

# Tackling the Techniques Tandem Mass Spectrometry

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NHS Foundation Trust



*Directorate of Laboratory Medicine*

# Introduction

- Background and history of MS/MS
- How MS/MS works
- Common errors and troubleshooting
- Examples

# Mass Spectrometry - History

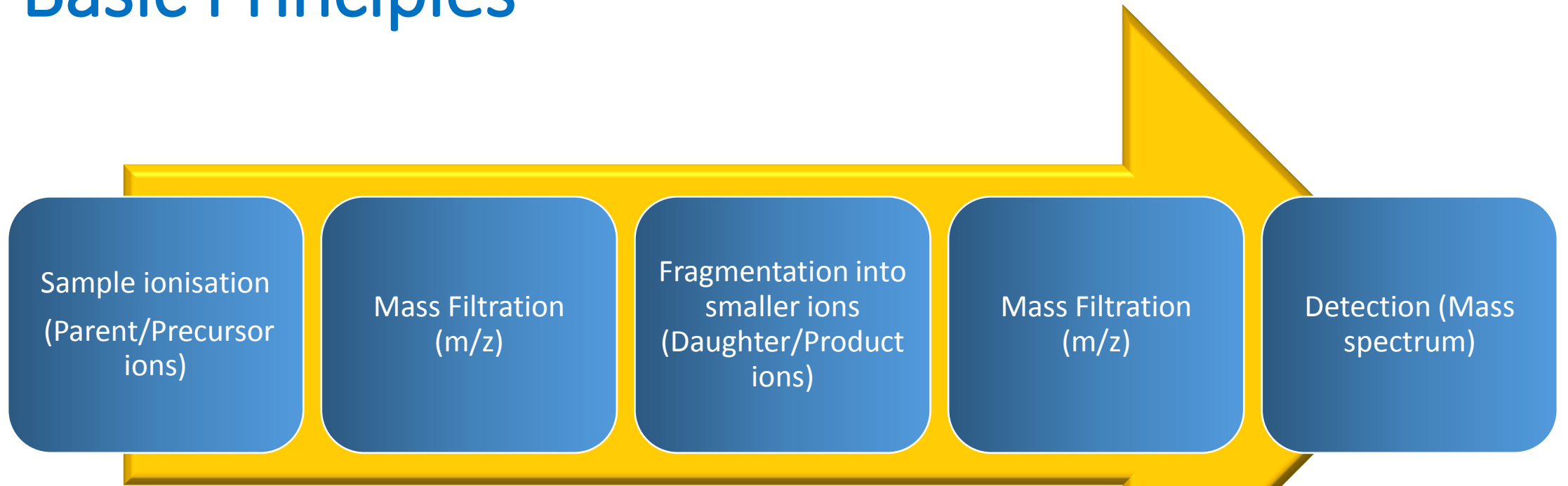
*“I feel sure that there are many problems in Chemistry which could be solved with far greater ease by this than by any other method. The method is surprisingly sensitive – more so even than that of spectrum analysis – requires an infinitesimal amount of material and does not require this to be specially purified”...*

*(Sir J.J Thomson 1921)*

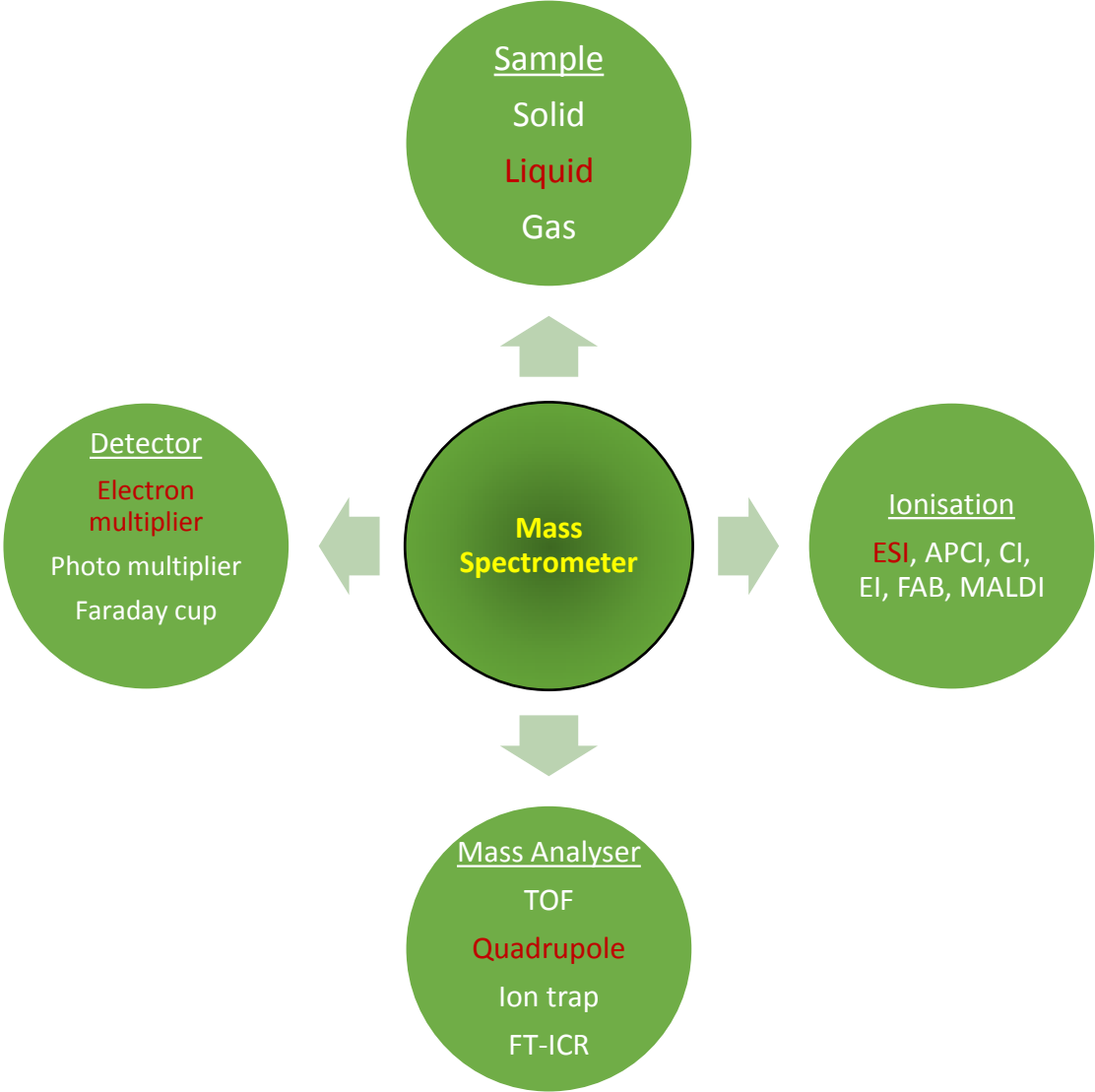
# Tandem Mass Spectrometry - History

- Most significant advance in metabolic disease/NBS screening
- Exponential increase in use to biosciences during 1980s
  - Development of ionisation methods (ESI, MALDI)
- First used in clinical setting in 1982
- Inherited metabolic diseases in early 1990s
- Short analysis time (2-3 mins)
- Broad spectrum – 1 test/ many disorders

# Basic Principles



# Types of Mass Spectrometers



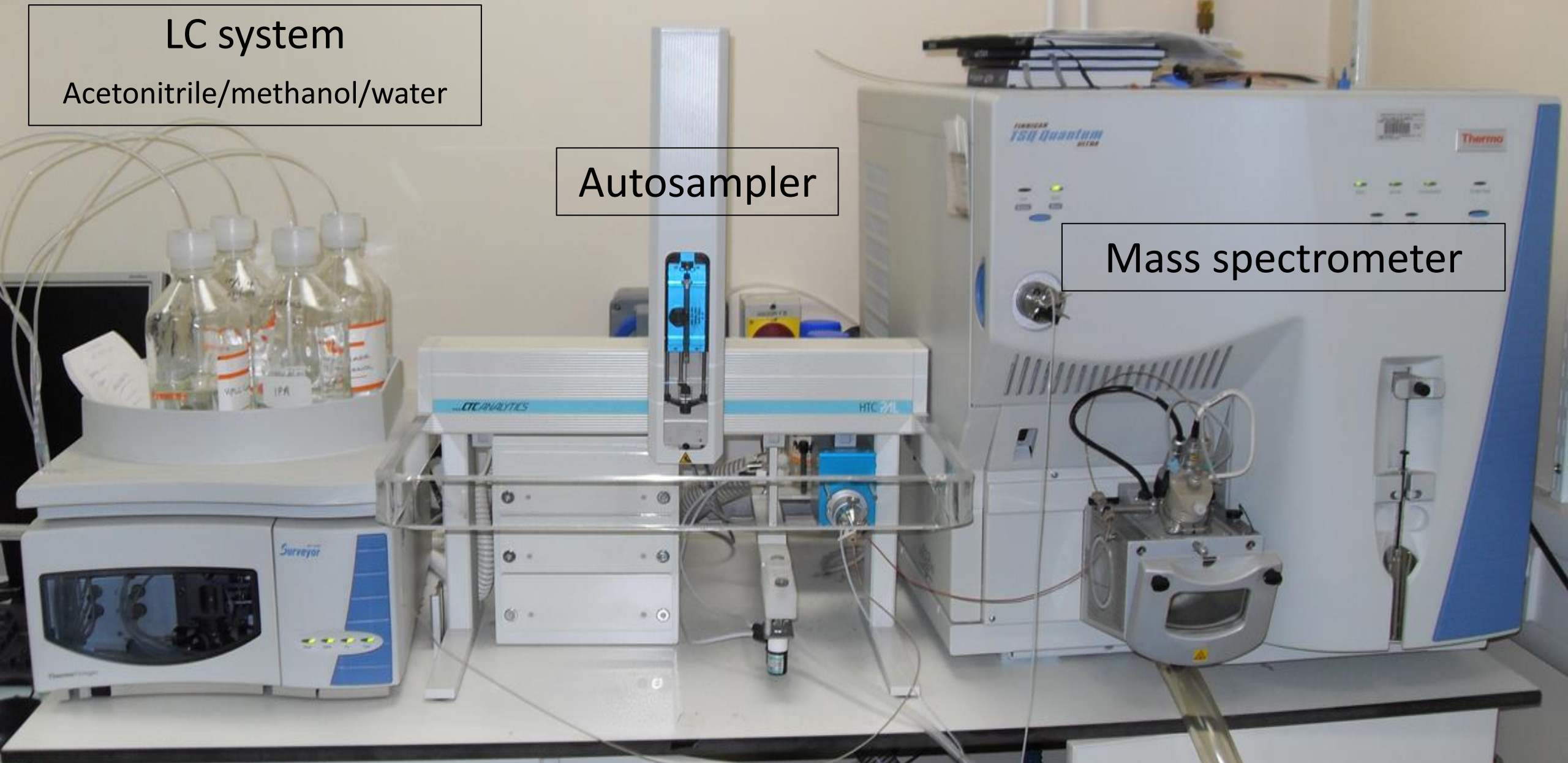
# ESI triple quadrupole tandem mass spectrometer

LC system

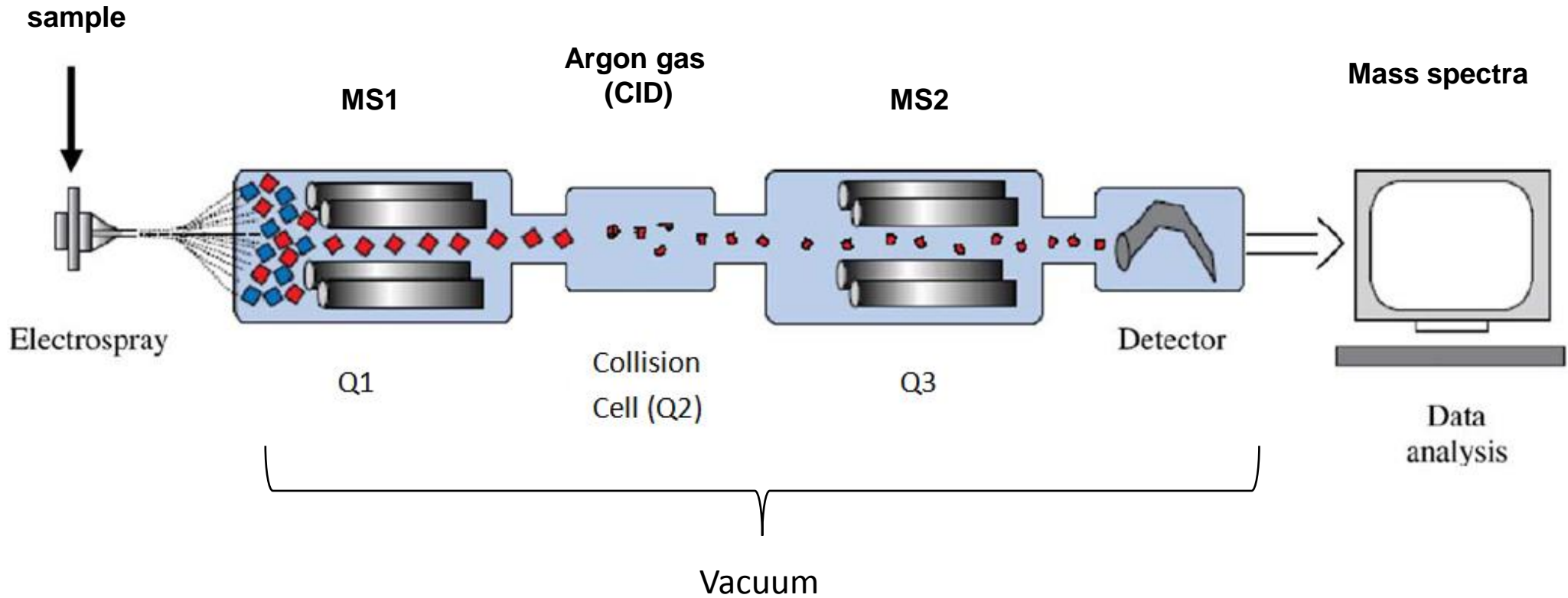
Acetonitrile/methanol/water

Autosampler

Mass spectrometer

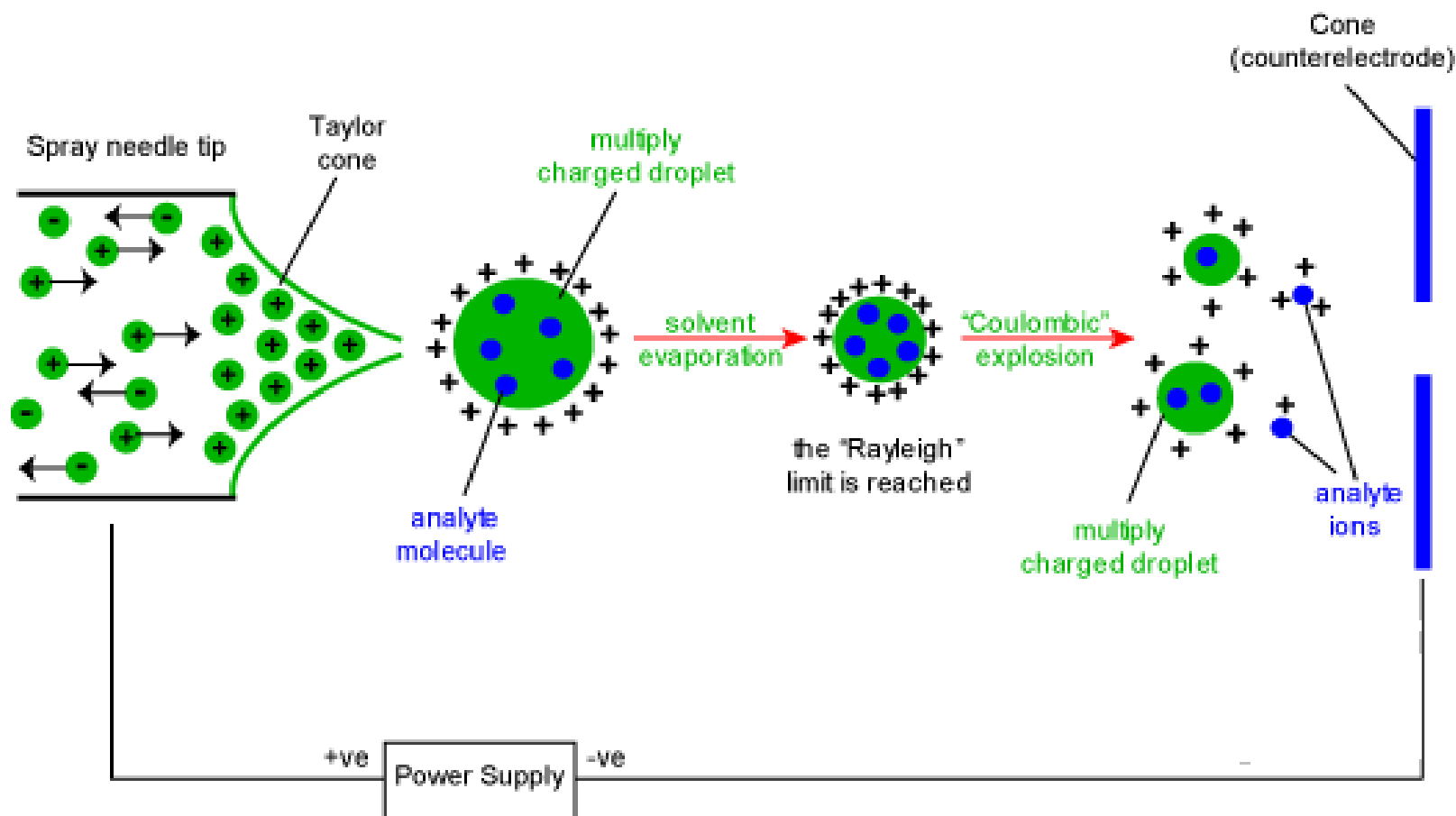
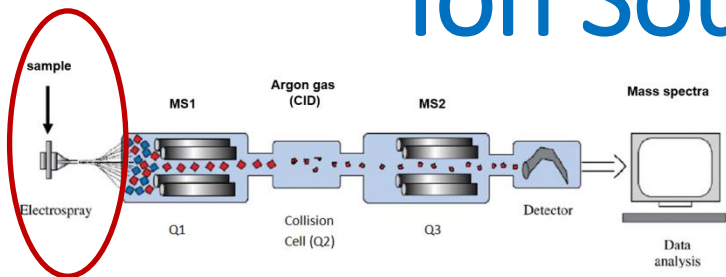


