Central Manchester University Hospitals NHS
NHS Foundation Trust

Gas Chromatography Mass Spectrometry (GC-MS) an overview

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GCMS

- Basic Principle:
- Sample Prep
- Inlet
- Separation
- Detection

GCMS

GCMS

Sample Prep

- Liquid/Liquid
- Solid Phase
- Derivatisation:
  - » Volatile
  - » Thermally Stable

Gas Chromatography

- Inlet: Vapourise (Split and Splitless Injections)
- Separation

Inert stationary phase or GC Column

(Variety of properties, depending on the application)

Inert Carrier Gas, eg. He

Separates Primarily according to BP

Mass Spectrometry

Detector

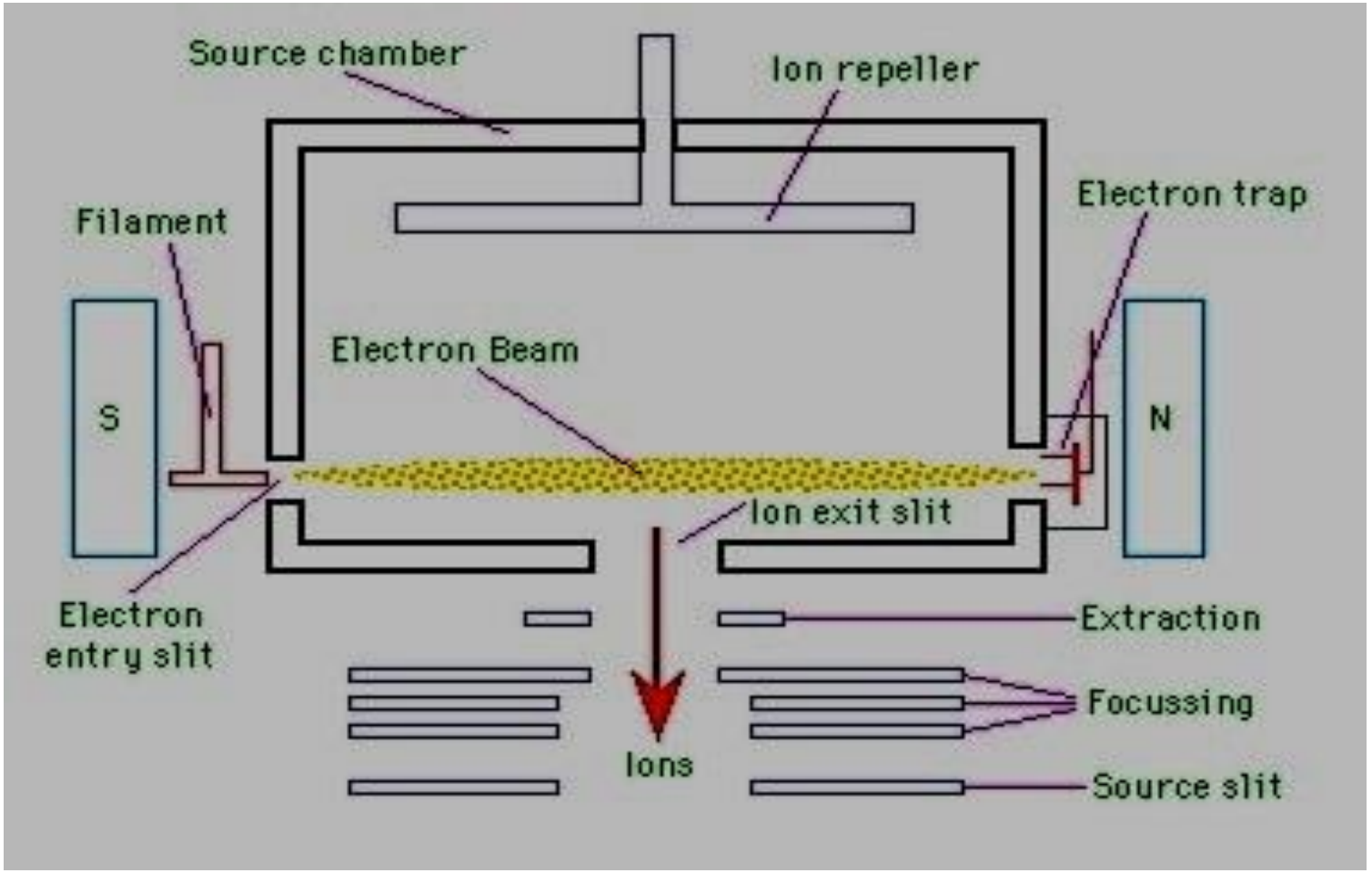
Ion Source:

Most Applications use Electronic Ionisation (EI)

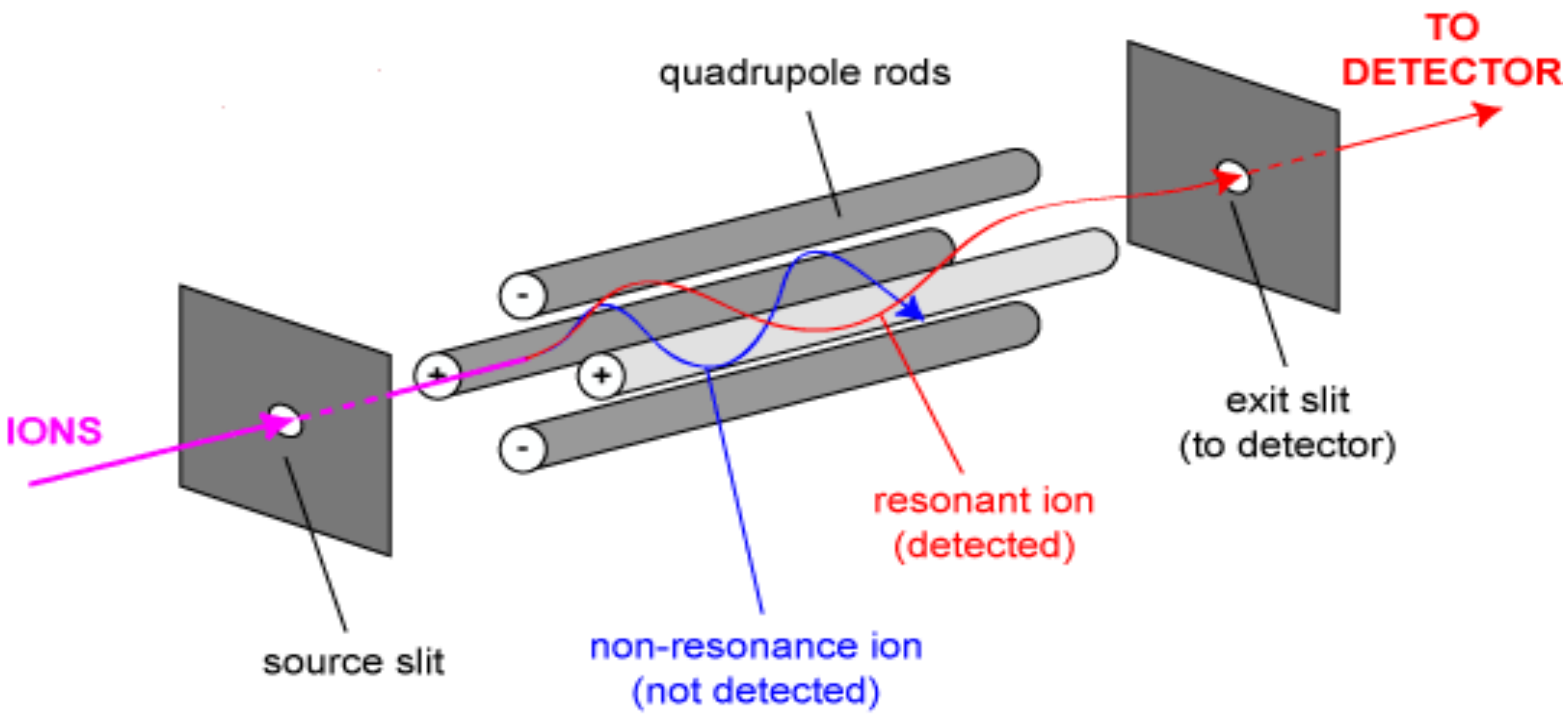
$$e^- \longrightarrow M \longrightarrow M^+ 2e^-$$

Collision energy causes fragmentation

Ion Source


Source chamber
Ion repeller
Filament
Electron trap
S
Electron Beam
N
Ion exit slit
Electron
entry slit
Extraction
Focussing
Ions
Source slit

Quadrupole


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

Analysis Type

Scan

Scans all ions and masses

In order to avoid overloading the detector, this requires a split flow upon injection.

Analysis Type (Cont.)

Selected Ion Monitoring (SIM)

Only allows Ions of a specific mass to pass through the quadrupole

Typically runs with a splitless injection

Can run multiple time windows and masses in the same run.

Quantitation

- Calibrant of known concentration e.g. 1mM
- Add Internal Standard.
- This generates a response, which in turn generates a specific concentration i.e. 1mM
- The Internal Standard is then added to each sample

Quantitation (Cont.)

- Concentration of the species in patient sample generates a response when measured with the internal standard.

Quantiation (Cont)

- Calibration

Single level or Multipoint

- Internal Standard

Stable Isotope Dilution or Non natural occurring species.

