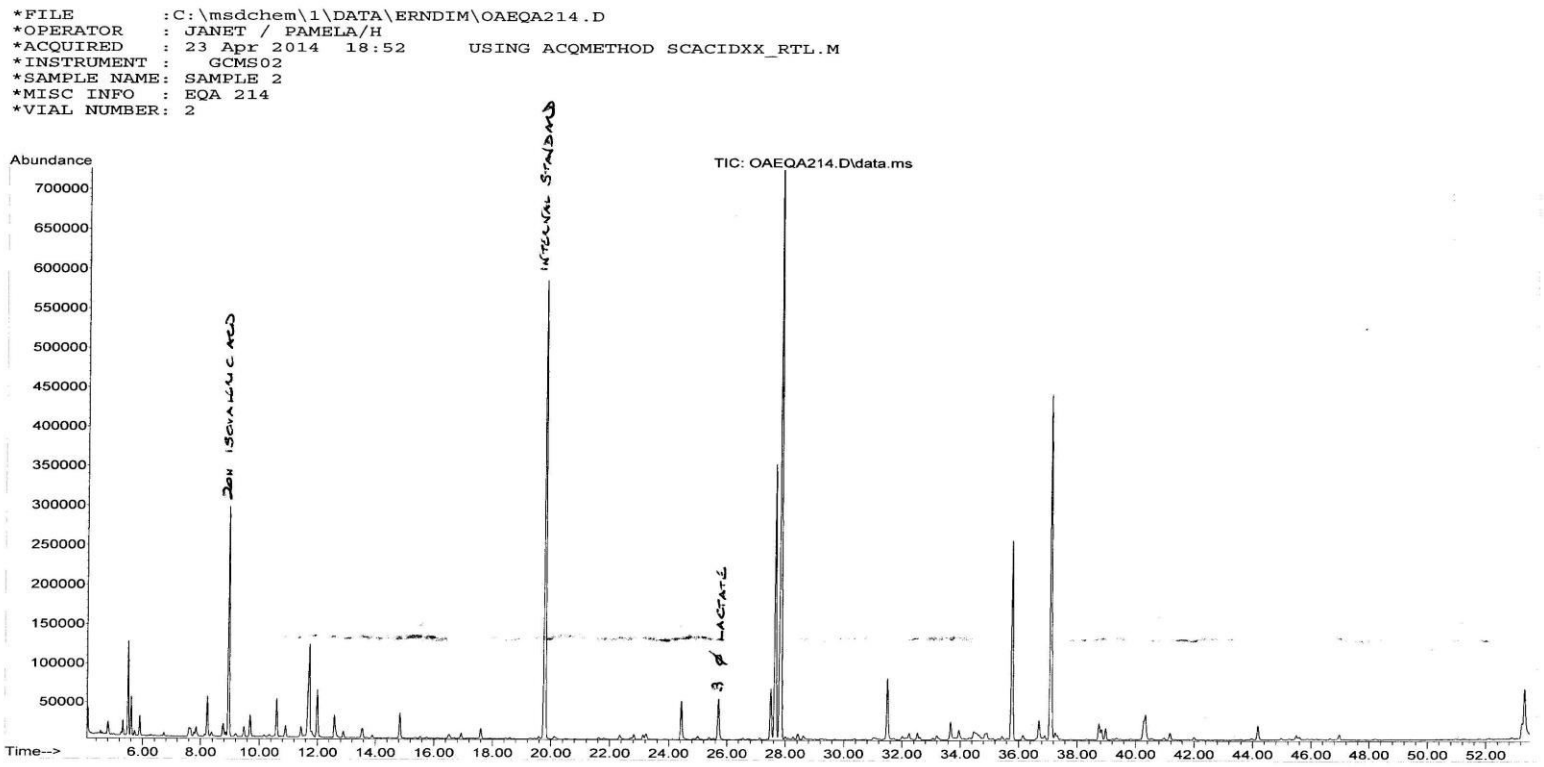
# Isovaleric aciduria



# Methylmalonic acidura

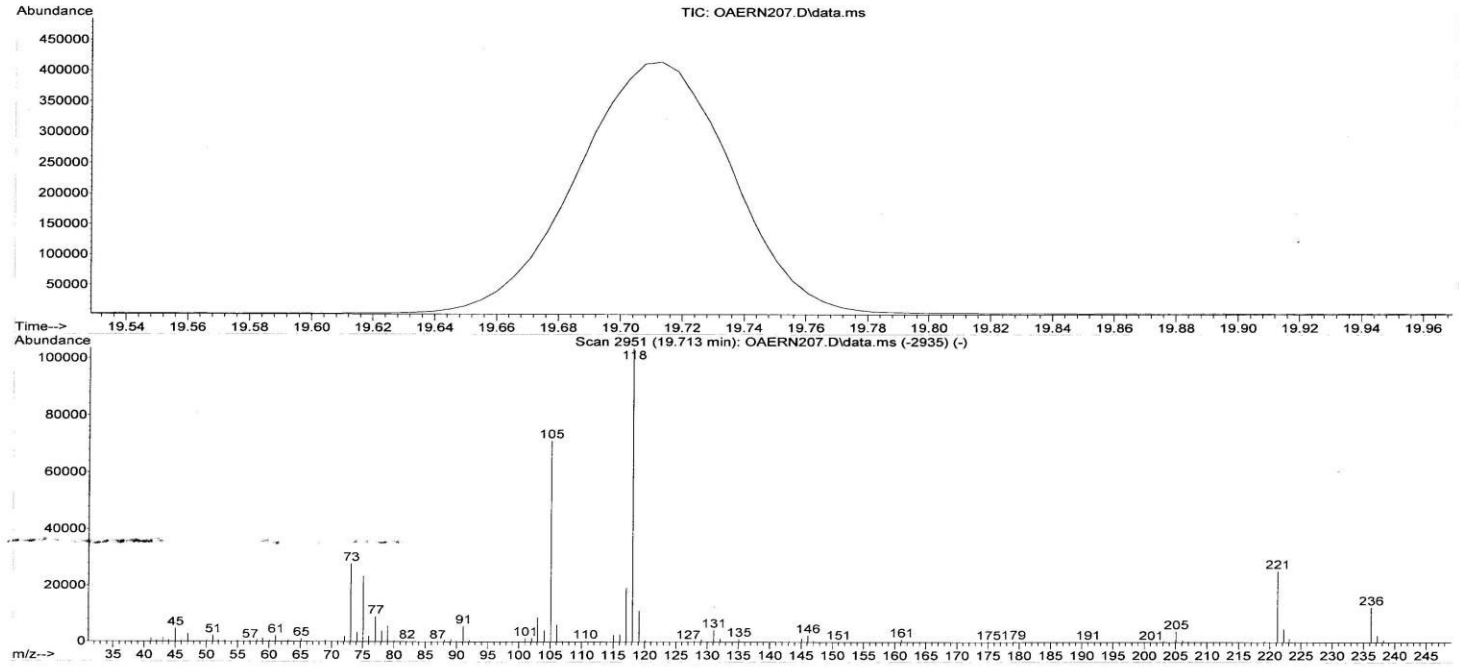


# MSUD

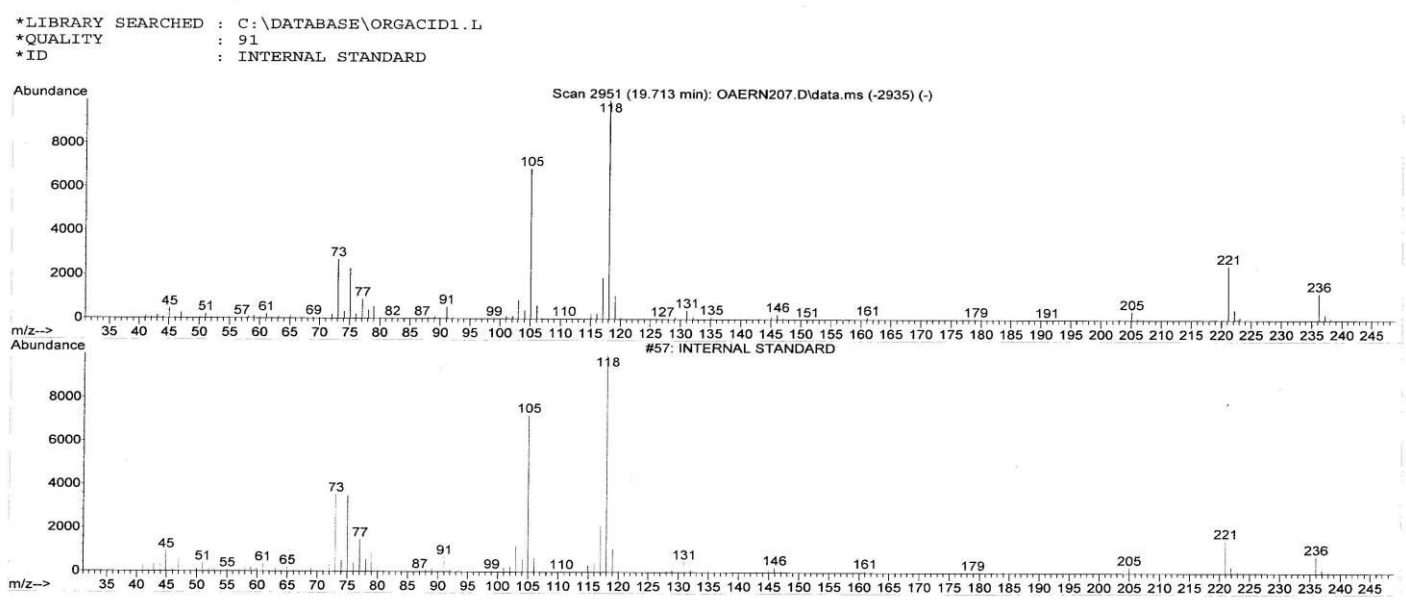

*FILE :C:\msdchem\1\DATA\ERNDIM\OAEQA214.D
*OPERATOR : JANET / PAMELA/H
*ACQUIRED : 23 Apr 2014 18:52 USING ACQMETHOD SCACIDXX_RTL.M
*INSTRUMENT : GCMS02
*SAMPLE NAME: SAMPLE 2
*MISC INFO : EQA 214
*VIAL NUMBER: 2
Abundance
TIC: OAEQA214.D\data.ms
2ow ISOVALERIC ACID
INTERNAL STANDARD
3 β LACTATE
Time-->

# Mass spectrum

```
*FILE       :C:\msdchem\1\DATA\ERNDIM\OAERN207.D
*OPERATOR   : BRENDA
*ACQUIRED   : 19 Apr 2013  17:45     USING ACQMETHOD SCACIDXX_RTL.M
*INSTRUMENT :     GCMS02
*SAMPLE NAME: SAMPLE 04
*MISC INFO  : ERNDIM 207
*VIAL NUMBER: 69
```