# ESI triple quadrupole tandem mass spectrometry

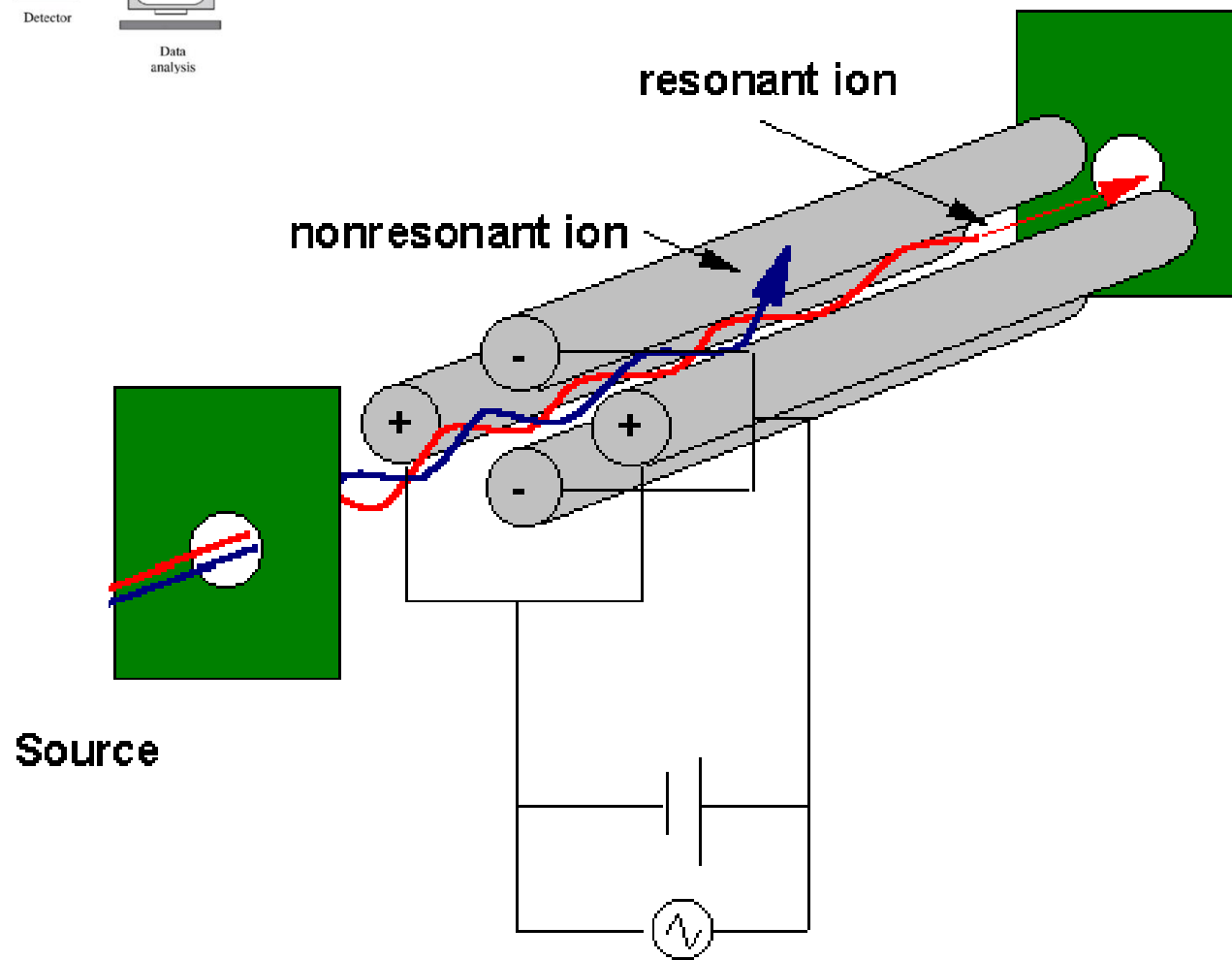
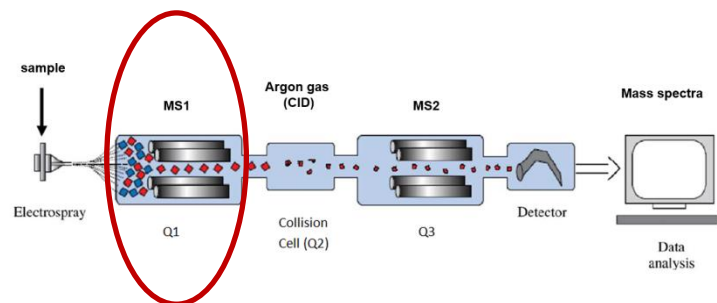




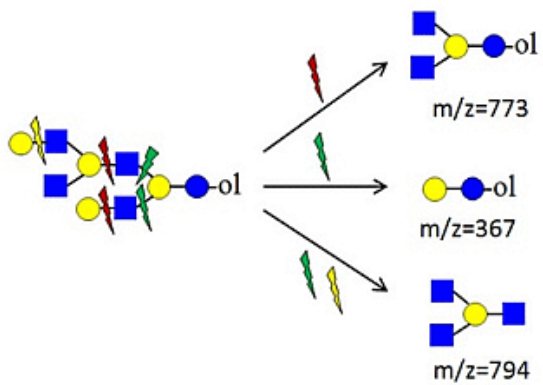
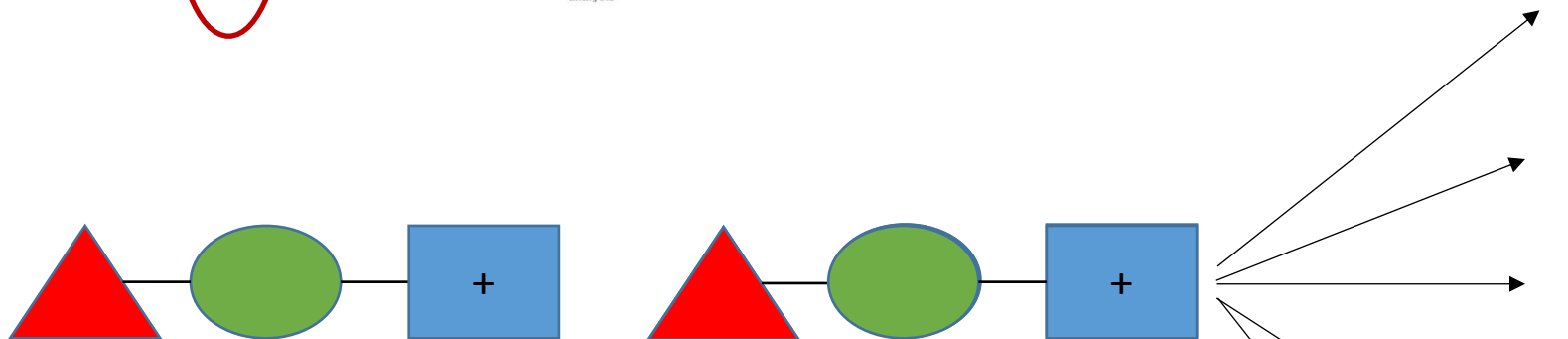
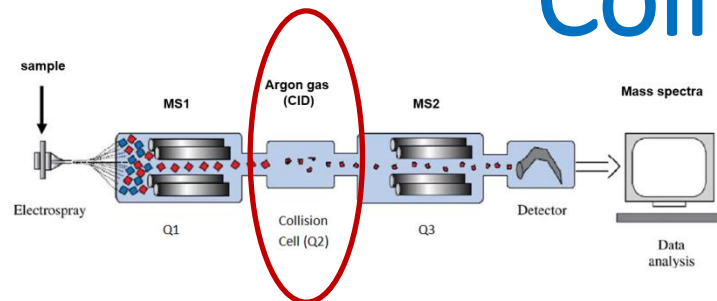
# Ion Source – Electrospray Ionisation



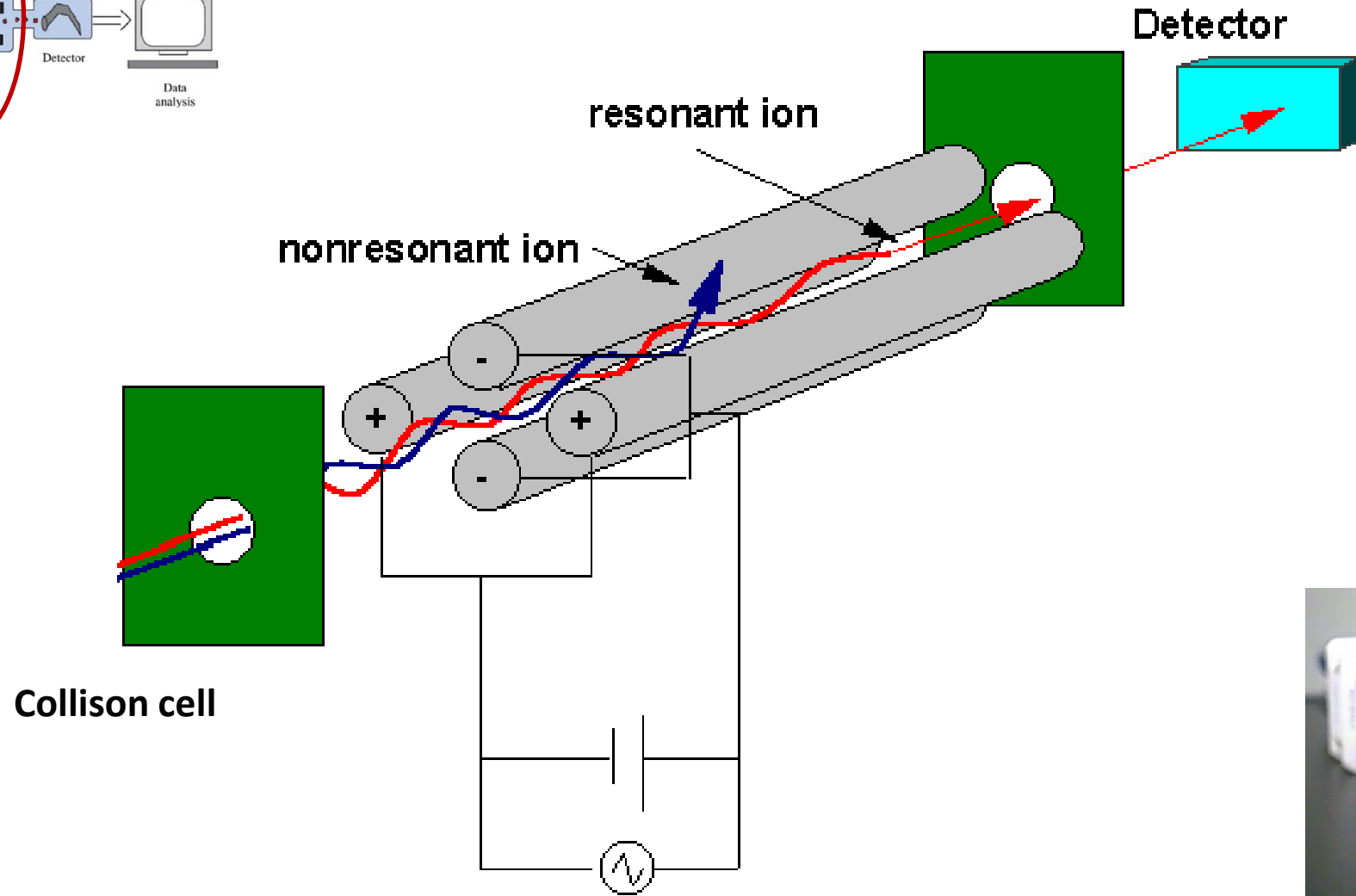
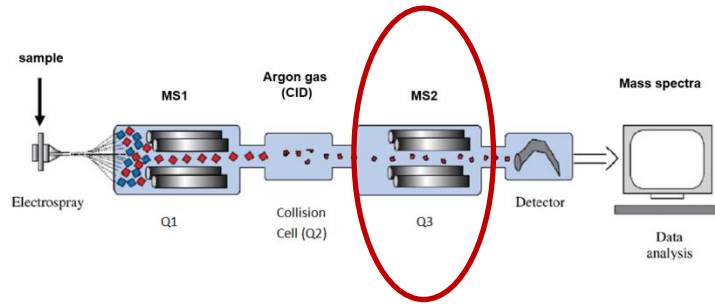
# Quadrupoles – MS1



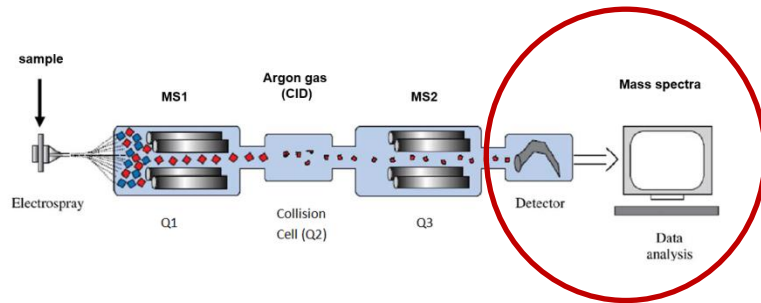
# Collision-induced Dissociation



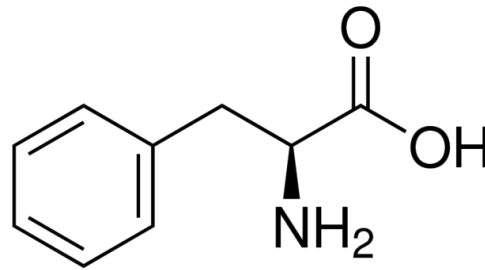
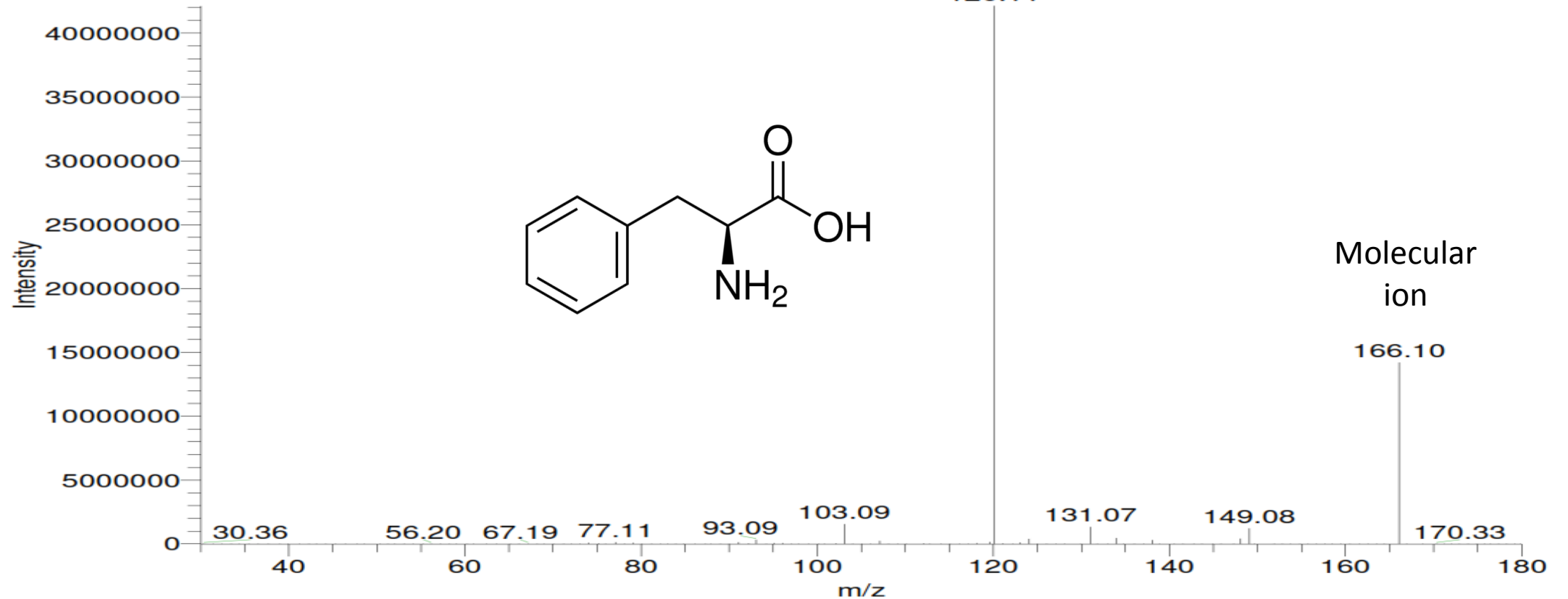
# Quadrupoles – MS2



# Detection – Mass spectra

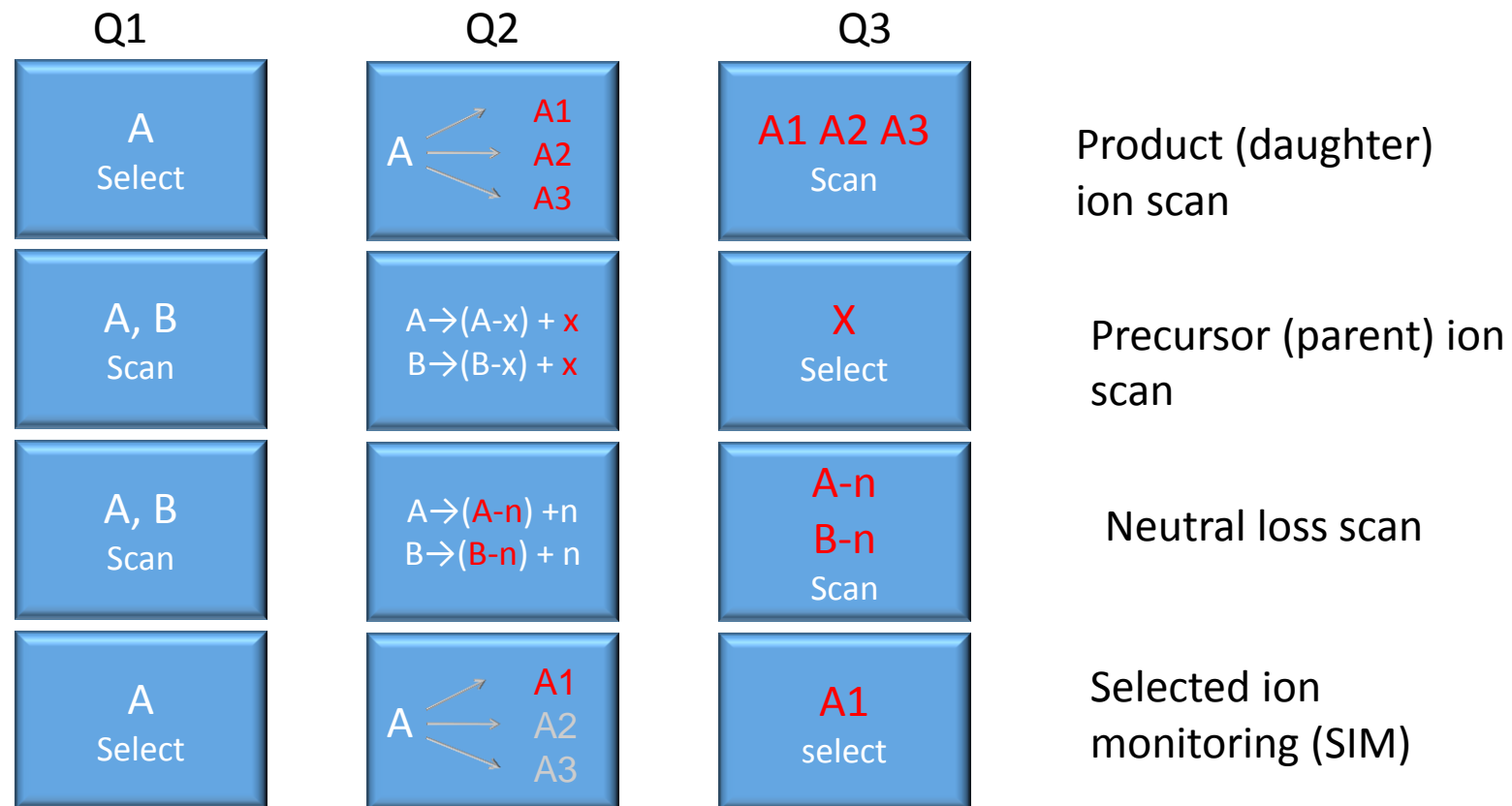


Phe\_120609 #6 RT: 0.20 AV: 1 NL: 4.21E7  
T: + c Full ms2 166.10@-10.00 [ 30.00-180.00]