Applications

- Scan

Organic Acids

- SIM

VLCFA

Phytanic Acid

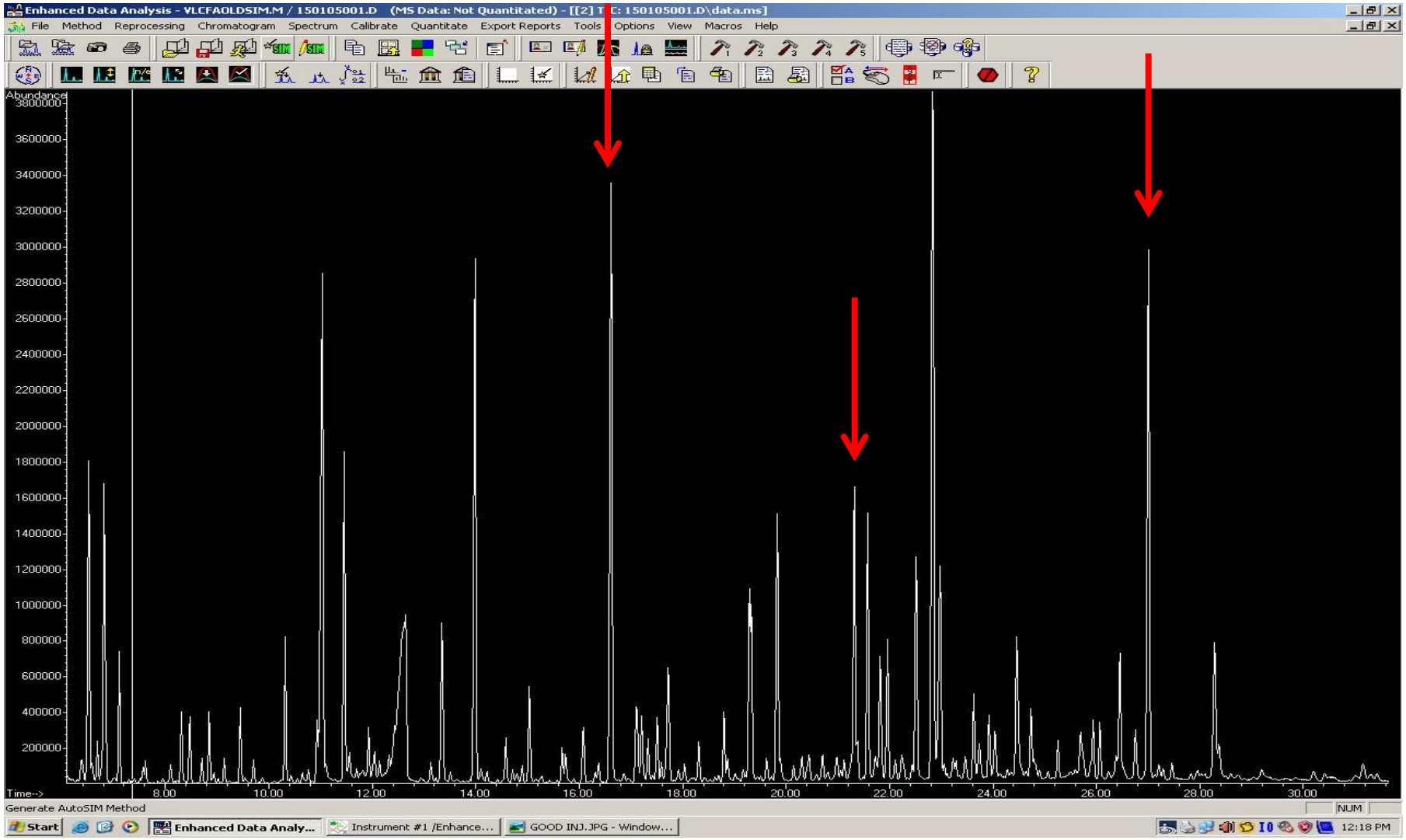
7DHC

MMA

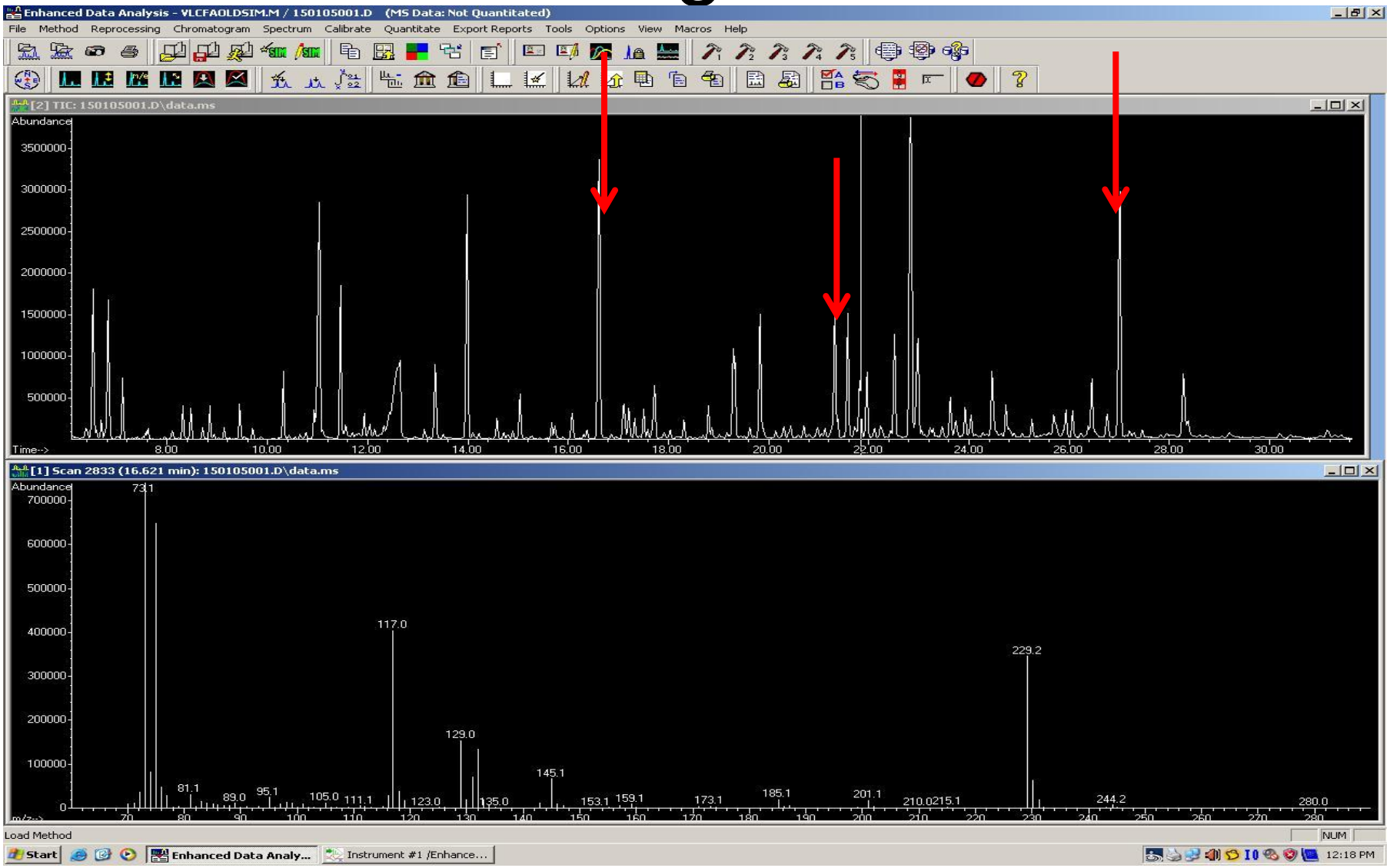
Orotic Acid

Organic Acid Examples

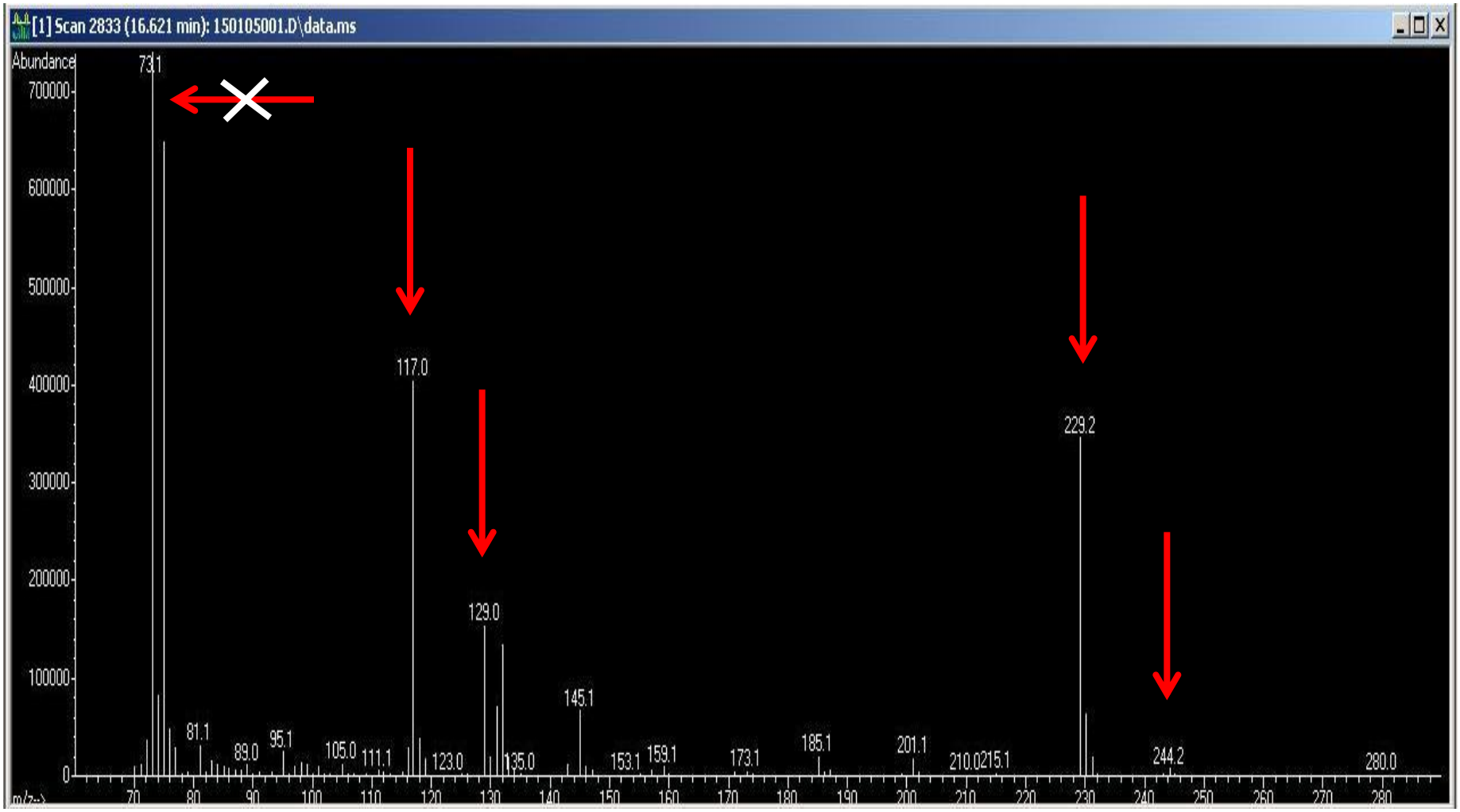