# Library matching



# Cases: Very Long Chain Fatty Acids

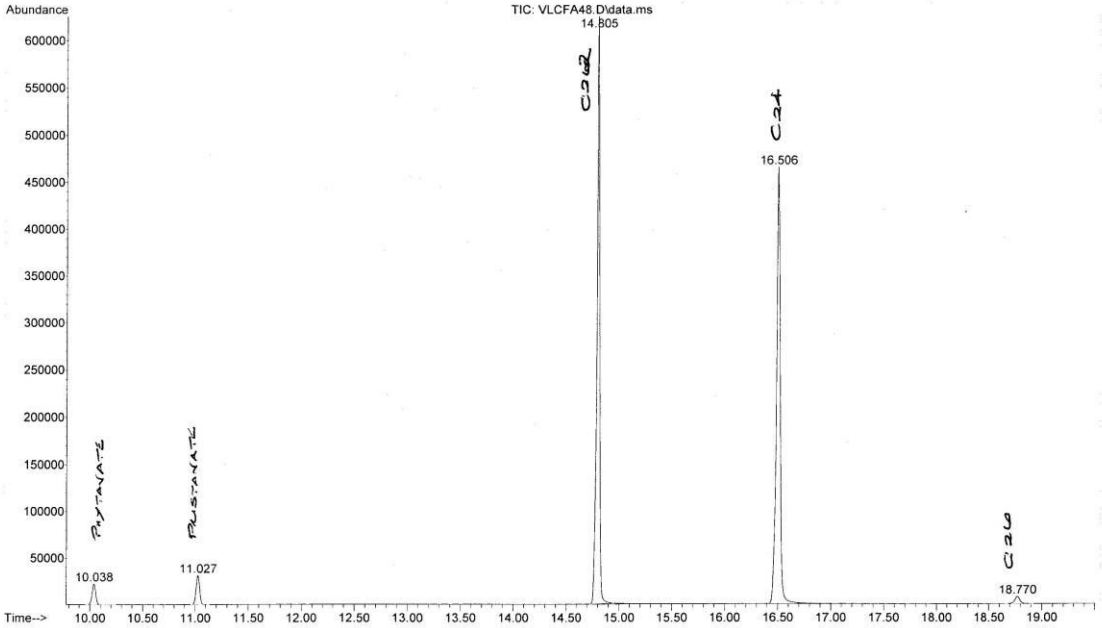
- Peroxisomal disorders:eg Zellwegers

ALD adrenaleukodystrophy

- C26
- C22/24
- C24/C26
- Phytanate ( Refsums disease)
- Pristanic acid

# VLCFA using SIM

```
File       :D:\MSDCHEM\1\DATA\VLCFA48.D
Operator   : JANET 02/04/15
Acquired   :  2 Apr 2015  19:29      using AcqMethod MARK2.M
Instrument :   GCMS03
Sample Name: SAMPLE 06
Misc Info  : S5
Vial Number: 6
```



# Quantitation of MMA- stable isotope dilution

- B12 deficient patients
- Cobalamin disorders
- B12 responsive Methylmalonicacidaemia

# Urine steroid profiles

- Viapath @ Kings (Dr. Norman Taylor)

  Cortisol and cortisone metabolites

  Androgens and androgen metabolites (age related reference ranges).

# Tackling techniques

Thank You.

Questions?

# Logical troubleshooting:

- Gain information from the GCMS system

i. define the problem

ii. check the front panels

iii. refer to the log book, recent maintenance etc

Isolate the problem…….

# Tuning and troubleshooting:

- Standard spectra autotune – be aware of common contaminant ions
- Status checks
- Vacuum status
- Diagnostics
- Customer care lines: Agilent Remote adviser: technical help over telephone.
- Engineer assistance

# Troubleshooting : tips

- System considerations:

Gases: regulators, pipeline maintenance

Traps

Column :score cleanly , break- inspect for jagged edges or burrs! Use the correct ferrules for the job! Do NOT overtighten!

Injection port: septum,liner/O-ring/gold seal/washer

Sample – ? contamination. Screen your sample first.Sample clean up. Inject the smallest volume possible

# Troubleshooting : tips continued

- Vacuum
- pump oil, pump fan
- MSD – ion source : filaments
- Tool box, carry spares: liners, septa, seals, columns, ferrules etc
- Discuss with knowledgable colleagues….. learning curve