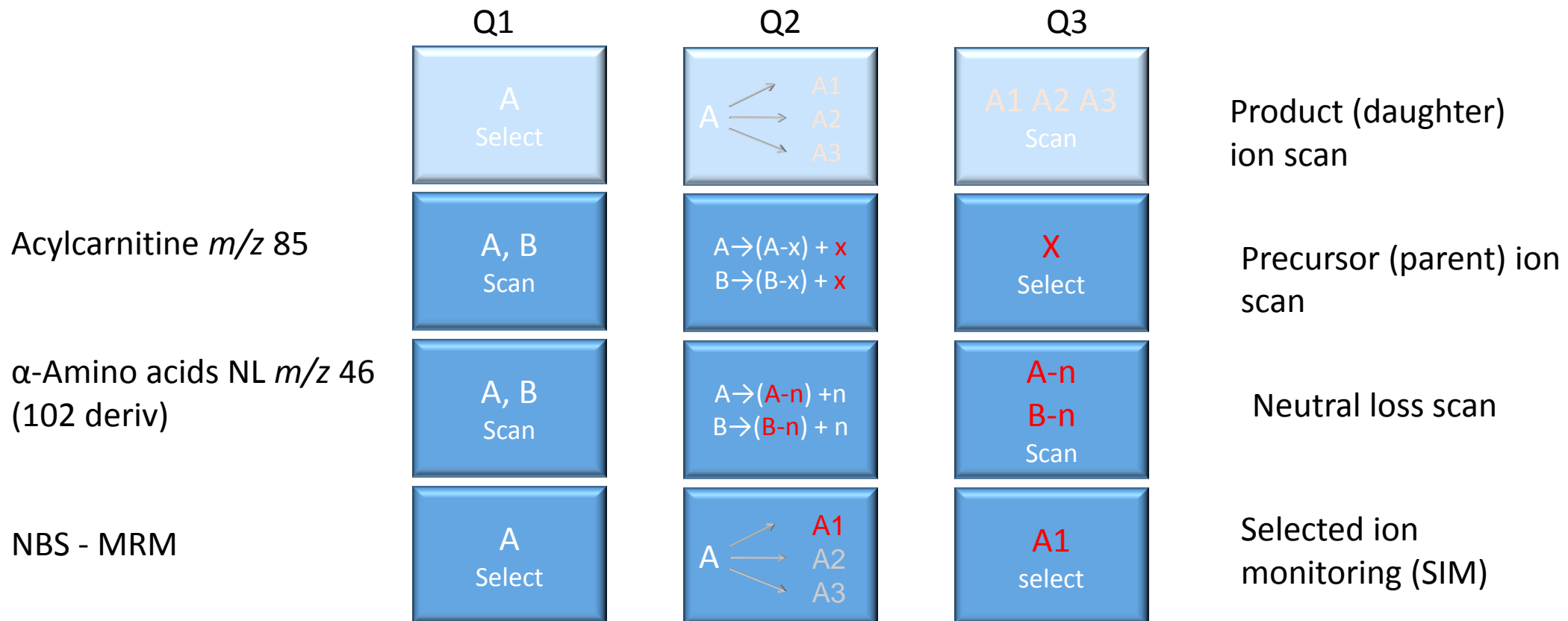
# ESI +ve triple quadrupole mass spectrometer

## ➤ Modes of MS/MS operation



# ESI +ve triple quadrupole mass spectrometer

## ➤ Modes of MS/MS operation

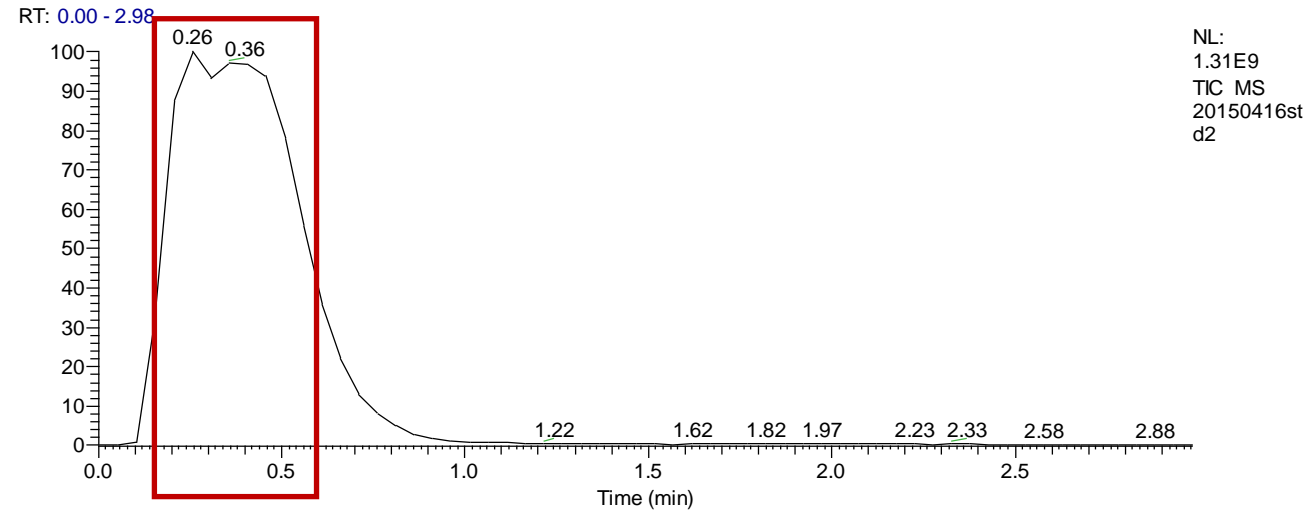


# Precursor of m/z 85 scan

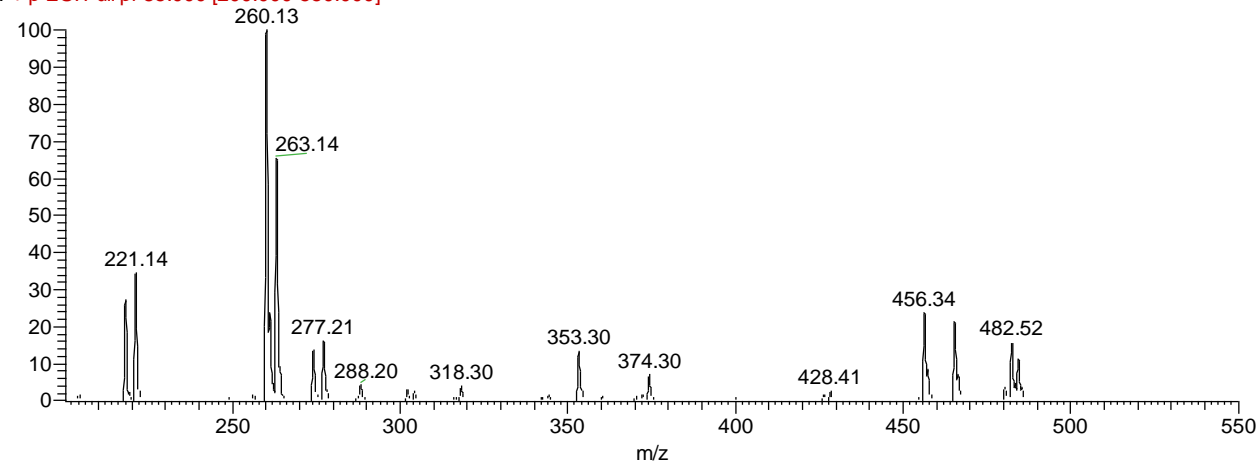
C:\Xcalibur...\2015\20150416std2

16/04/2015 17:14:13

CN Std - 020415



20150416std3 #6-15 RT: 0.26-0.59 AV: 5 SM: 7B NL: 3.52E6  
F: +p ESI Full pr 85.000 [200.000-550.000]







## Clinical Biochemistry

Volume 39, Issue 4, April 2006, Pages 315–332



Review

### Expanded newborn screening of inherited metabolic disorders by tandem mass spectrometry: Clinical and laboratory aspects

Uttam Garg<sup>a</sup>  , Majed Dasouki<sup>b</sup>



Clin Biochem Rev. 2003 Feb; 24(1): 3–12.

PMCID: PMC1853331

### Electrospray Ionisation Mass Spectrometry: Principles and Clinical Applications

[CS Ho](#), [CWK Lam](#),\* [MHM Chan](#), [RCK Cheung](#), [LK Law](#), [LCW Lit](#), [KF Ng](#), [MWM Suen](#), and [HL Tai](#)

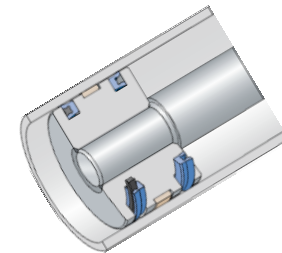
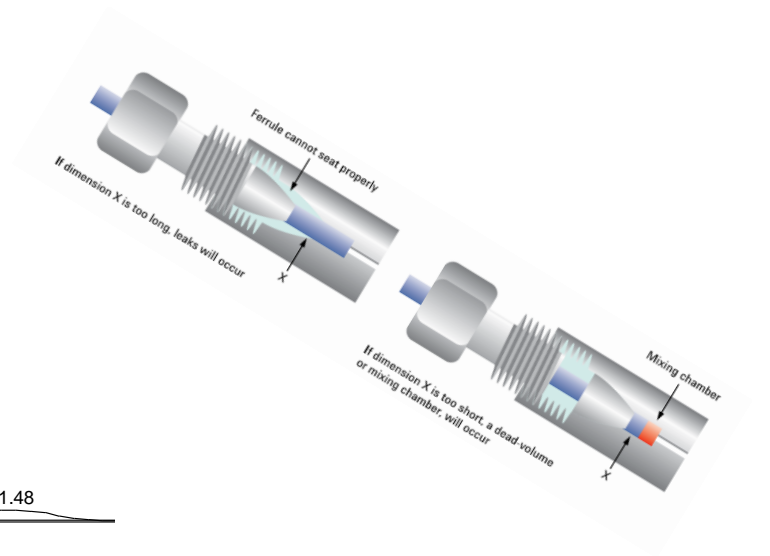
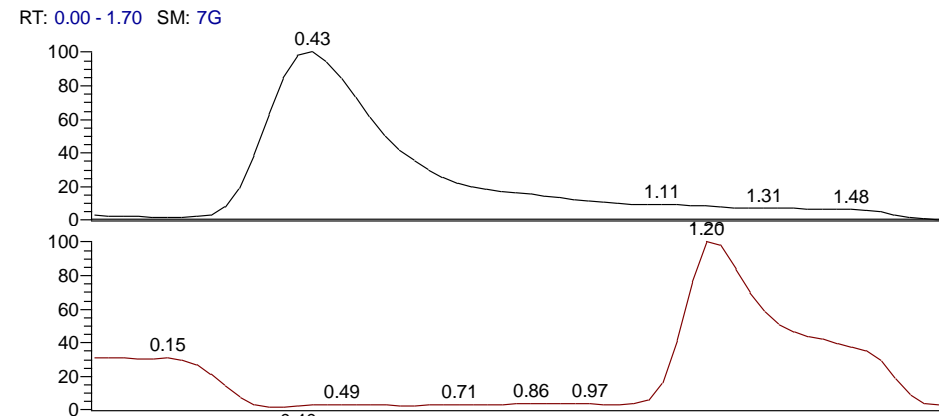
# Common Errors & Troubleshooting

# Sample Preparation

- Appropriate extraction, internal standard and derivatisation performed
- Eluent compatible with mobile phase
- Good laboratory practice - accuracy



# LC Pressure System



# Autosampler

- Common symptom is Low intensity
- Syringe
- Blockages - sample port/stata valve
- Leaks – rotor seal
- Wrong sample injection – user error!



# Contamination

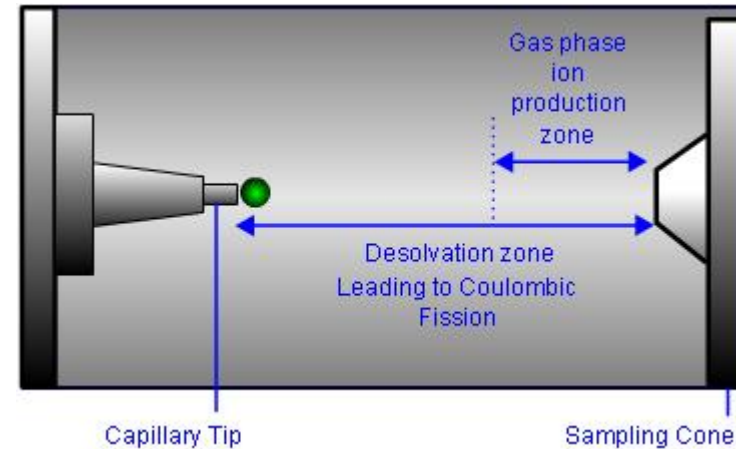
- Highly sensitive instrument
- Contamination may results from
  - Sample preparation – other reagents and handcreams
  - Bad laboratory practice
  - Mobile phase – microbe growth in water
  - Dirt build up in source
  - Improper cleaning



# Mass spectrometer

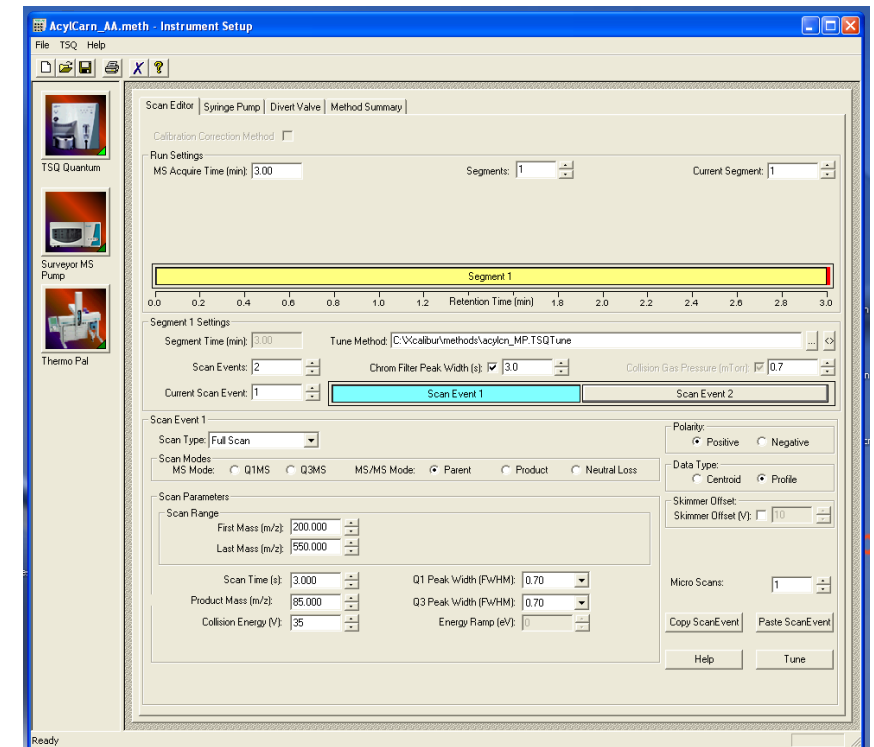
- **Physical**

- Clean cone shield and ion transfer tube – reduced signal intensity
- Probe correct position from the cone – proper nebulization



# Mass spectrometer

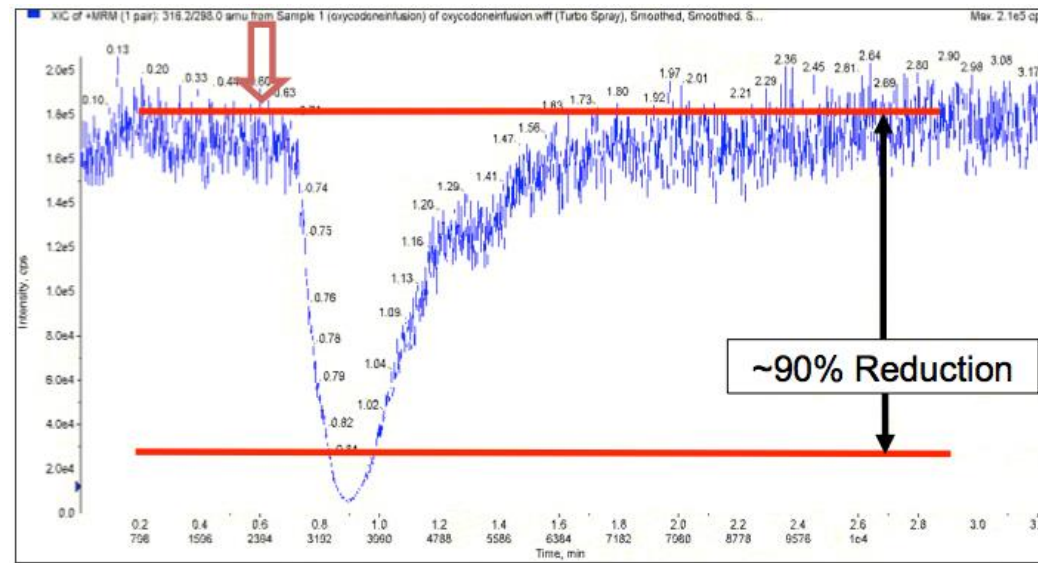
- Physical
- **Acquisition Parameters**
  - ESI Probe/source settings – temperature and gas flow optimised per compound (polar mobile phases need high temperatures)
  - Collision energy too high or too low –  
no product ions formed
  - Incorrect transitions or scan mode
  - Incorrect flow rates