Good Injection Profile



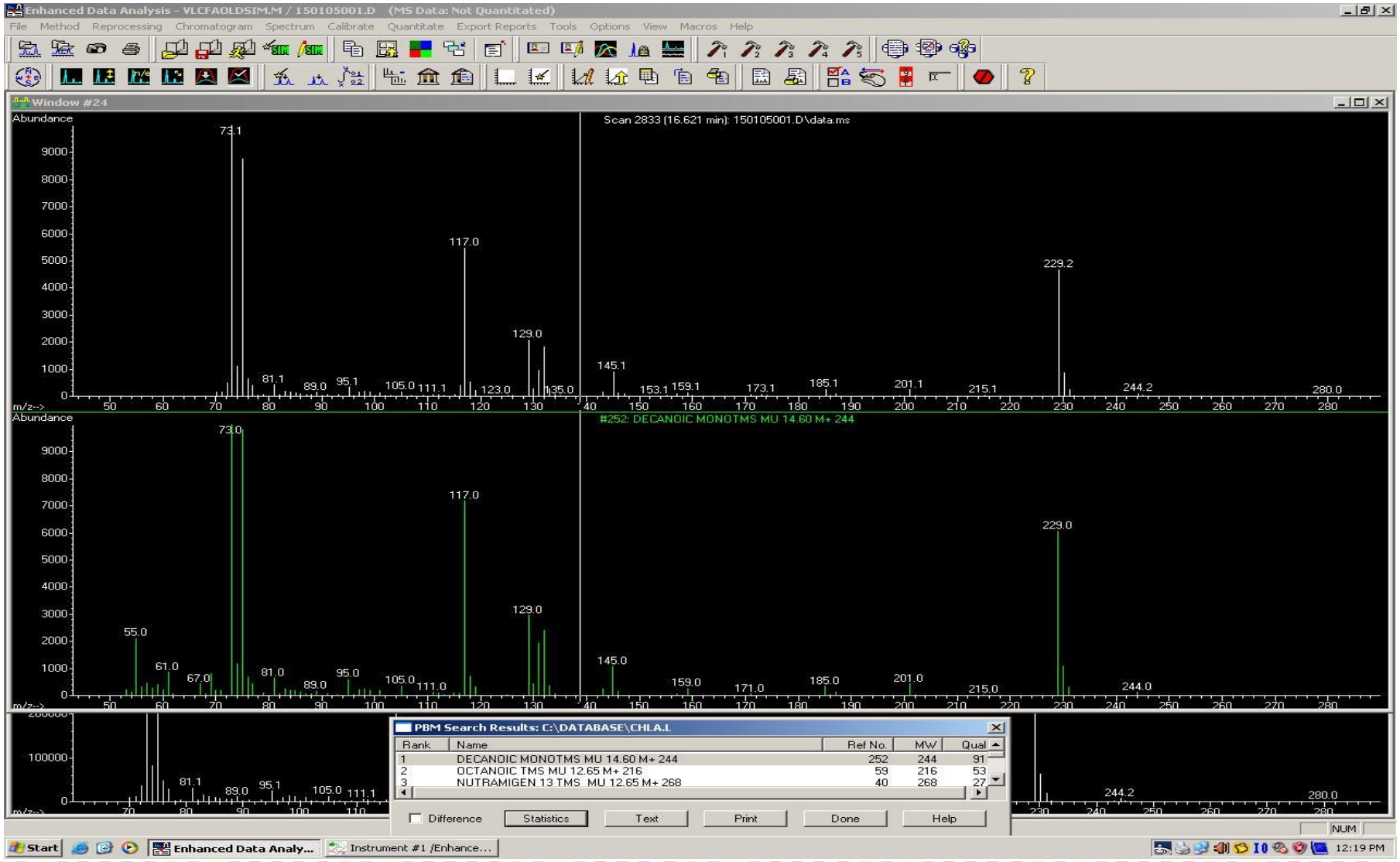
Chromatogram + MS



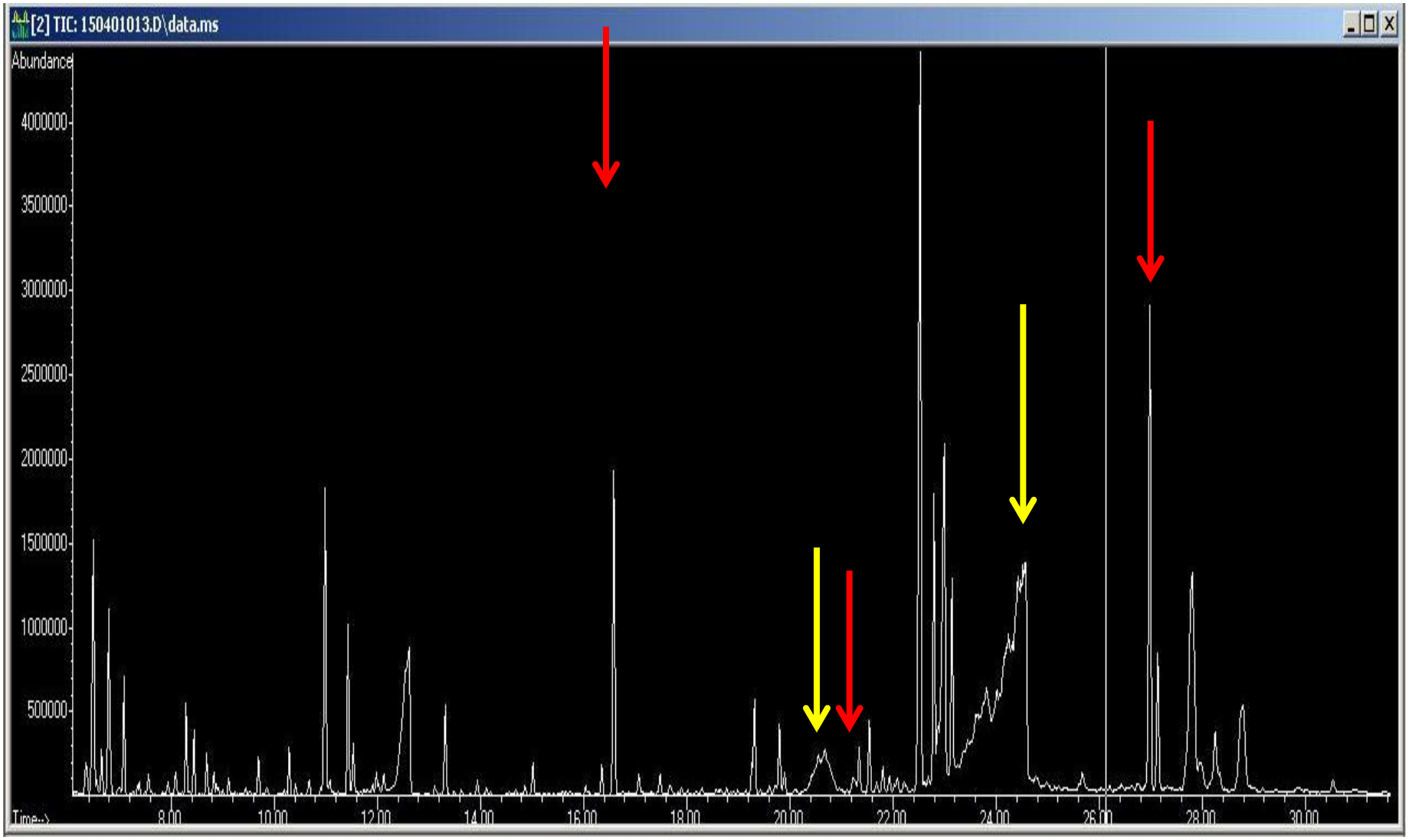
Mass Spectrum



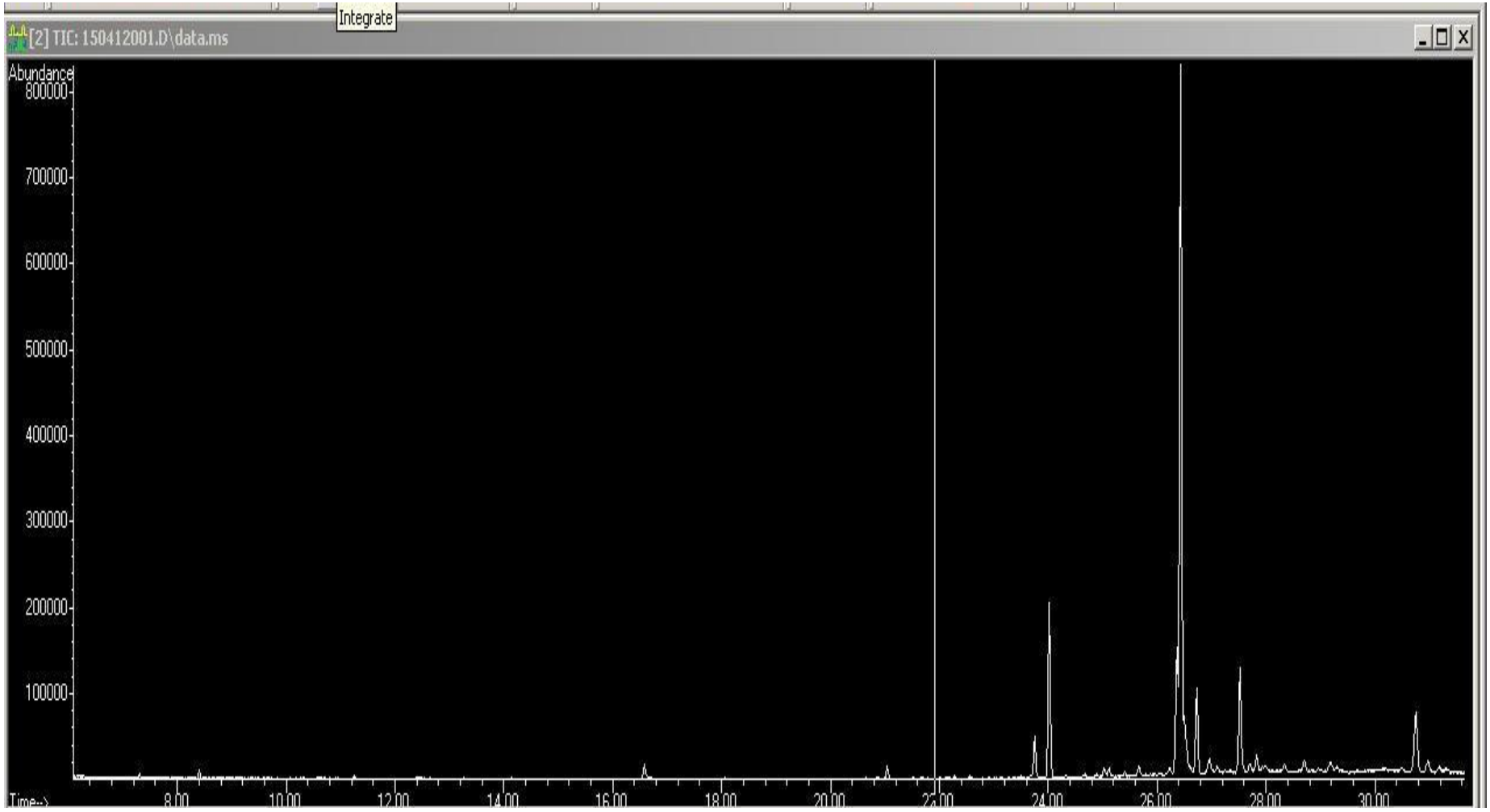
Library Search