# Mass spectrometer

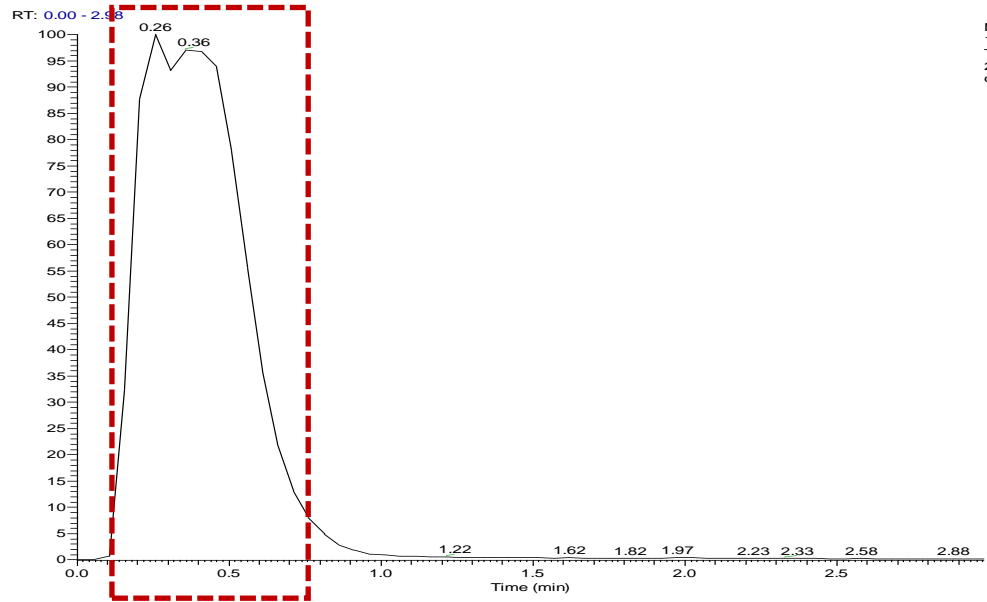
- Physical
- Acquisition Parameters
- **Ion suppression**
  - Sample matrix and mobile phase additives (acids, salts) alter signal of target analyte.



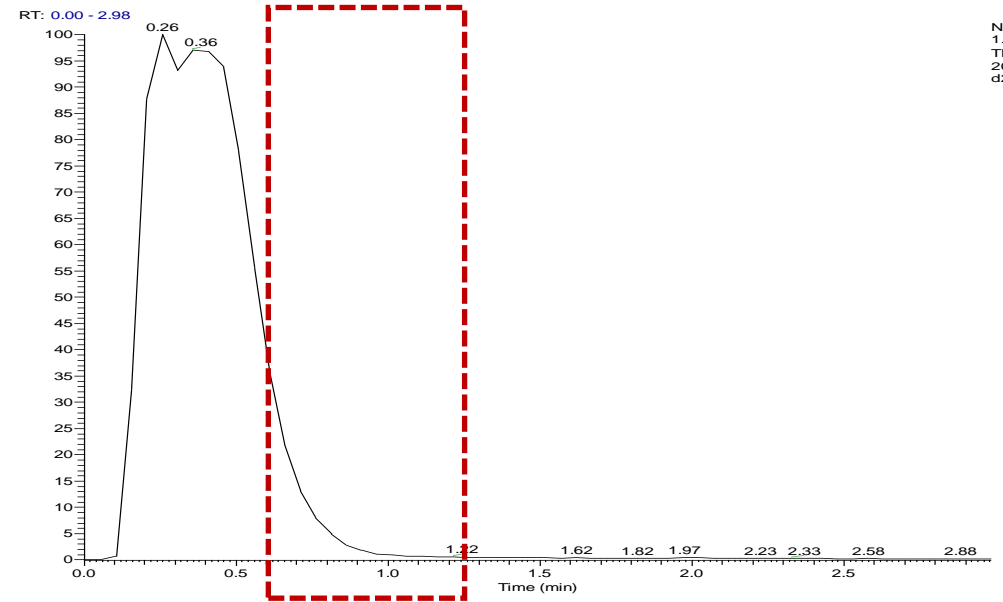
# Mass spectrometer

- Physical
- Acquisition Parameters
- Ion suppression
- **Data Processing**

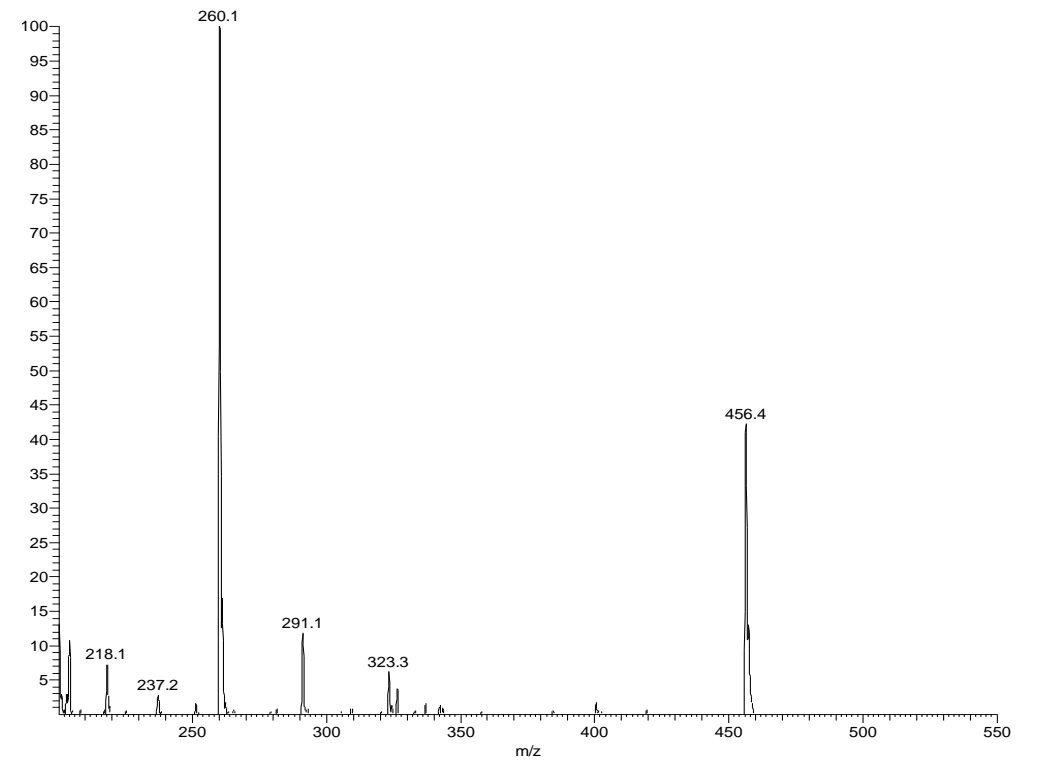
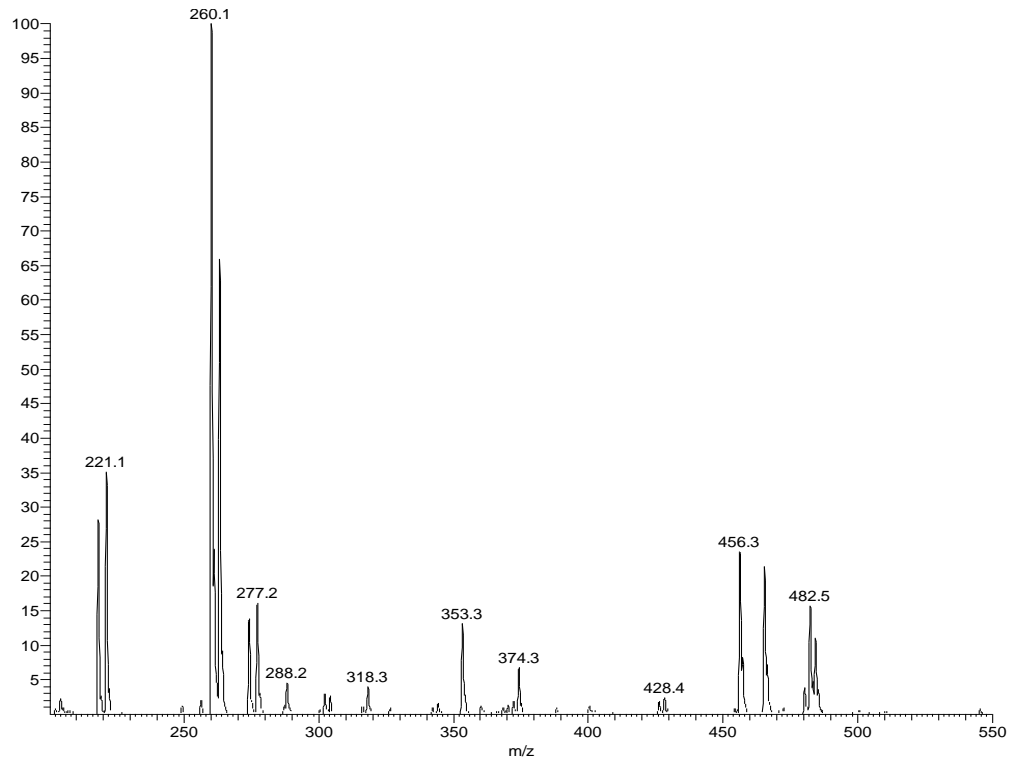
ARE YOU LOOKING AT THE RIGHT THING??



NL:  
1.31E9  
TIC MS  
20150416st  
d2



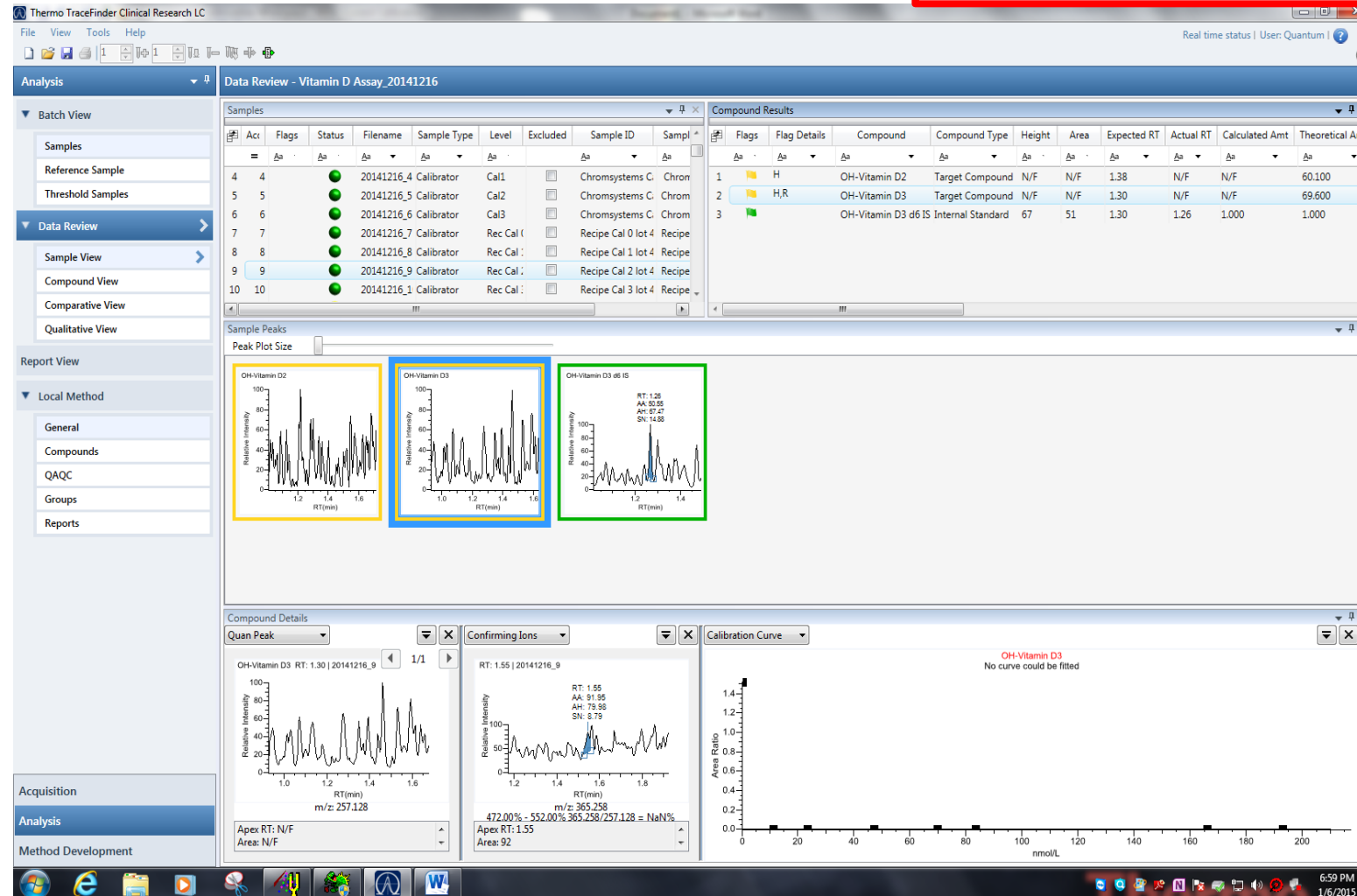
NL:  
1.31E9  
TIC MS  
20150416st  
d2



# Examples

# Example 1

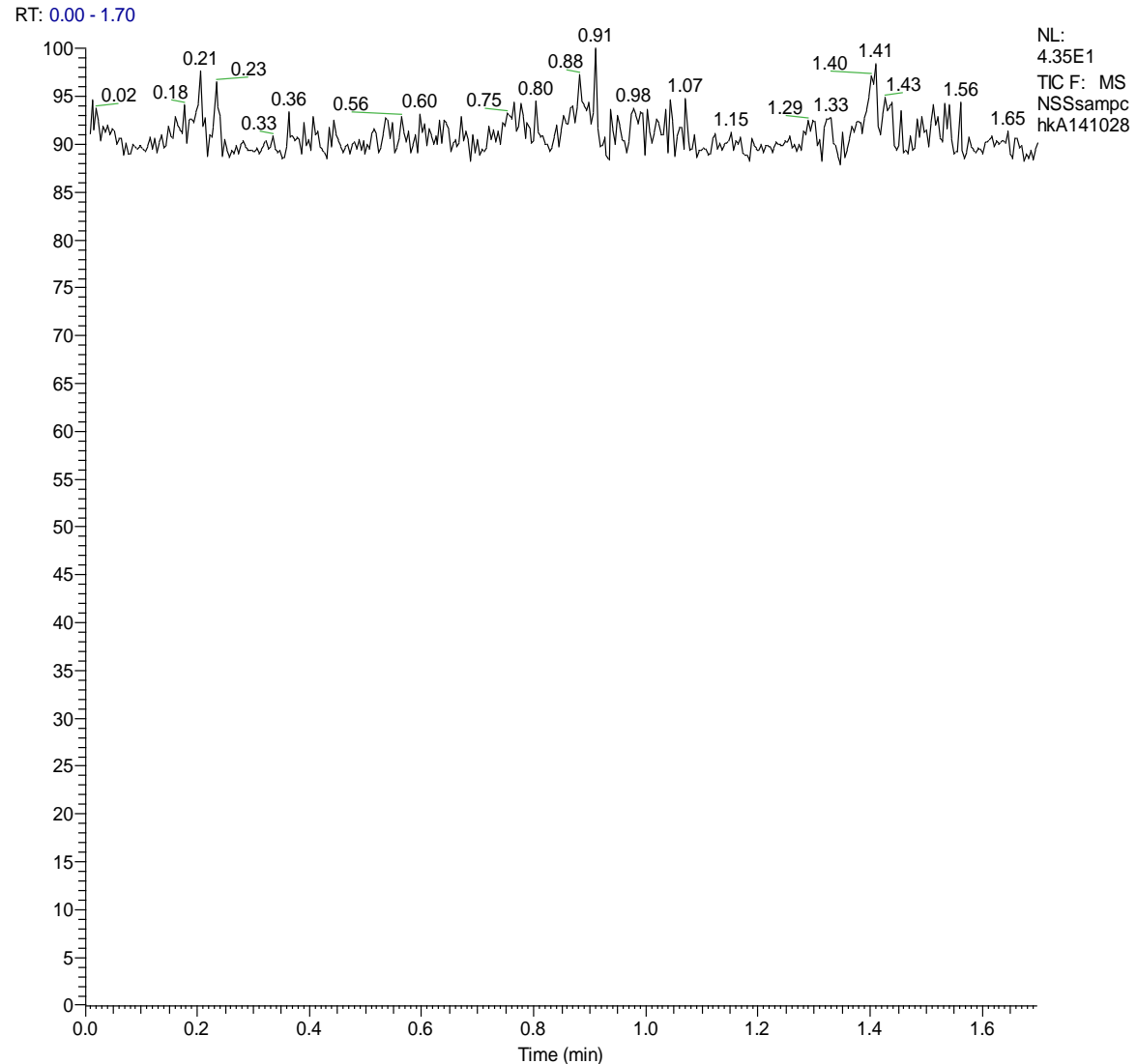
Misaligned microtitre plate



Courtesy of Liverpool

# Example 2

- Plate positioned correctly ✓
- Sample in well ✓
- LC-solvents correct ✓
- No Leaks/blocks ✓
- Correct acquisition parameters ✓



Thermo TSQ Tune Master - Instrument Method Development Workspace - ESI - acylcn\_MP - Current Calibration

File Workspace View Control Scan Parameters Display Setup Help

Scan Type: Full Scan SIM SRM

Scan Mode: MS Q1MS Q3MS MS/MS Parent Product Neutral Loss

Scan Parameters:

Scan Range: Entry Mode: FM/LM First Mass: 30.000 Last Mass: 600.000 Scan Time: 1.000 Product Mass: 85.000 Collision Energy: 10 Energy Ramp: 0 AutoSIM

Peak Width: Q1: 0.70 Q3: 0.70

Charge State: Parent Ion: 1 Product Ion: 1 Number of Peaks: 10 Weight Factor: 0.0

Skimmer Offset: 10 Data Processing: Average Chrom. Filter: 0.7 Q2 CID Gas: Micro Scans: 1

Thermo Pal Surveyor MS Pump

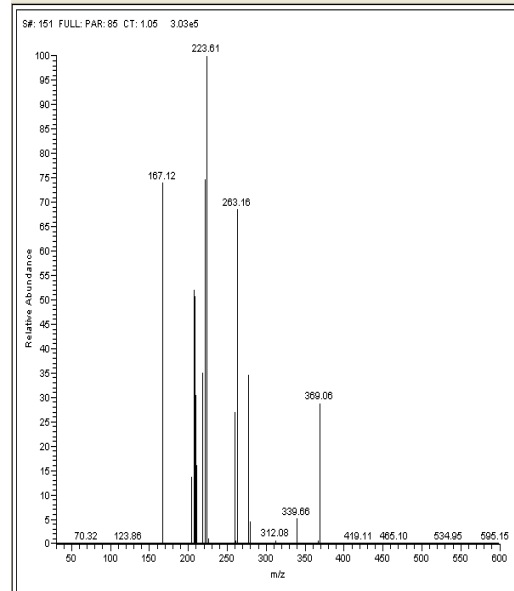
Pump 1 Pump 2

Take pump under control

Flow: 100.0 100.0 µl/min Time: 0.20 min (00:00:12) Inlet A: 25.00 25.00 % Inlet B: 0.00 0.00 % Inlet C: 75.00 75.00 % Inlet D: 0.00 0.00 % Pressure: 0.1 bar

Pump ready  Release injection

Apply Abort



Thermo TSQ Tune Master - Instrument Method Development Workspace - ESI - acylcn\_MP - Current Calibration

File Workspace View Control Scan Parameters Display Setup Help

Scan Type: Full Scan SIM SRM

Scan Mode: MS Q1MS Q3MS MS/MS Parent Product Neutral Loss

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Thermo Pal Surveyor MS Pump

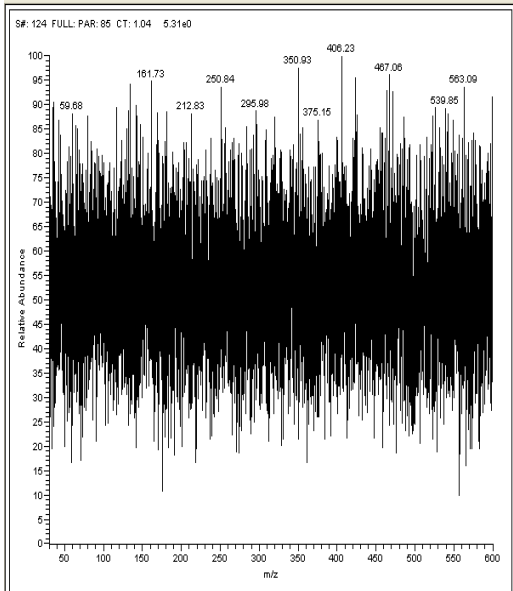
Pump 1 Pump 2

Take pump under control

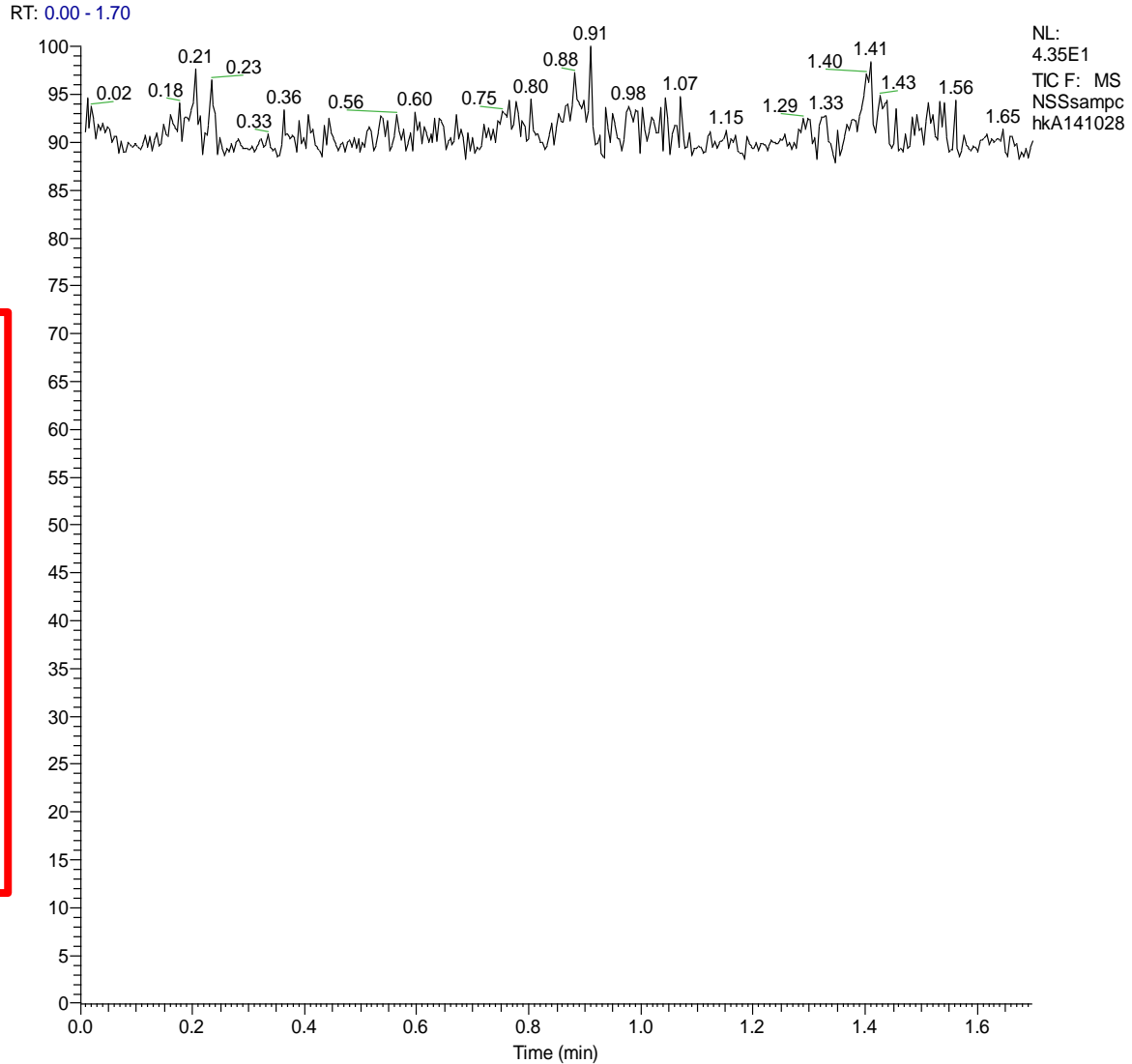
Flow: 100.0 0.0 µl/min Time: 0.00 min (00:00:00) Inlet A: 25.00 25.00 % Inlet B: 0.00 0.00 % Inlet C: 75.00 75.00 % Inlet D: 0.00 0.00 % Pressure: 0.0 bar