Bad Injection Profile



Really Bad Injection



Troubleshooting

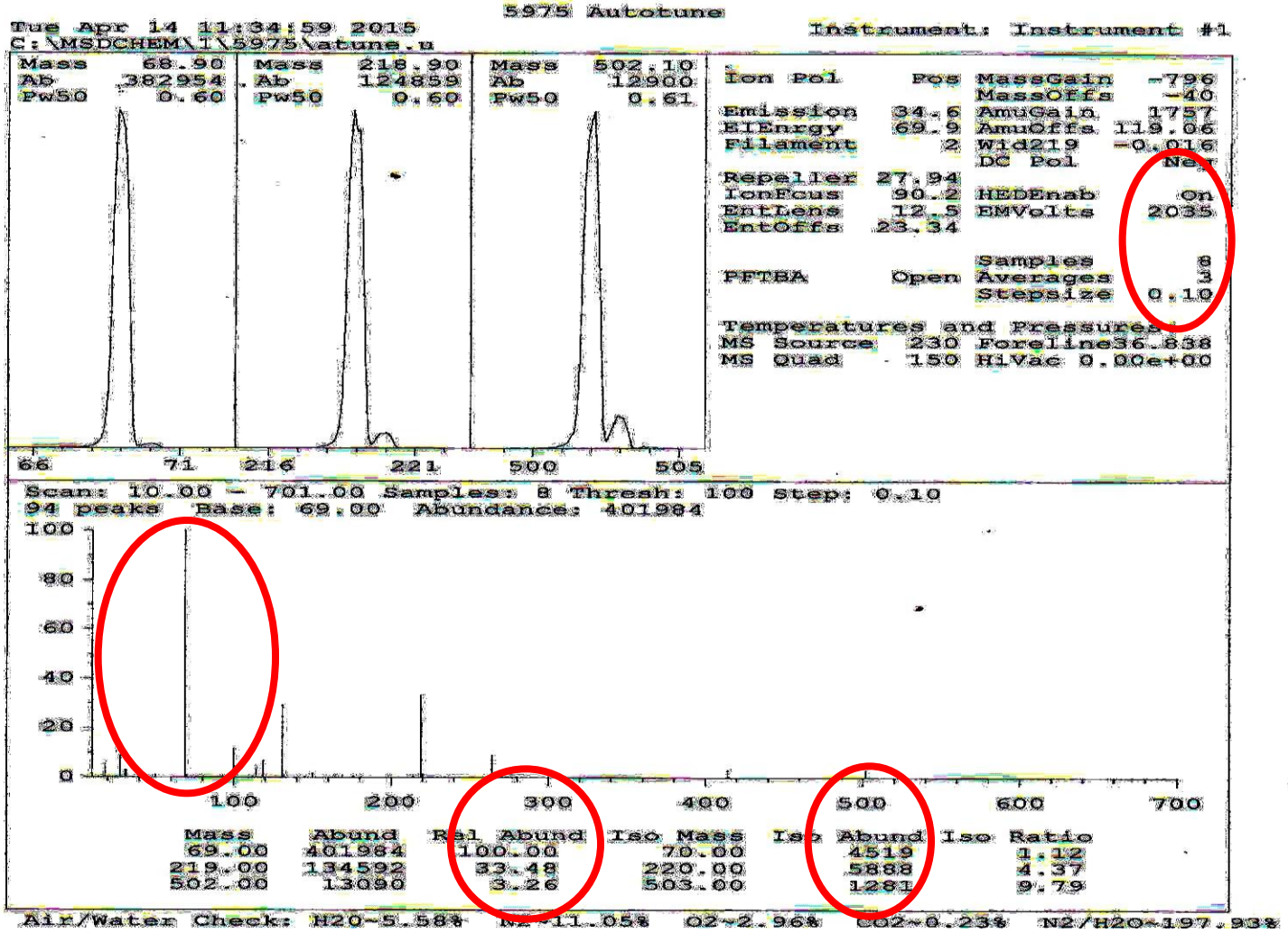
- Maintenance
  - Inlet Septa
  - Inlet Liner
  - Column
  - Blocked Syringe
  - Insufficient Solvent

Further Troubleshooting

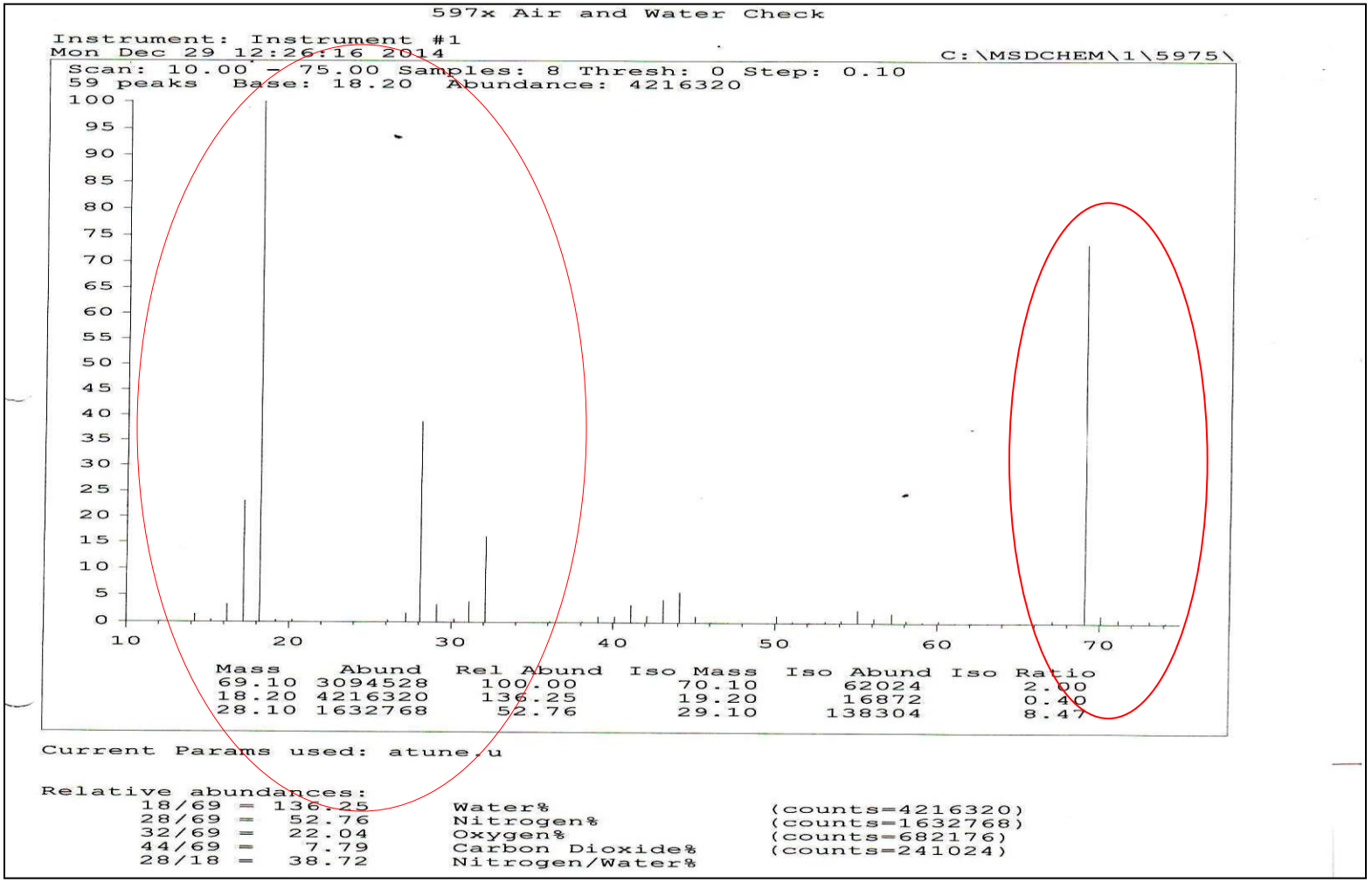
- Reduced Sensitivity
  - Air Leak
  - Dirty Ion Source

How do we know about this:

MS Tune


5975 Autotune
Tue Apr 14 11:34:59 2015
C:\MSDCHEM\1\5975\atune.u
Instrument: Instrument #1
Mass 68.90
Ab 382954
Pw50 0.60
Mass 218.90
Ab 124859
Pw50 0.60
Mass 502.10
Ab 12900
Pw50 0.61
66 71 216 221 500 505
Ion Pol Pos
Emission 34.6
EIEnergy 69.9
Filament 2
Repeller 27.94
IonFocus 90.2
EntLens 12.5
EntOffs 23.34
PFTBA Open
MassGain -796
MassOffs -40
AmuGain 1757
AmuOffs 119.06
Wid219 -0.016
DC Pol Neg
HEDEnab On
EMVolts 2035
Samples 8
Averages 3
Stepsize 0.10
Temperatures and Pressures
MS Source 230 Foreline36.858
MS Quad 150 HiVac 0.00e+00
Scan: 10.00 - 701.00 Samples: 8 Thresh: 100 Step: 0.10
94 peaks Base: 69.00 Abundance: 401984
100
80
60
40
20
0
100 200 300 400 500 600 700
Mass Abund Rel Abund Iso Mass Iso Abund Iso Ratio
69.00 401984 100.00 70.00 4519 1.12
219.00 134592 33.48 220.00 5888 4.37
502.00 13090 3.26 503.00 1281 9.79
Air/Water Check: H2O~5.58% N2~11.05% O2~2.96% CO2~0.23% N2/H2O~197.93%

Air/Water Check

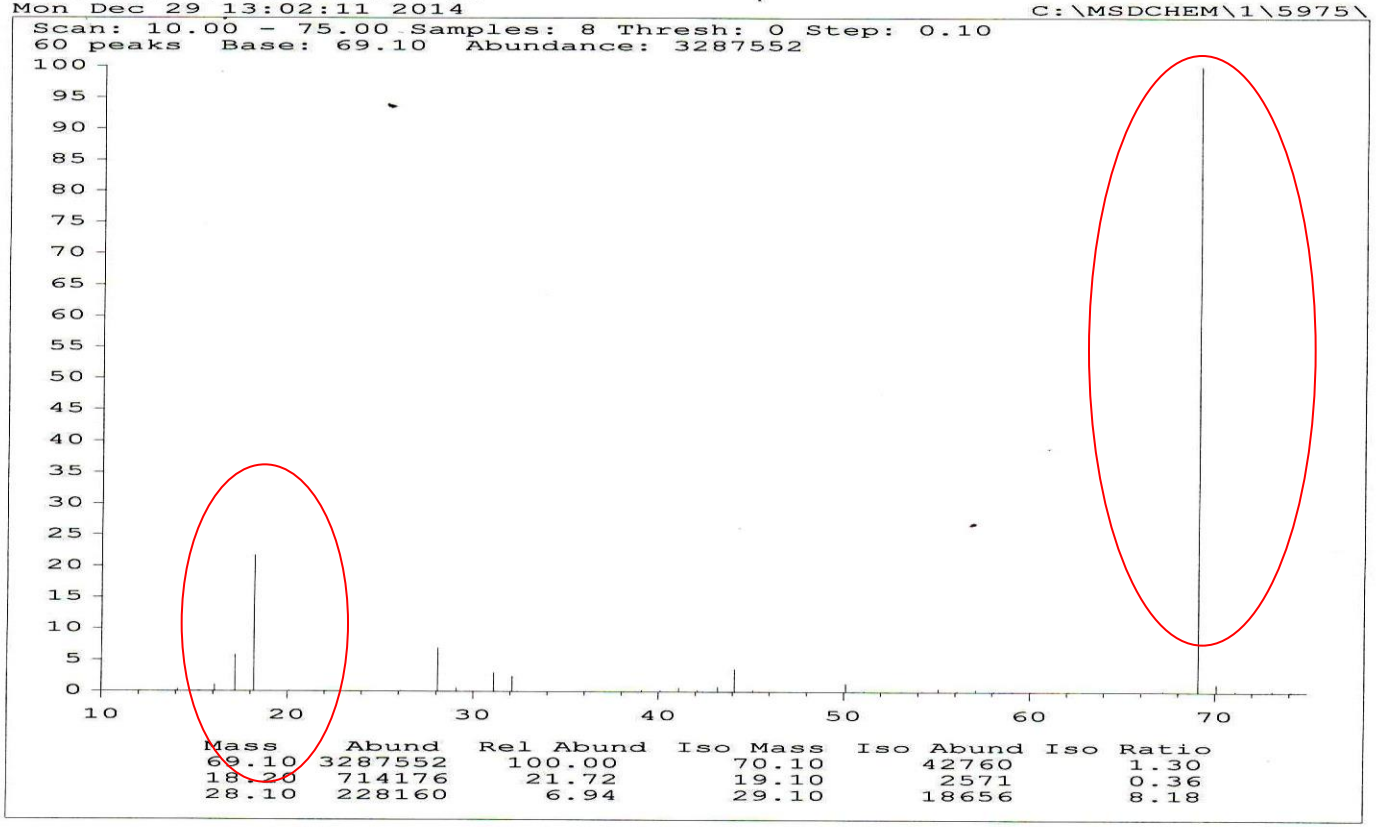


Air/Water Check

597x Air and Water Check

Instrument: Instrument #1
Mon Dec 29 13:02:11 2014 C:\MSDCHEM\1\5975\

Scan: 10.00 - 75.00 Samples: 8 Thresh: 0 Step: 0.10
60 peaks Base: 69.10 Abundance: 3287552