Pump ready  Release injection

Apply Abort



# Example 2



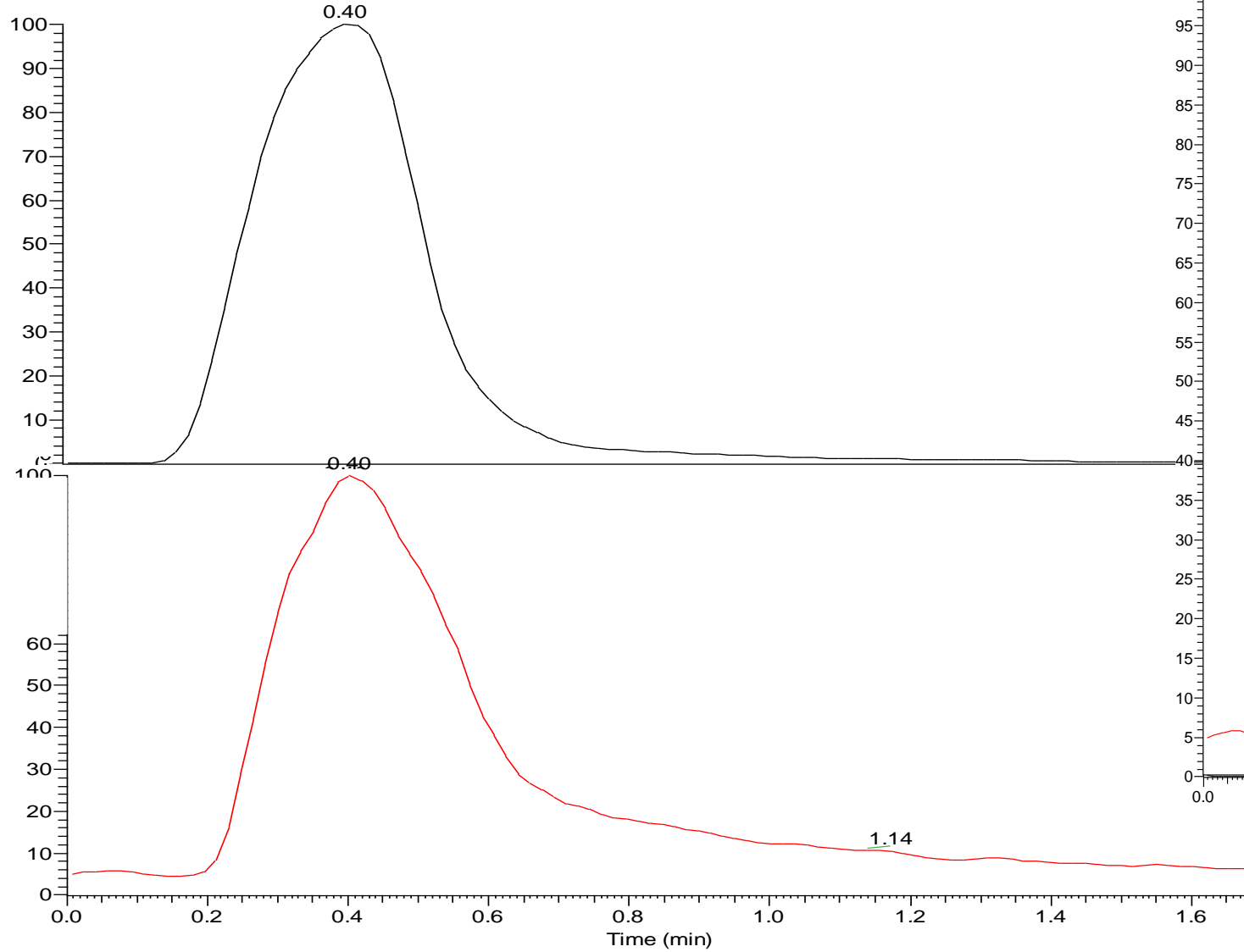
**Power supply 3  
assembly failure**

**Call an engineer**

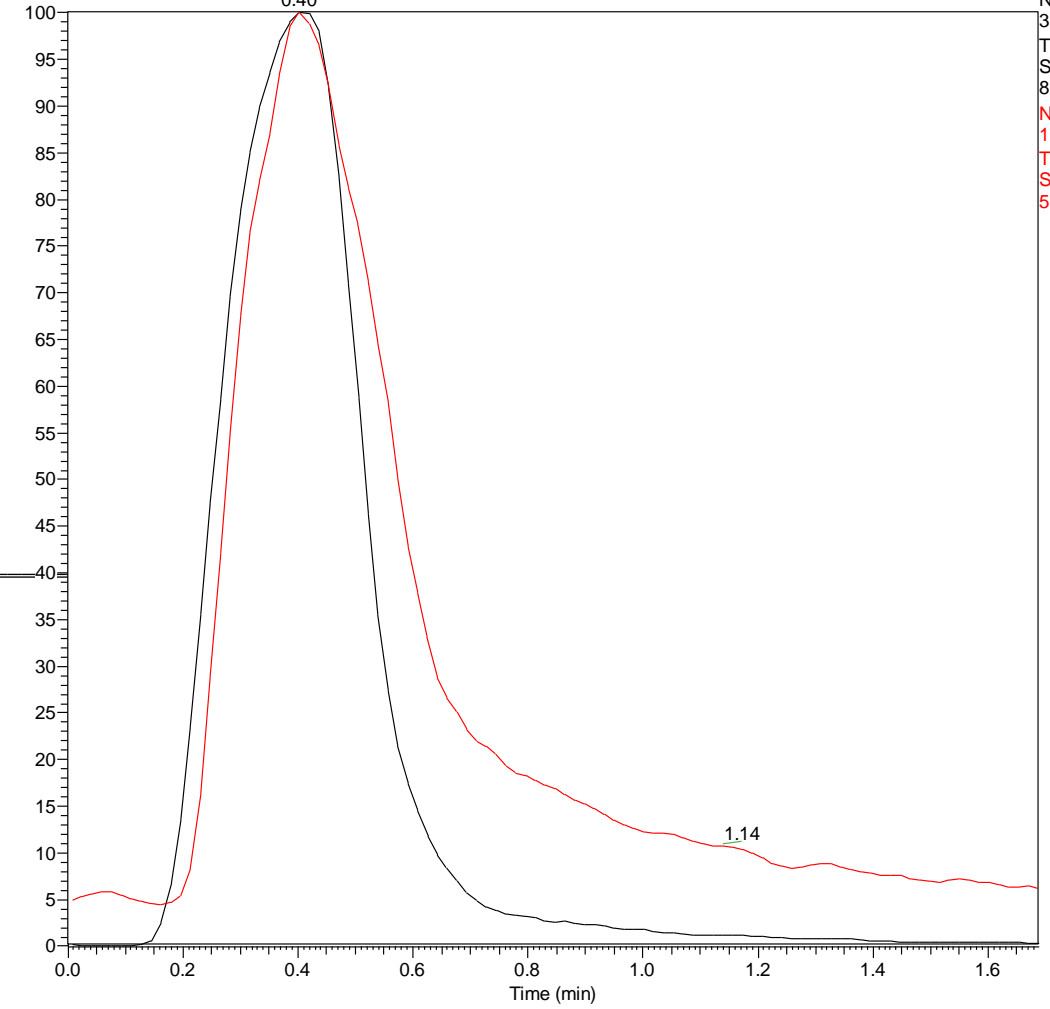


# Example 3

RT: 0.00 - 1.69 SM: 5B



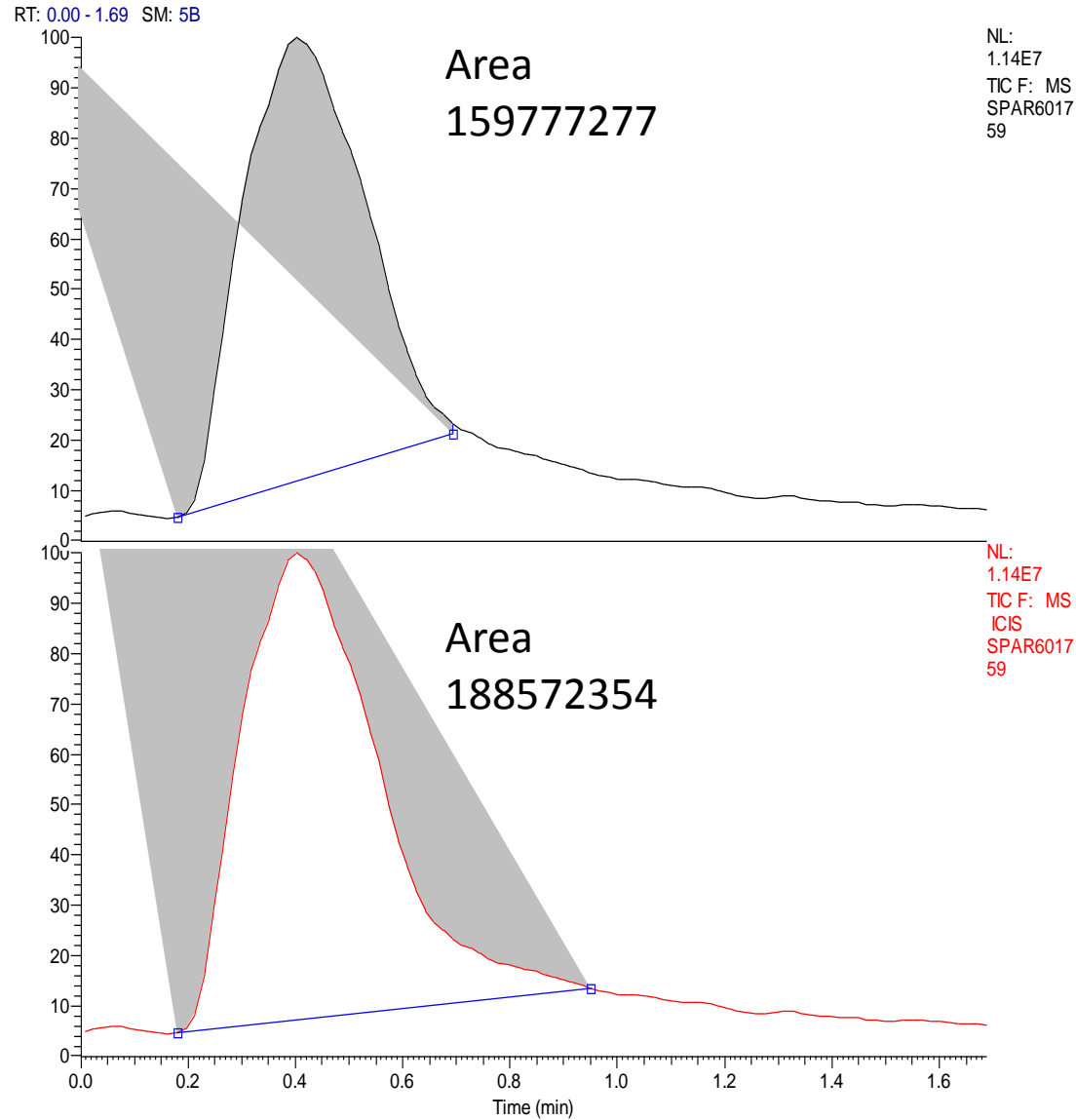
RT: 0.00 - 1.69 SM: 5B



NL: 3.45E7  
TIC F: MS SPA14010 8RC1  
NL: 1.14E7  
TIC F: MS SPAR6017 59

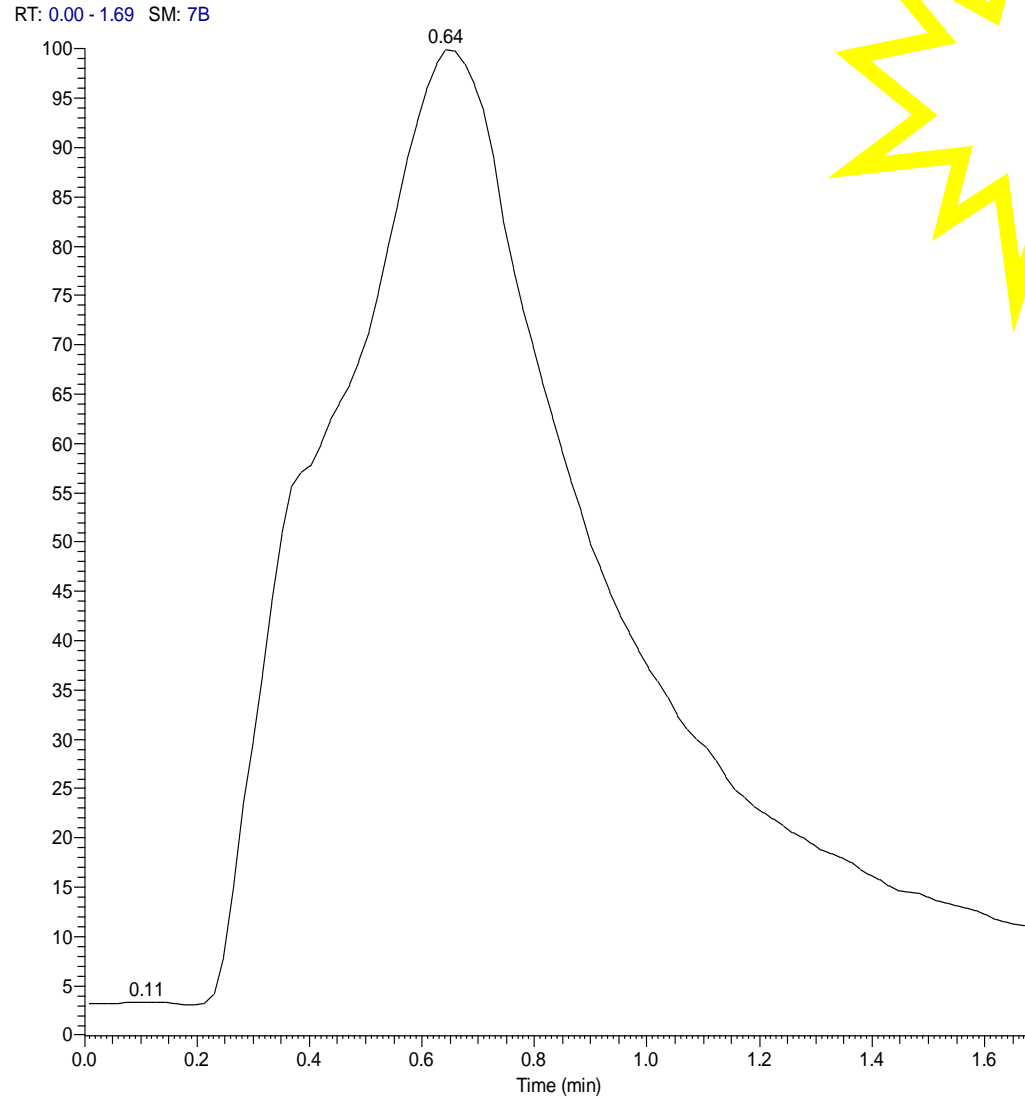
# Example 3

**Incorrect seating  
of ferrule  
causing dead  
volume**



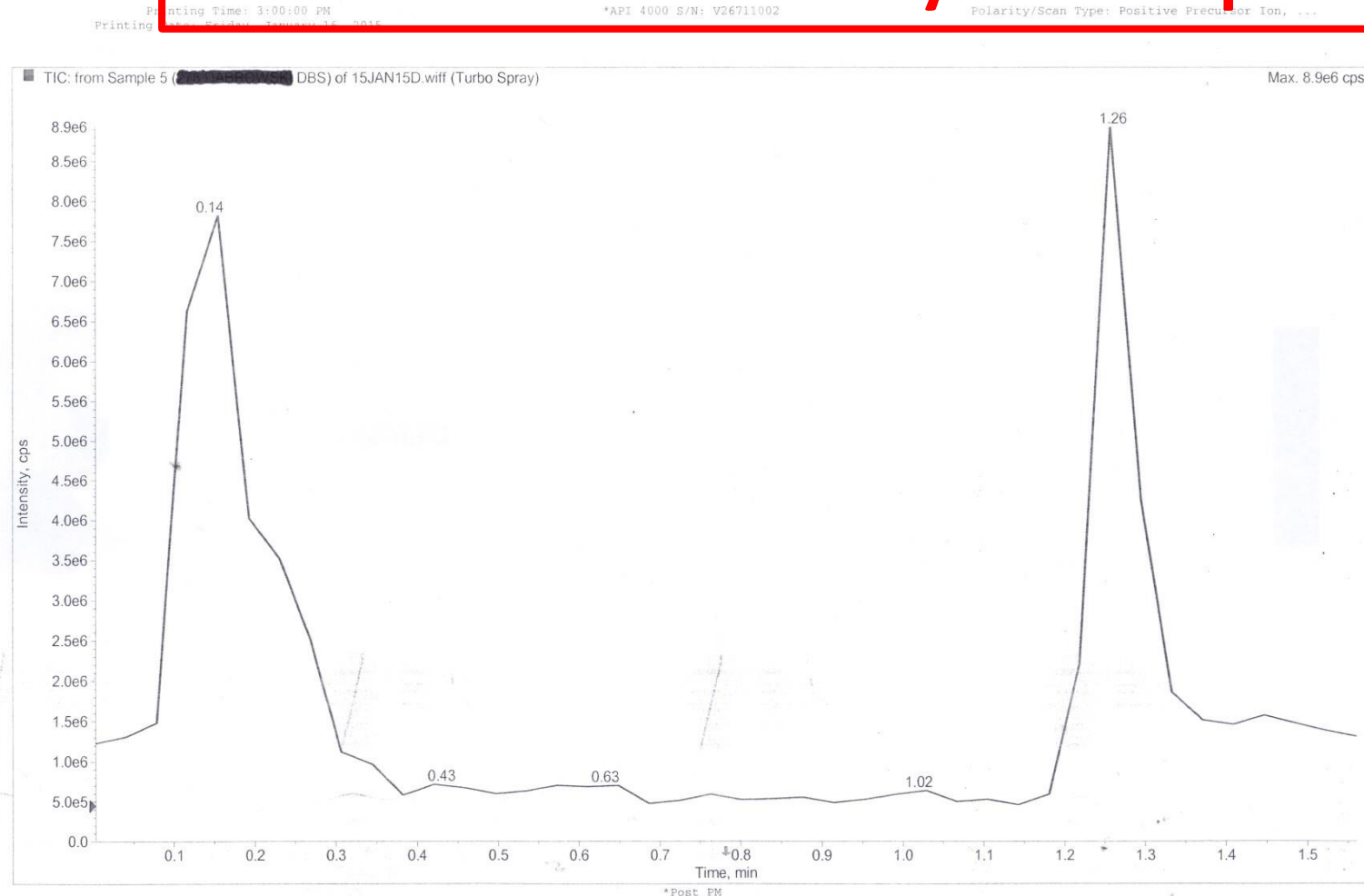
# Example 4

**Sample  
overload**

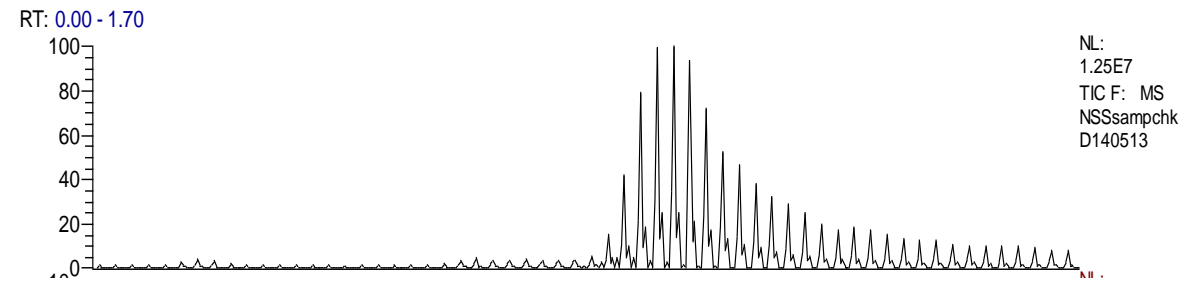


# Example 5

**Improper nebulization due to ESI capillary misaligned following maintenance.  
Did not extend beyond the probe tip**

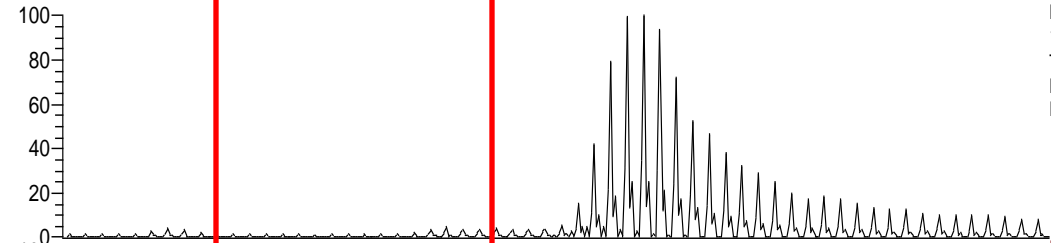


# Example 6

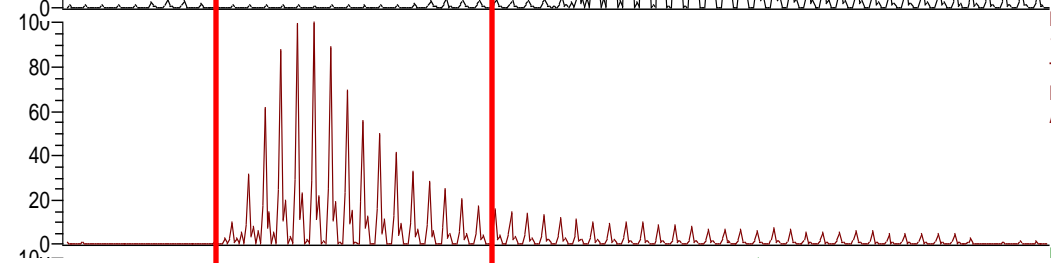


# Example 6

RT: 0.00 - 1.70



NL:  
1.25E7  
TIC F: MS  
NSSsampchk  
D140513

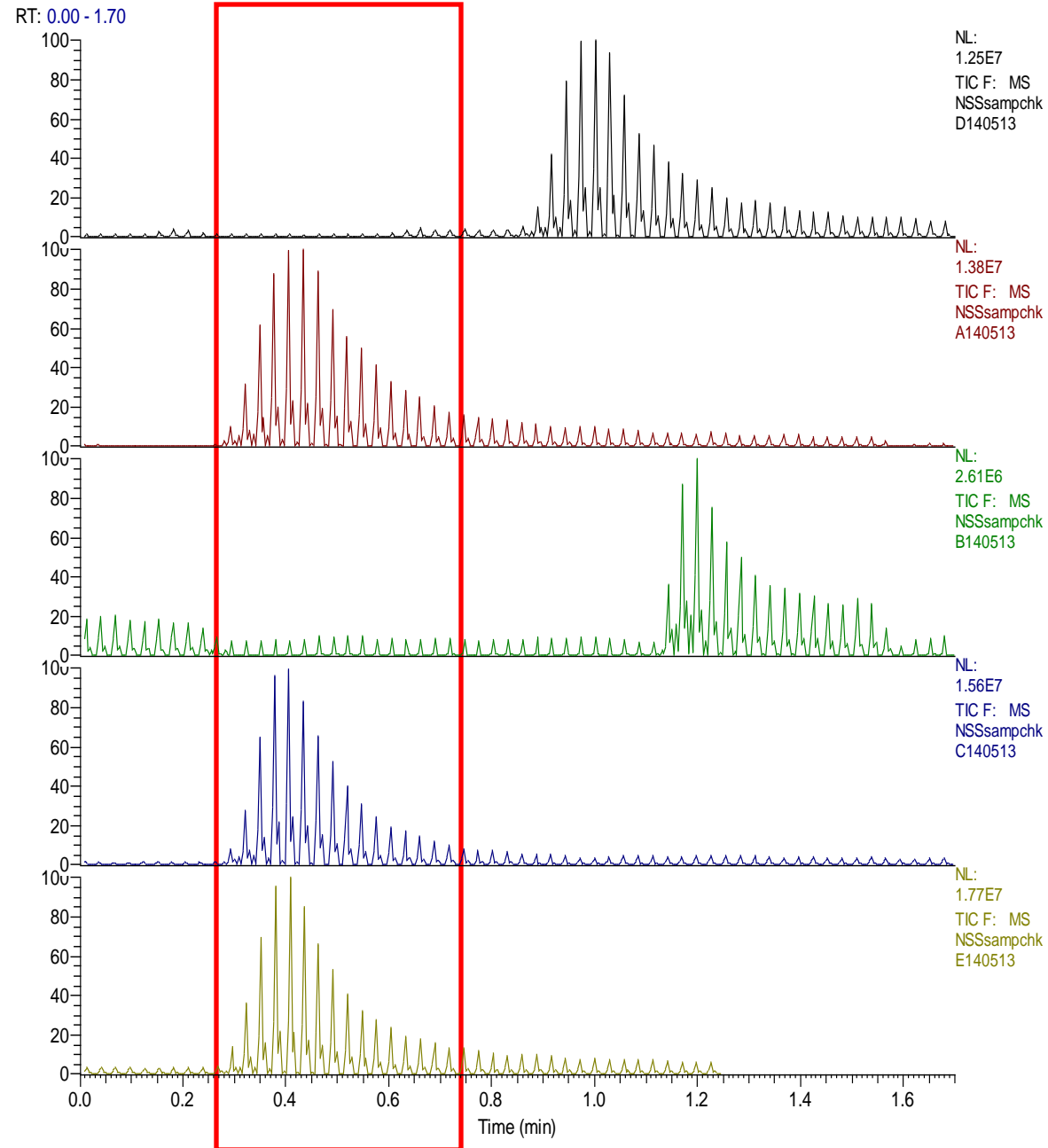


NL:  
1.38E7  
TIC F: MS  
NSSsampchk  