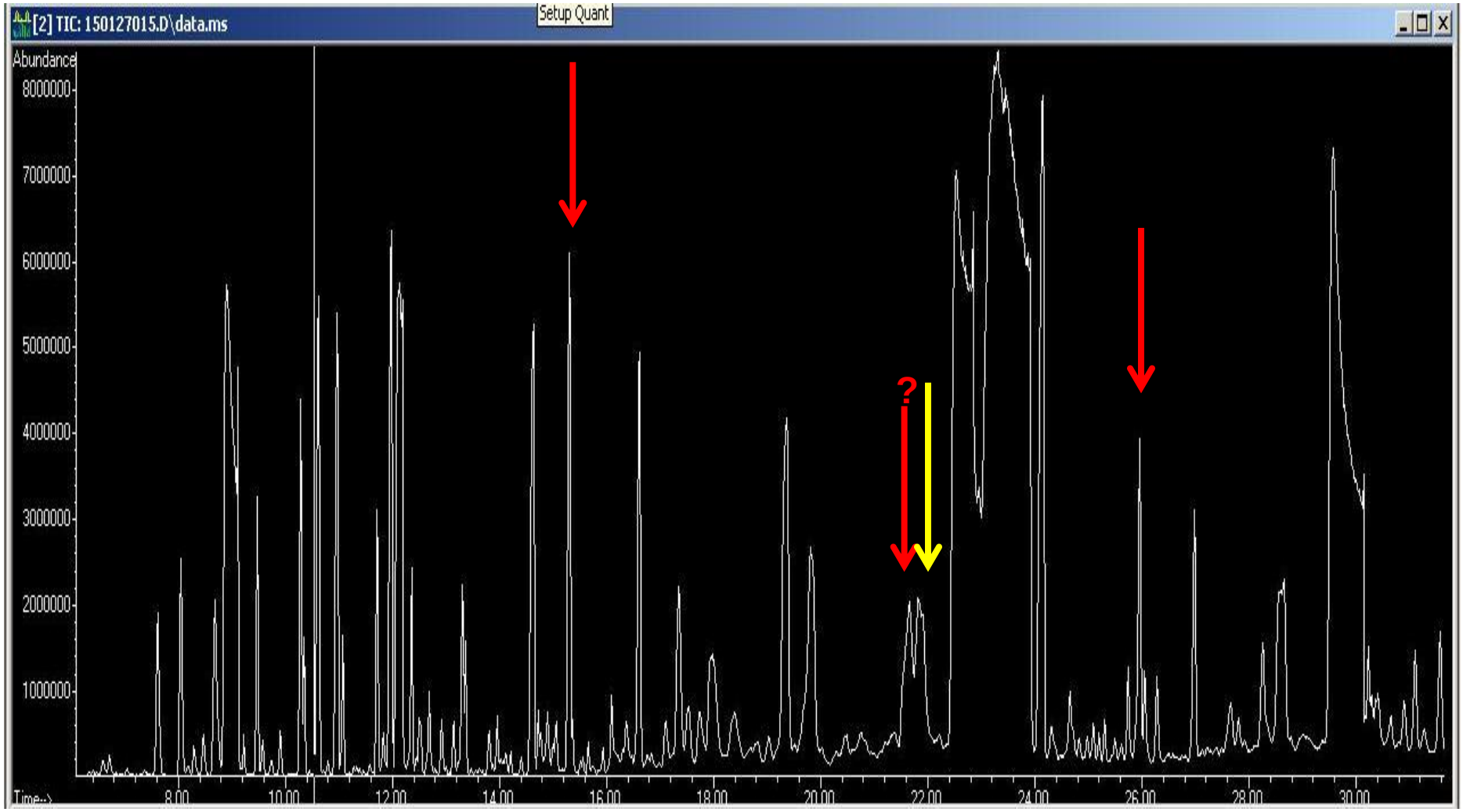
Mass	Abund	Rel Abund	Iso Mass	Iso Abund	Iso Ratio
69.10	3287552	100.00	70.10	42760	1.30
18.20	714176	21.72	19.10	2571	0.36
28.10	228160	6.94	29.10	18656	8.18

Current Params used: atune.u

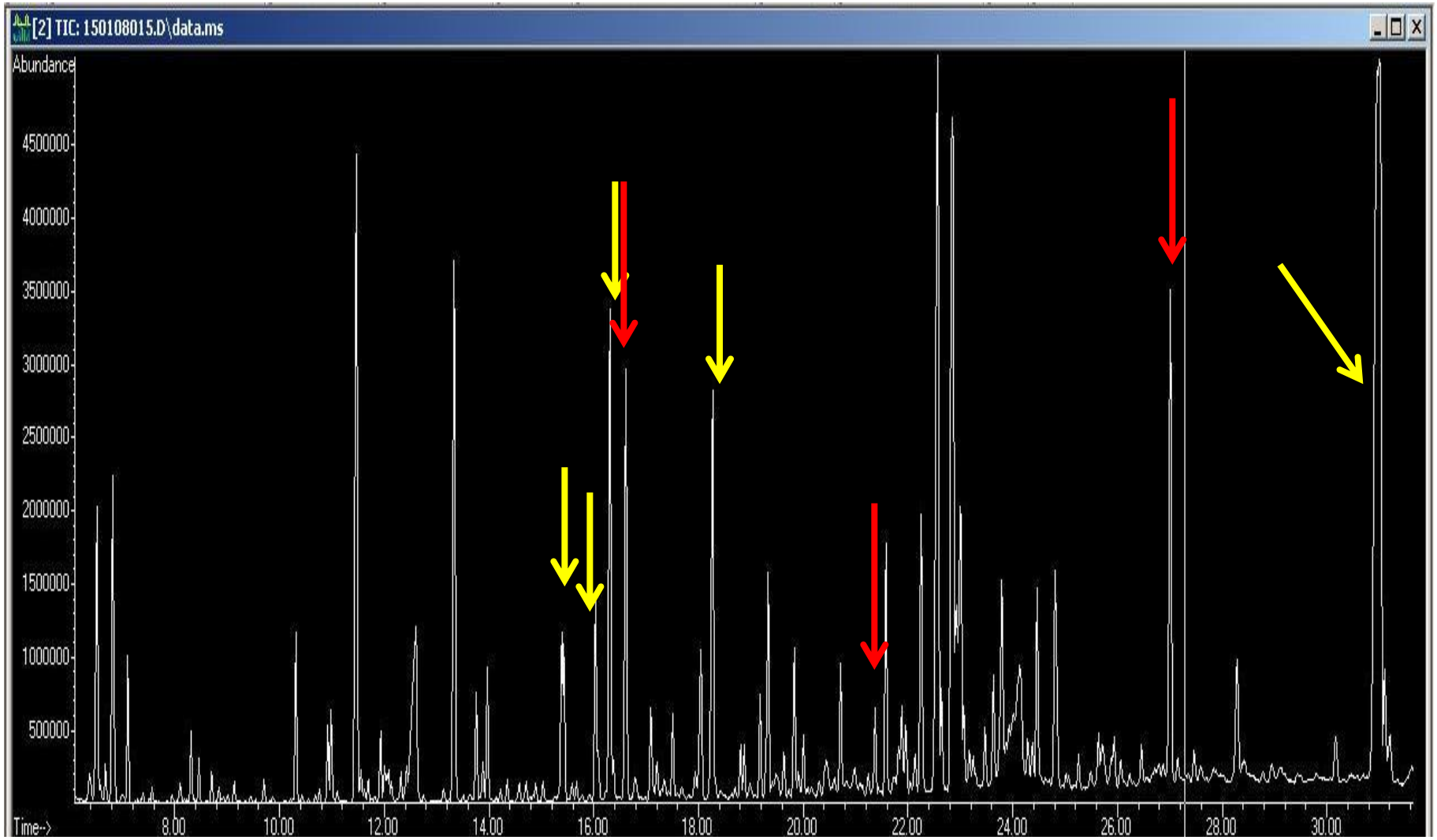
Relative abundances:

18/69 =	21.72	Water%	(counts=714176)
28/69 =	6.94	Nitrogen%	(counts=228160)
32/69 =	2.48	Oxygen%	(counts=81456)
44/69 =	3.65	Carbon Dioxide%	(counts=119872)
28/18 =	31.95	Nitrogen/Water%	