A140513

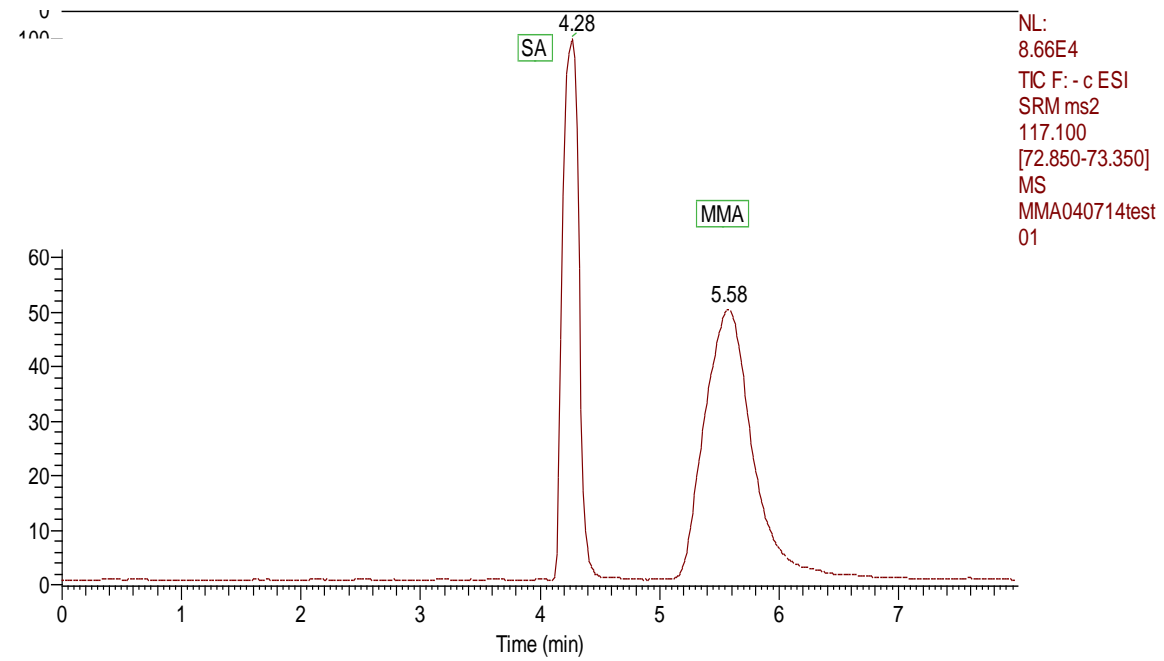
NL

# Example 6

**Pump running  
at low flow  
rate 50ul/min.  
Fix = Back  
pressure/retention  
loop (2m length  
peek) added**



# Example 7

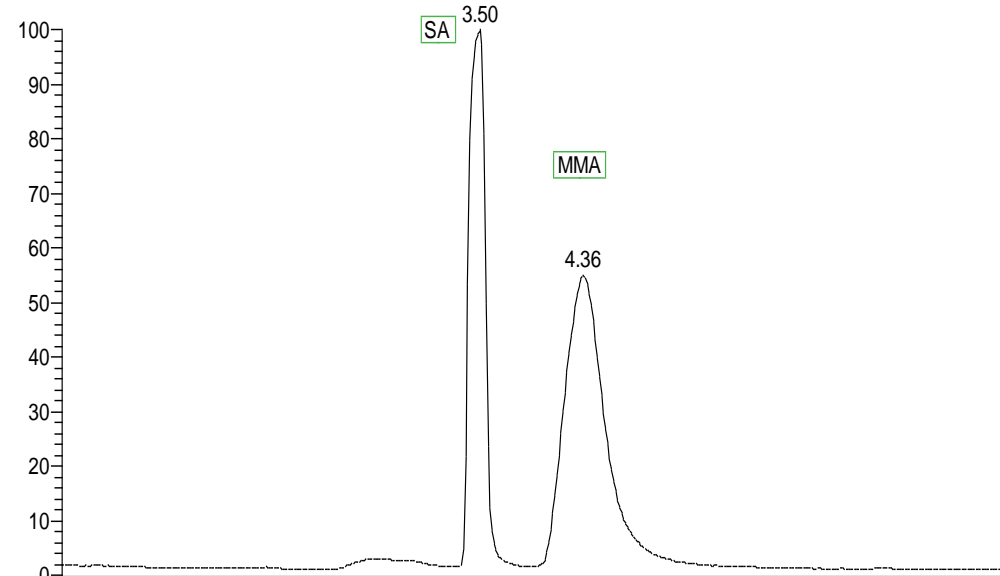




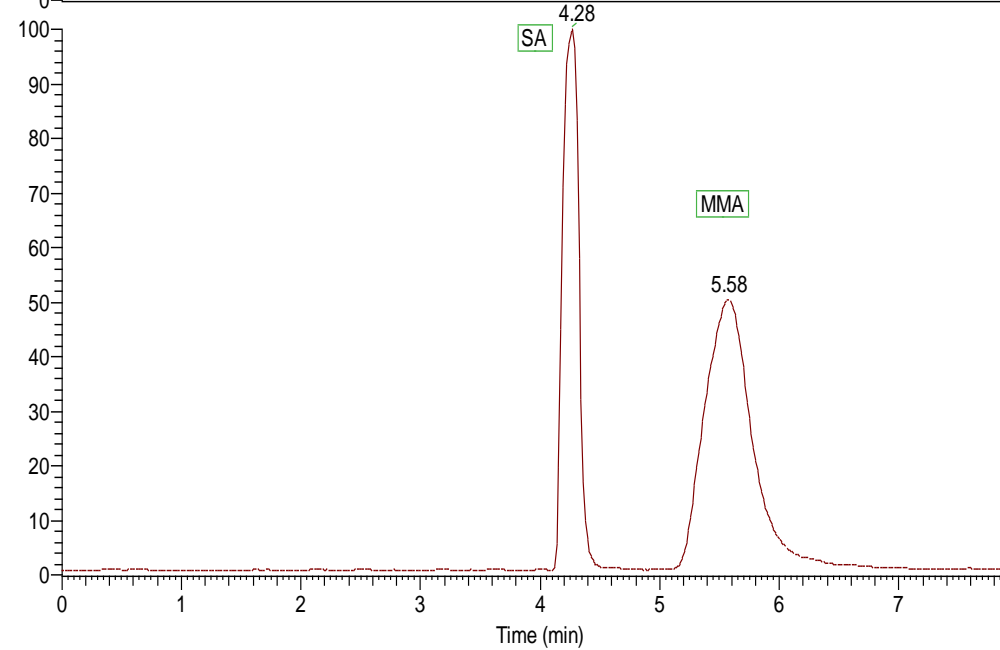
# Example 7

**Mobile phase composition**

RT: 0.00 - 8.00 SM: 9B



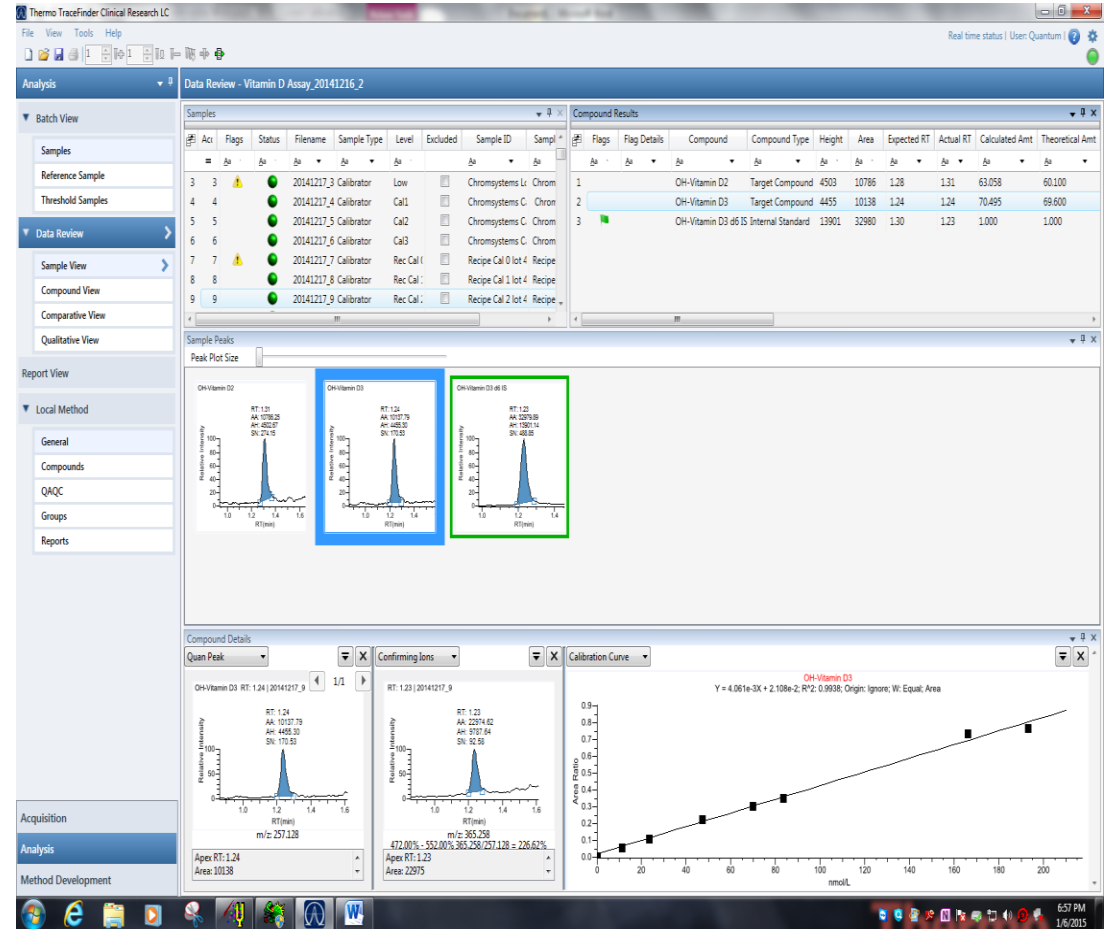
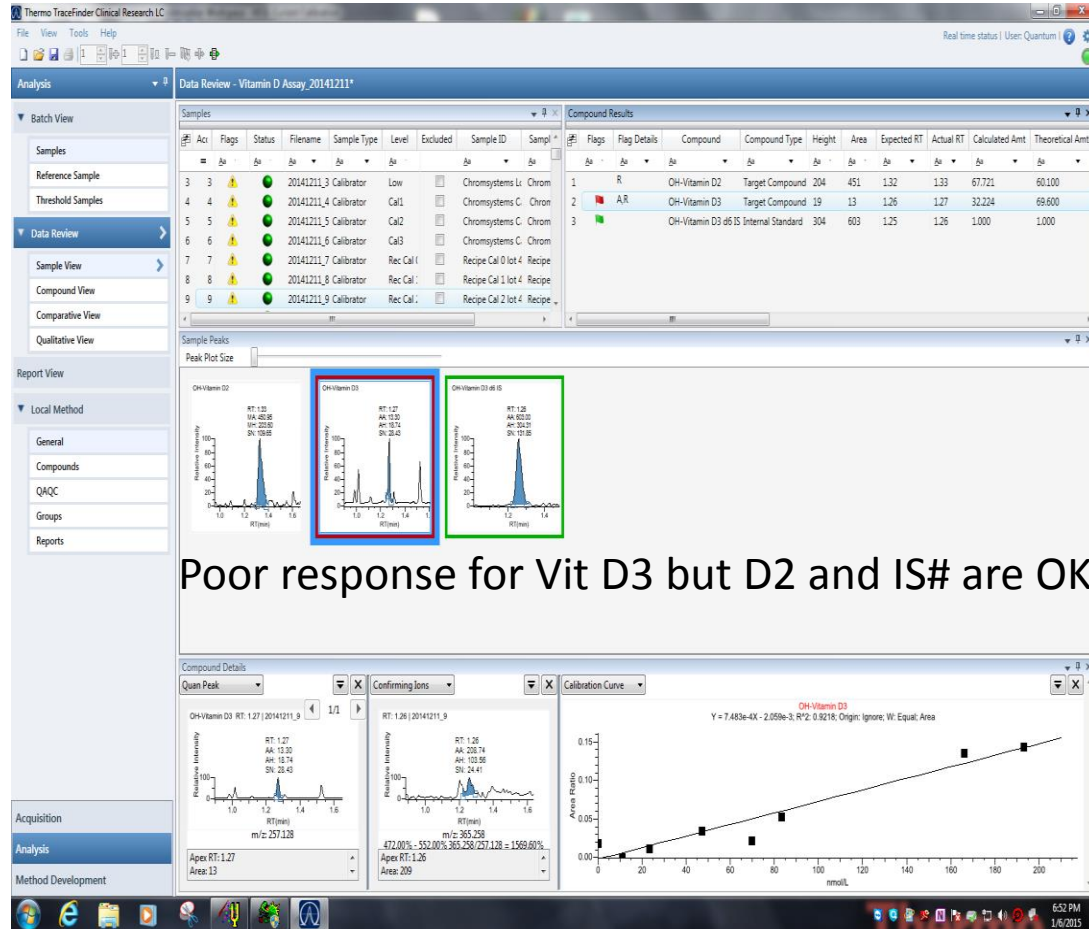
NL:  
1.09E5  
TIC F: - c ESI  
SRM ms2  
117.100  
[72.850-73.350]  
MS  
MMA040714test  
22



NL:  
8.66E4  
TIC F: - c ESI  
SRM ms2  
117.100  
[72.850-73.350]  
MS  
MMA040714test  
01

# Example 8

**Fresh mobile phase....Formic acid in methanol should be SHAKEN not STIRRED!**



Courtesy of Liverpool

# Example 9

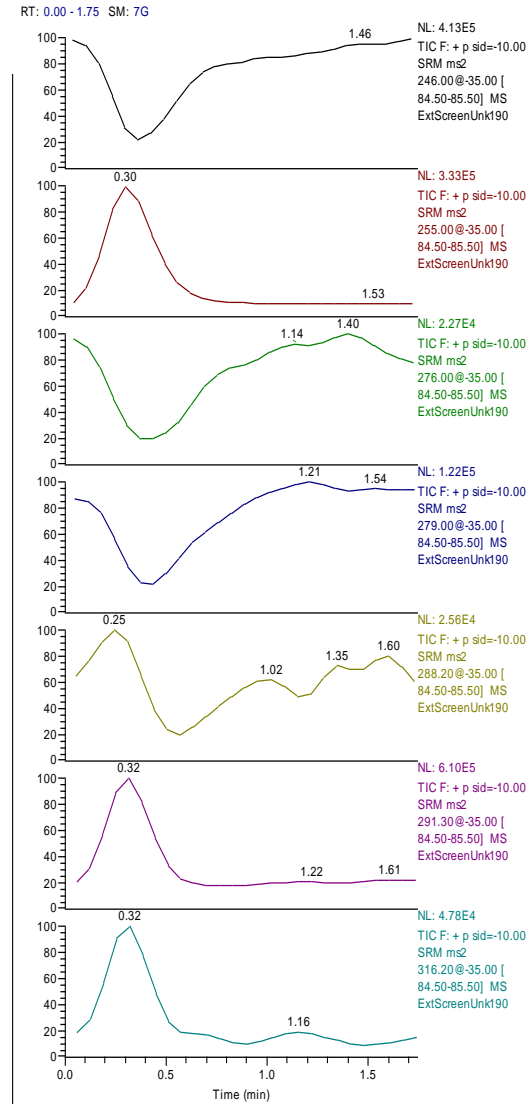
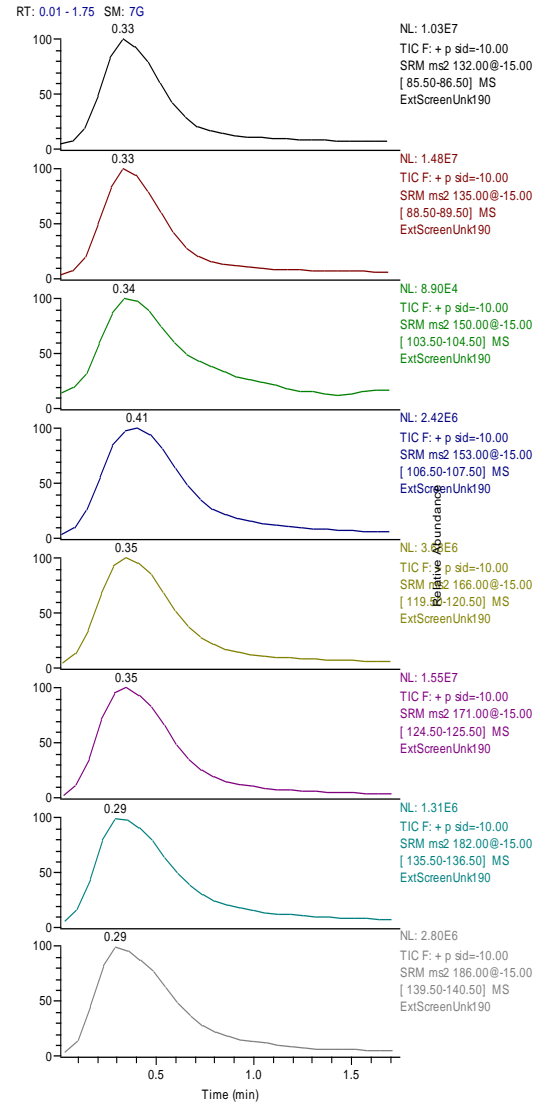
## Amino acids

## Acylcarnitines

C:\Xcalibur...\Sept\08\ExtScreenUnk190

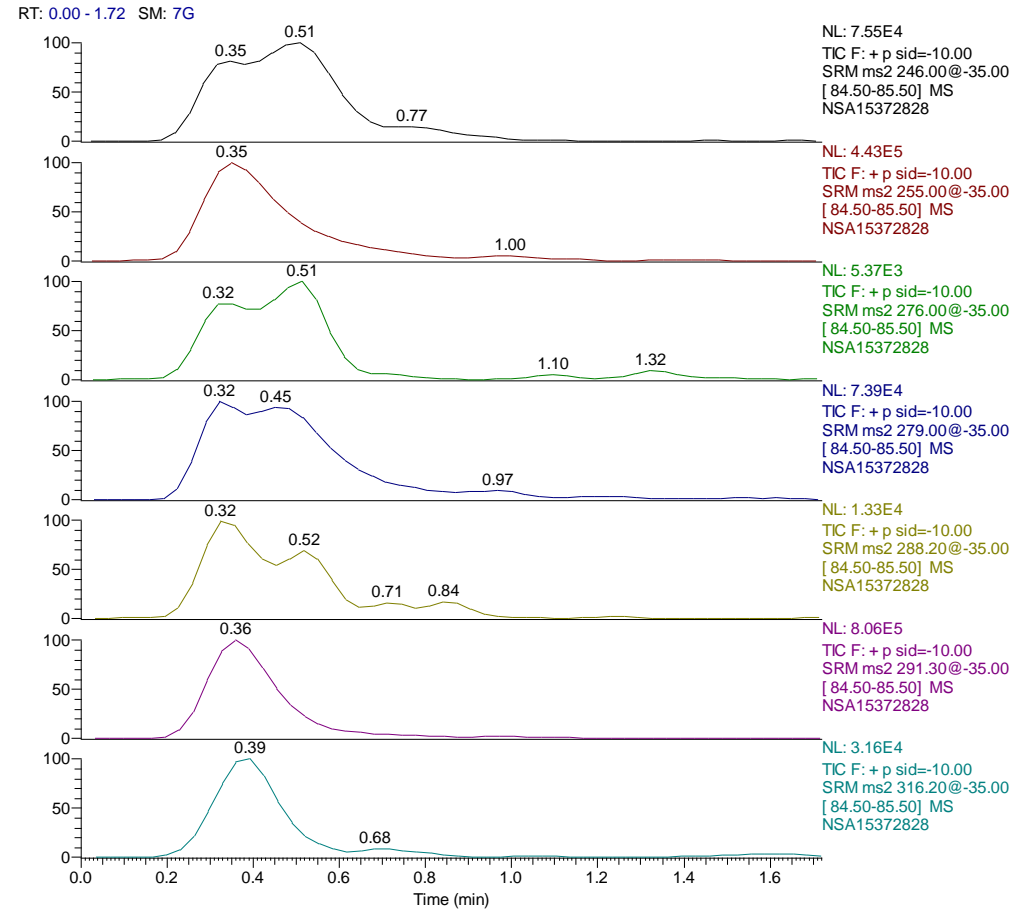
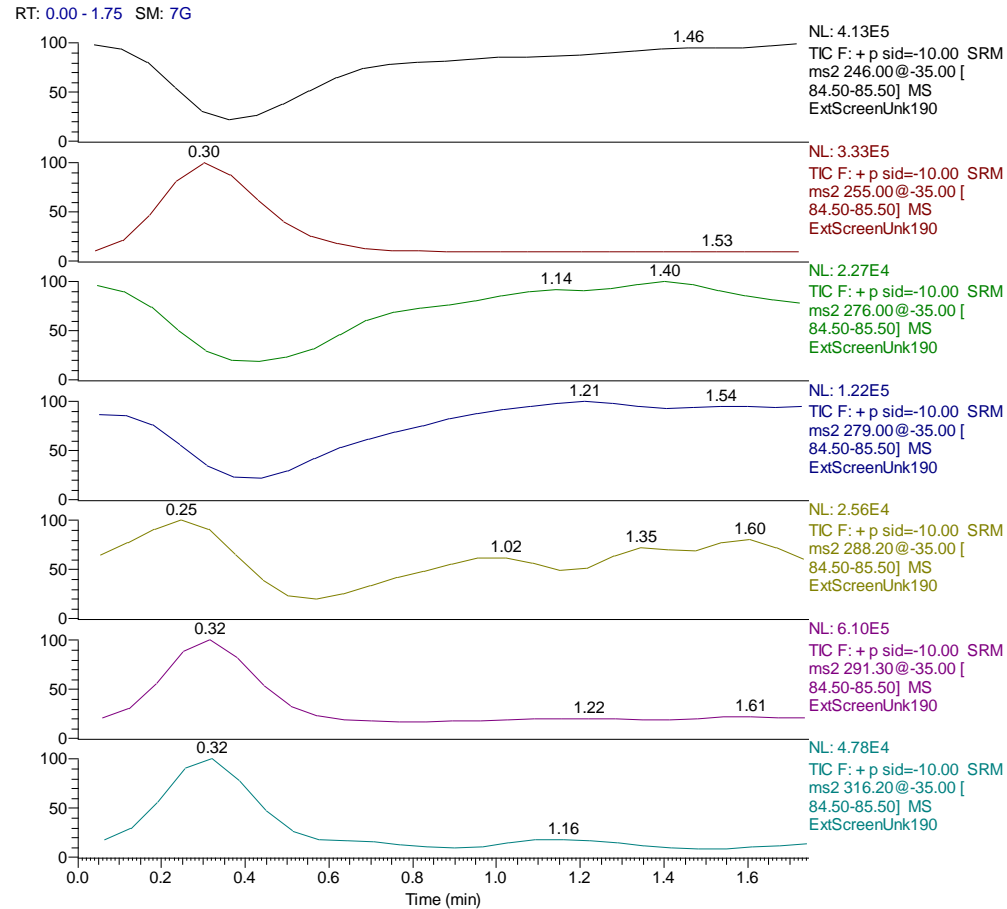
09/09/21

Unk190



# Example 9

## Formic acid in sample NOT mobile phase



# Example 10

## Contamination of reconstituting reagent (ACn) with Internal standard

AB SCIEX API 4000

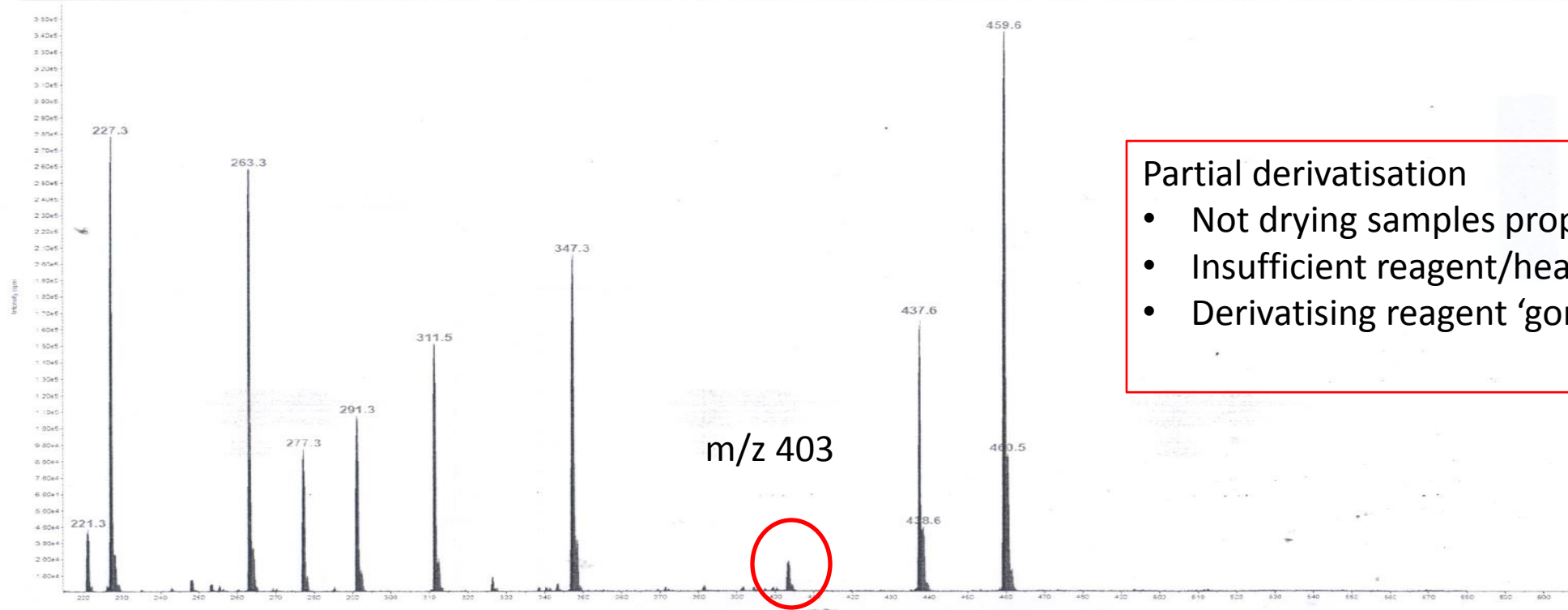
Sample: IS

Sample: IS

Sample

Data File	D:\Analyst Data\Projects\Acylcarnitines\Data\171214D.wiff		
Sample Number	1	Date	12/18/2014 8:47:04 AM
Sample Type	DBS	Vial	1
Processing Method	Acylcarnitines - Blood Spots	Plate	1

Birmingham Children's Hospital NHS Foundation Trust  
Created with Analyst Reporter 3.0  
Printed: 12/18/2014 8:48:04 AM



Partial derivatisation

- Not drying samples properly
- Insufficient reagent/heating
- Derivatising reagent 'gone off'

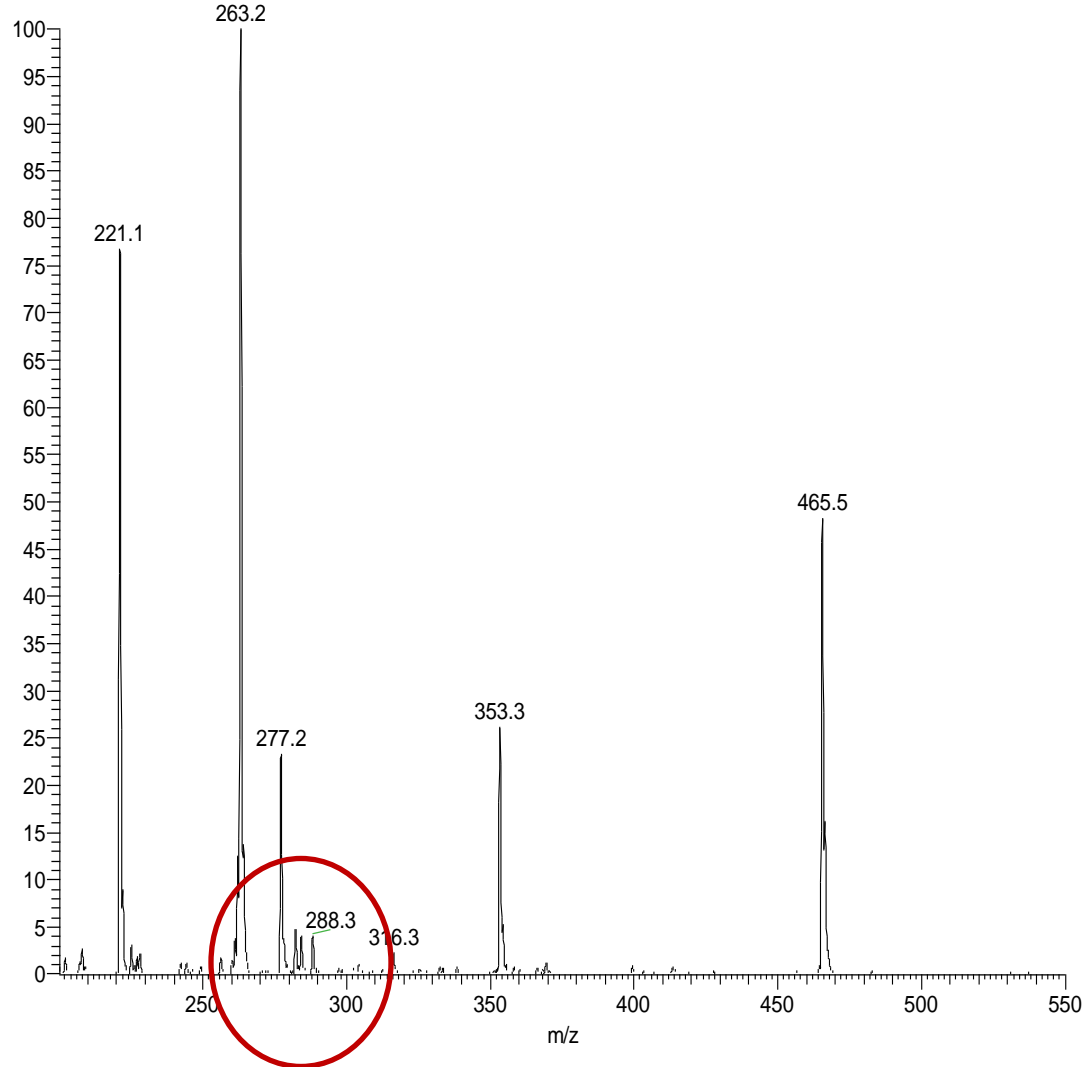
Courtesy of Birmingham

# Example 11

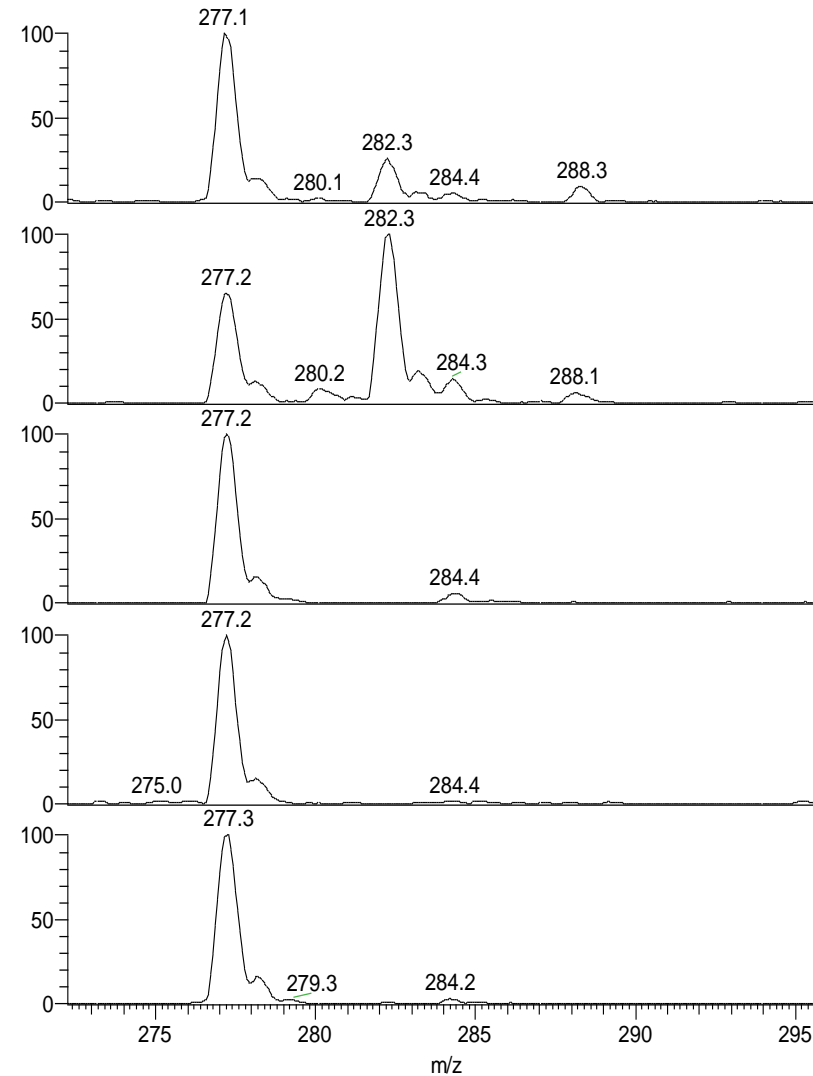
## Contamination from



20140721002 #6-17 RT: 0.26-0.68 AV: 6 SM: 7B NL: 2.89E6  
T: + p ESI Full pr 85.000 [200.000-550.000]



SM: 7B



NL: 5.46E5  
20140722023#6-17 RT:  
0.26-0.68 AV: 6 T: + p ESI  
Full pr 85.000  
[200.000-550.000]

NL: 7.20E5  
20140724002#6-17 RT:  
0.26-0.68 AV: 6 T: + p ESI  
Full pr 85.000  
[200.000-550.000]

NL: 7.25E5  
20140722027#6-17 RT:  
0.26-0.68 AV: 6 T: + p ESI  
Full pr 85.000  
[200.000-550.000]

NL: 5.75E5  
20140722029#6-17 RT:  
0.26-0.68 AV: 6 T: + p ESI  
Full pr 85.000  
[200.000-550.000]

NL: 8.44E5  
20140722025#6-17 RT:  
0.26-0.68 AV: 6 T: + p ESI  
Full pr 85.000  
[200.000-550.000]

# Conclusion

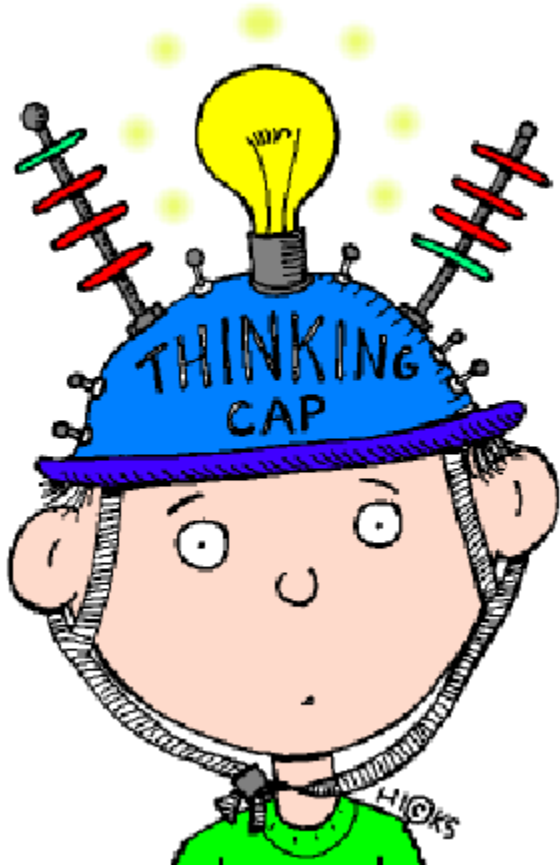
- MSMS are robust analytical instruments
- Sensitive to small changes
- Contamination will be detected – Good Lab Practice performed
- Common problems with leaks and blocks in peek tubing
- Take care with connection during maintenance

# Conclusion

- Contamination – sample preparation, dirty source
- Peak shifts and shape – blocks or leaks, mobile phase
- Low intensity- sample injection, clogged transfer capillary
- No peaks – acquisition parameters, sample prep/position



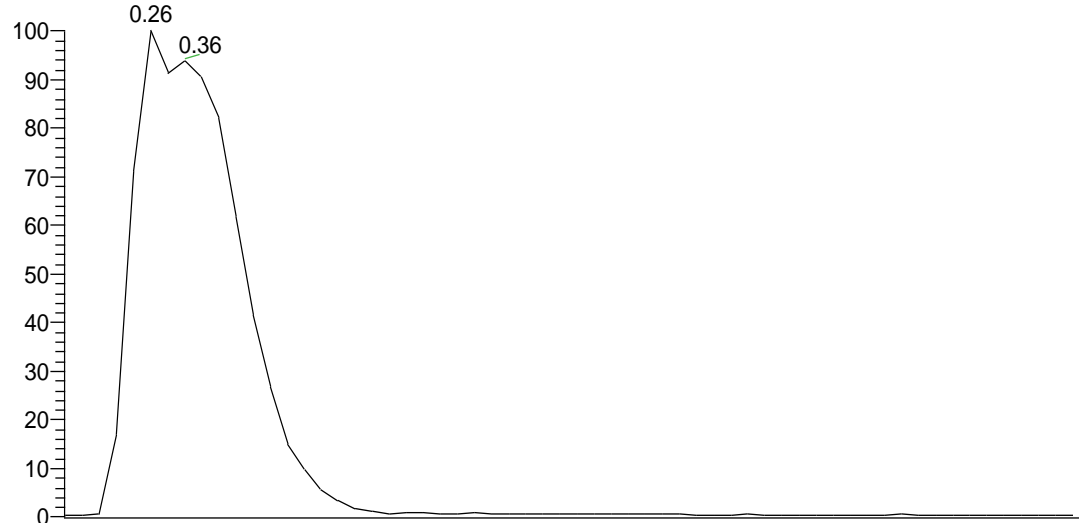
# Troubleshooting Strategy



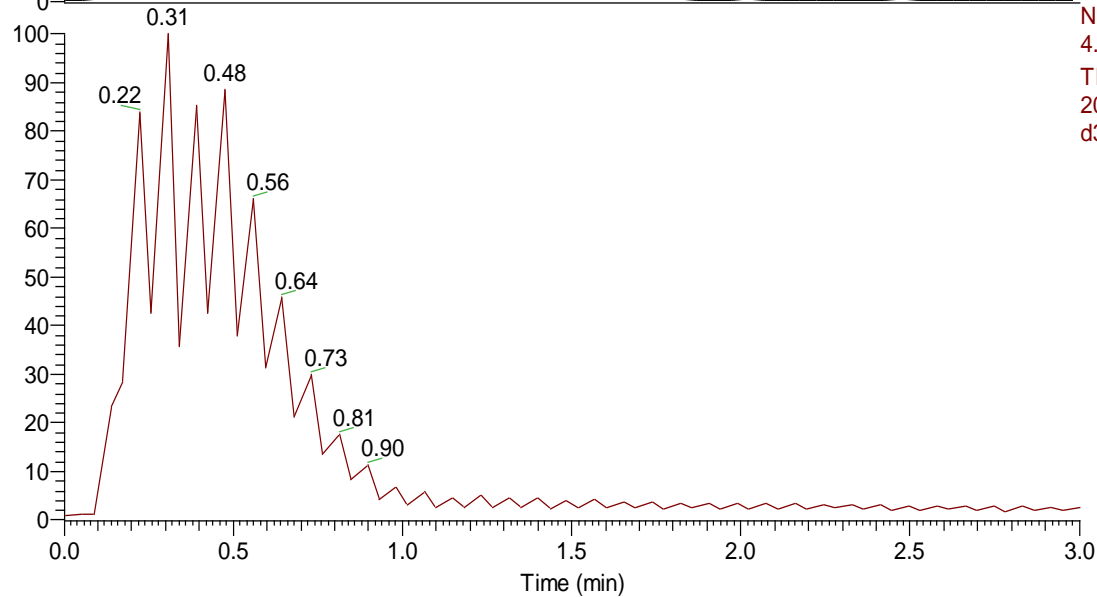
- Start from easiest to fix first
- Eliminate the obvious
- Examine your chromatographic profile

# Troubleshooting Strategy

RT: 0.00 - 3.00



NL:  
6.98E8  
TIC MS  
20150408st  
d1



NL:  
4.20E8  
TIC MS  
20150408st  
d3

- Familiarise yourself with YOUR TICs – tells you alot!
- If it doesn't look right.....Do something about it!



*"Okay—who put my lunch through the mass spectrometer..?"*