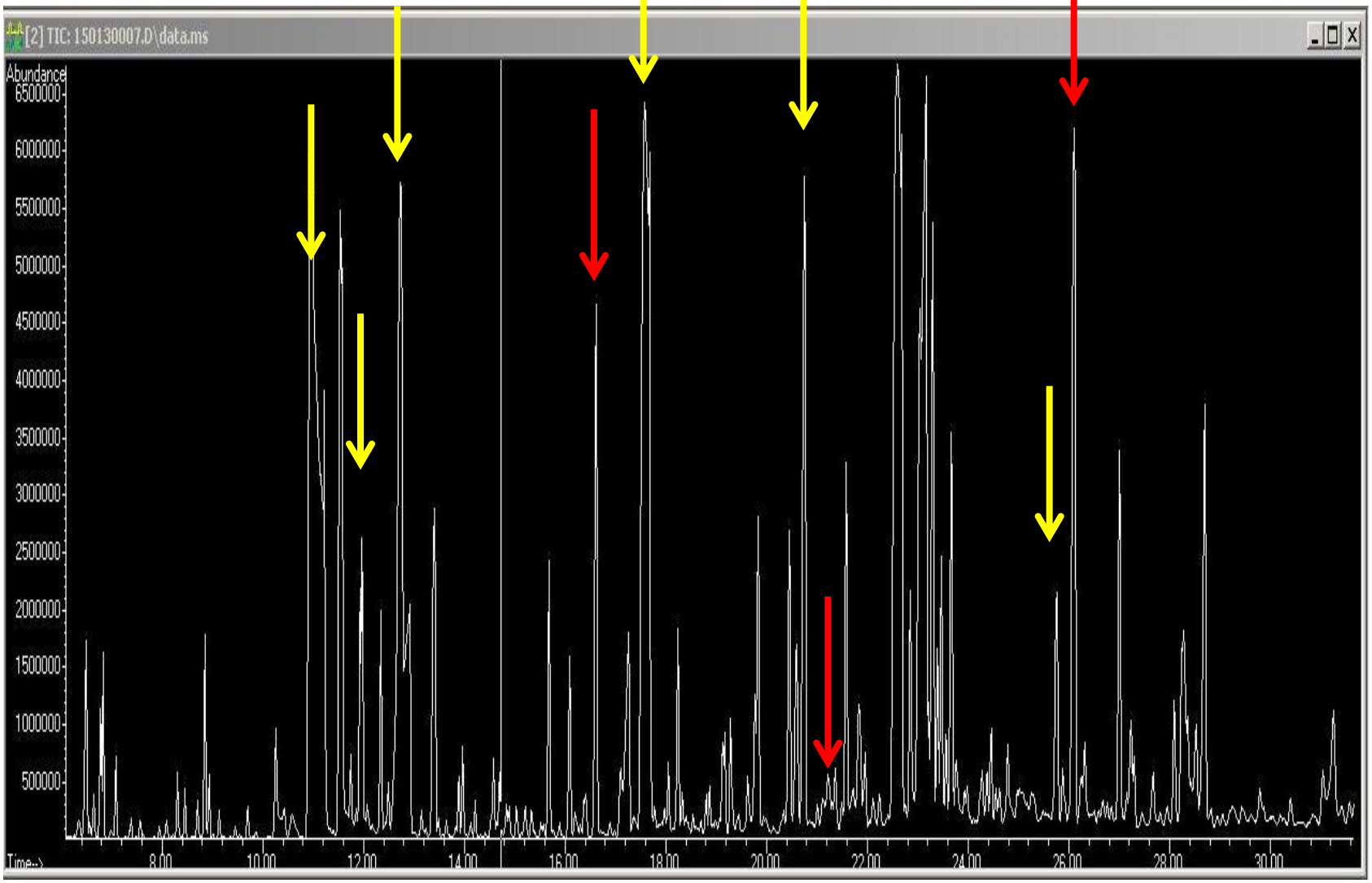
Benzoate


[2] TIC: 150127015.D\data.ms
Setup Quant
Abundance
8000000
7000000
6000000
5000000
4000000
3000000
2000000
1000000
?
Time
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00

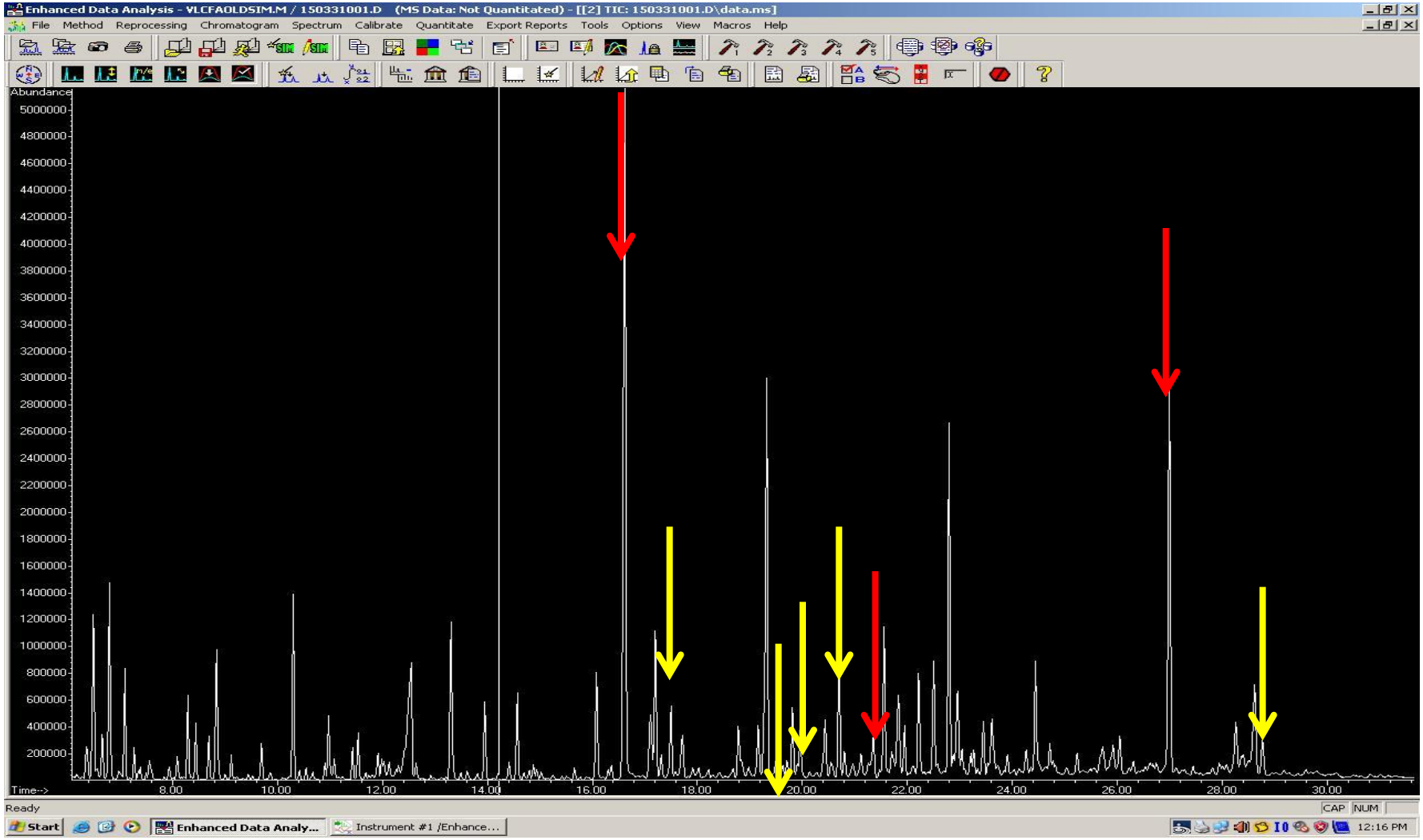
Valproate Interference



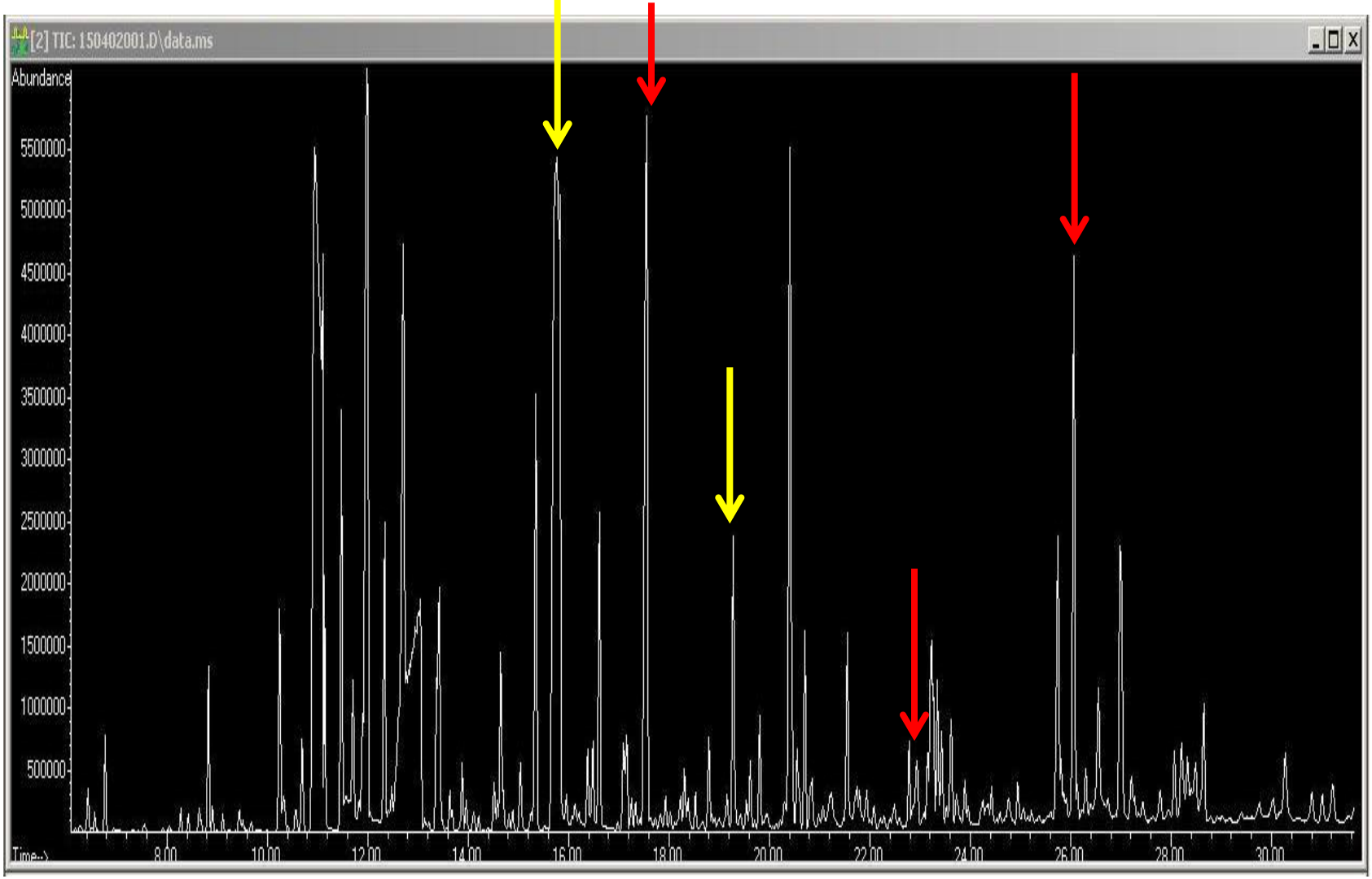
Abnormal 1


[2] TIC: 150130007.D\data.ms
Abundance
6500000
6000000
5500000
5000000
4500000
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
Time-->
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00

Abnormal 2


Enhanced Data Analysis - VLCFAOLDSIM.M / 150331001.D (MS Data: Not Quantitated) - [[2] TIC: 150331001.D\data.ms]
File Method Reprocessing Chromatogram Spectrum Calibrate Quantitate Export Reports Tools Options View Macros Help
Abundance
5000000
4800000
4600000
4400000
4200000
4000000
3800000
3600000
3400000
3200000
3000000
2800000
2600000
2400000
2200000
2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
Time-->
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
Ready
CAP NUM
Start
Enhanced Data Analy...
Instrument #1 /Enhance...
12:16 PM

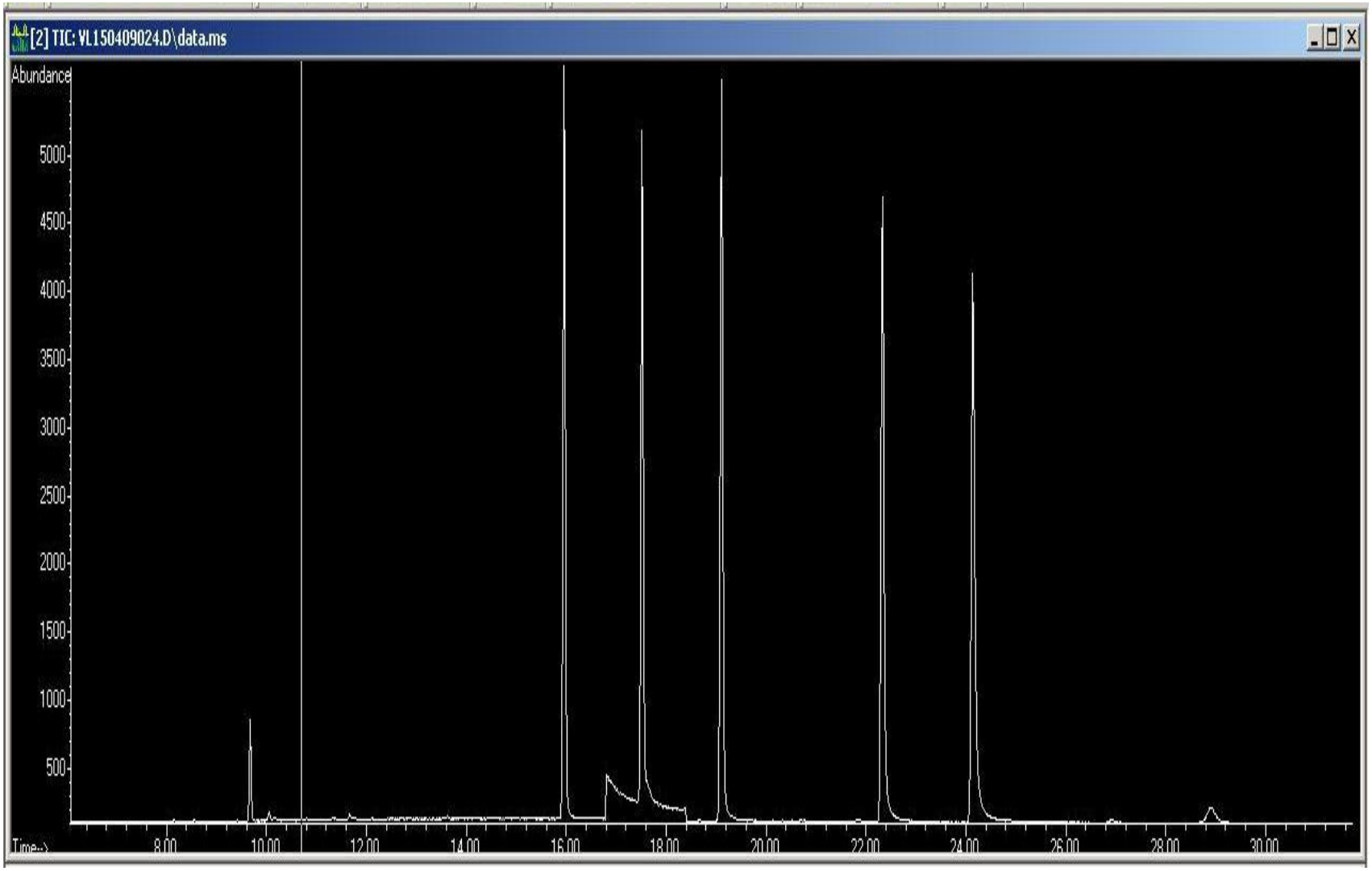
Abnormal 3


[2] TIC: 150402001.D\data.ms
Abundance
5500000
5000000
4500000
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
Time-->
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00

SIM Methods

Using VLCFA as an example

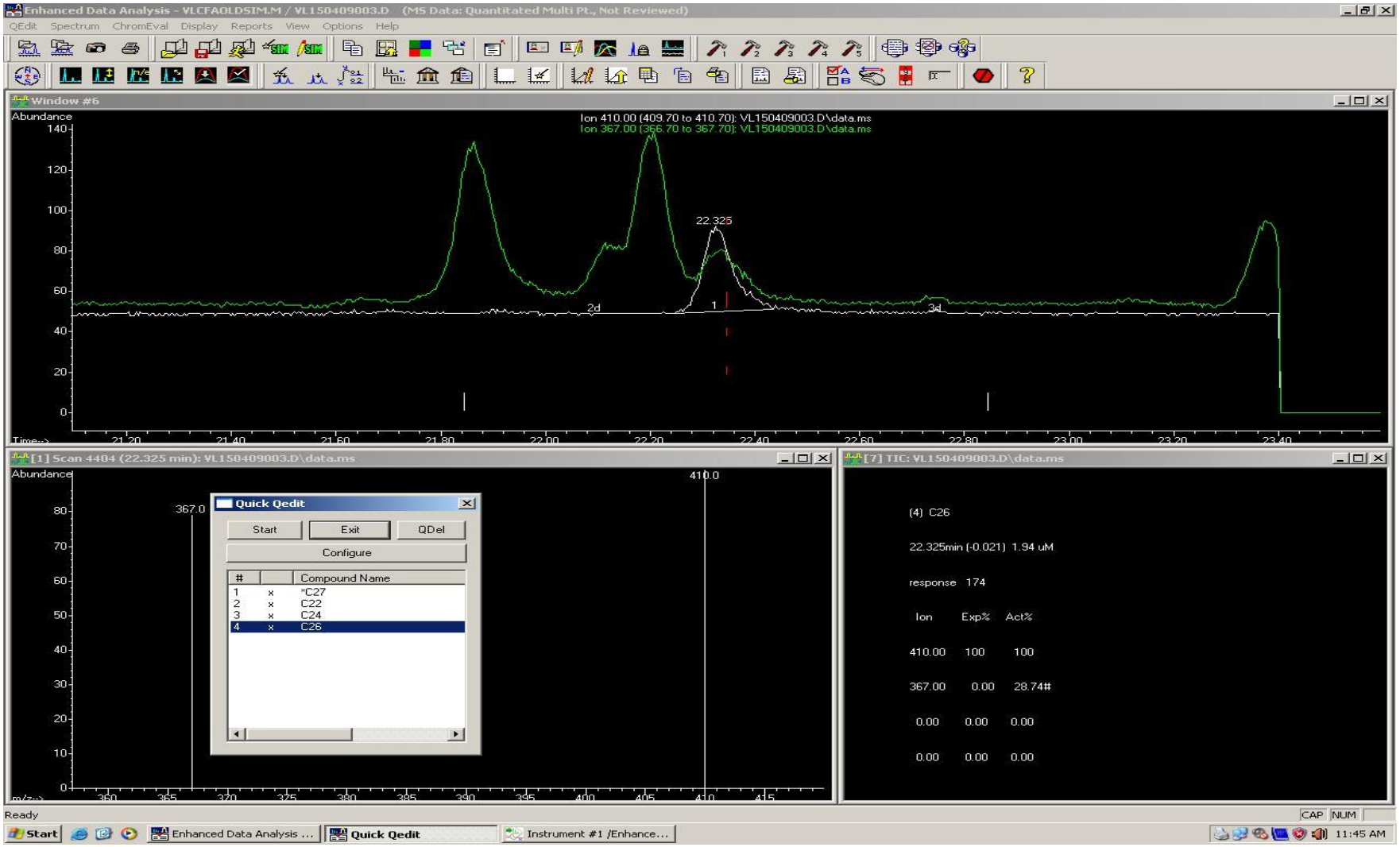
SIM Monitoring



Troubleshooting

- Ensure Time Windows are Correct
- If not run the standard on a scan method
- Reset Time Windows

Integration



Thank You For your Time

Any Questions