Mass Spectrometry in MCAL

- Two systems: GC-MS, LC-MS
  - GC seperates small, volatile, non-polar material
  - MS is detection devise (Agilent 320-MS TQ Mass Spectrometer)
    - Full scan monitoring
    - SIM single ion monitoring
    - MSMS monitoring
- Sample can be injected as a liquid, a gas or even a solid

Different Types of MS

- GC-MS - Gas Chromatography MS
  - separates volatile compounds in gas column and ID's by mass
- LC-MS - Liquid Chromatography MS
  - separates delicate compounds in HPLC column and ID's by mass
- MS-MS - Tandem Mass Spectrometry
  - separates compound fragments

How Does MS Measure Mass

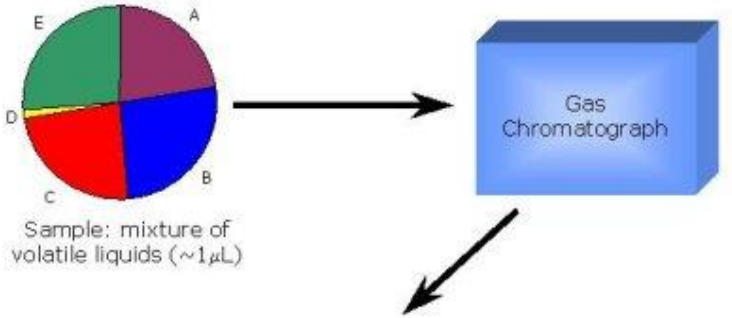
- Deflection of ions in electric or magnetic field which is related to mass of ion.

- Ions need to be charged. Can be either positive or negative.

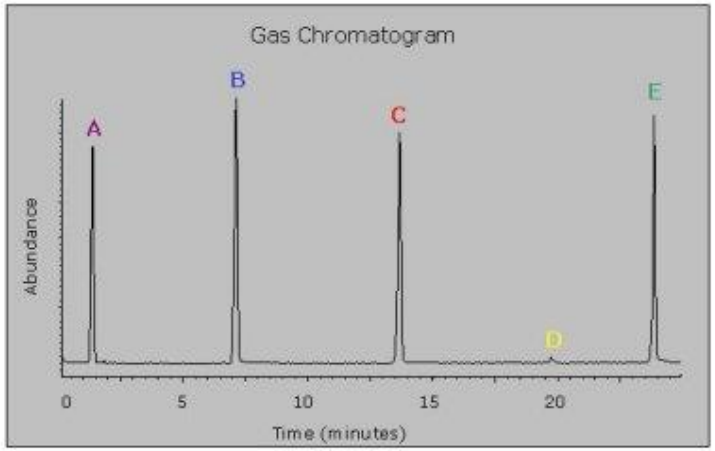
GC-MS

GC-MS

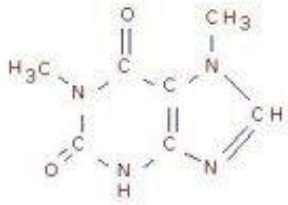
Gas Chromatography



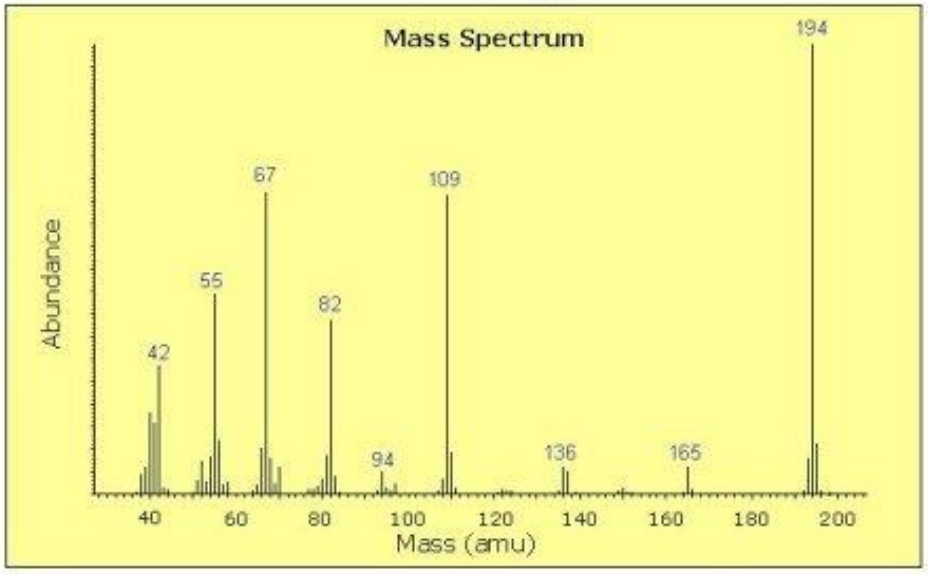
Sample: mixture of volatile liquids (~1μL)



Mass Spectrometry



Typical sample: isolated compound (~1 nanogram)



EI Fragmentation of CH_3OH

$$CH_3OH \longrightarrow CH_3OH^+$$

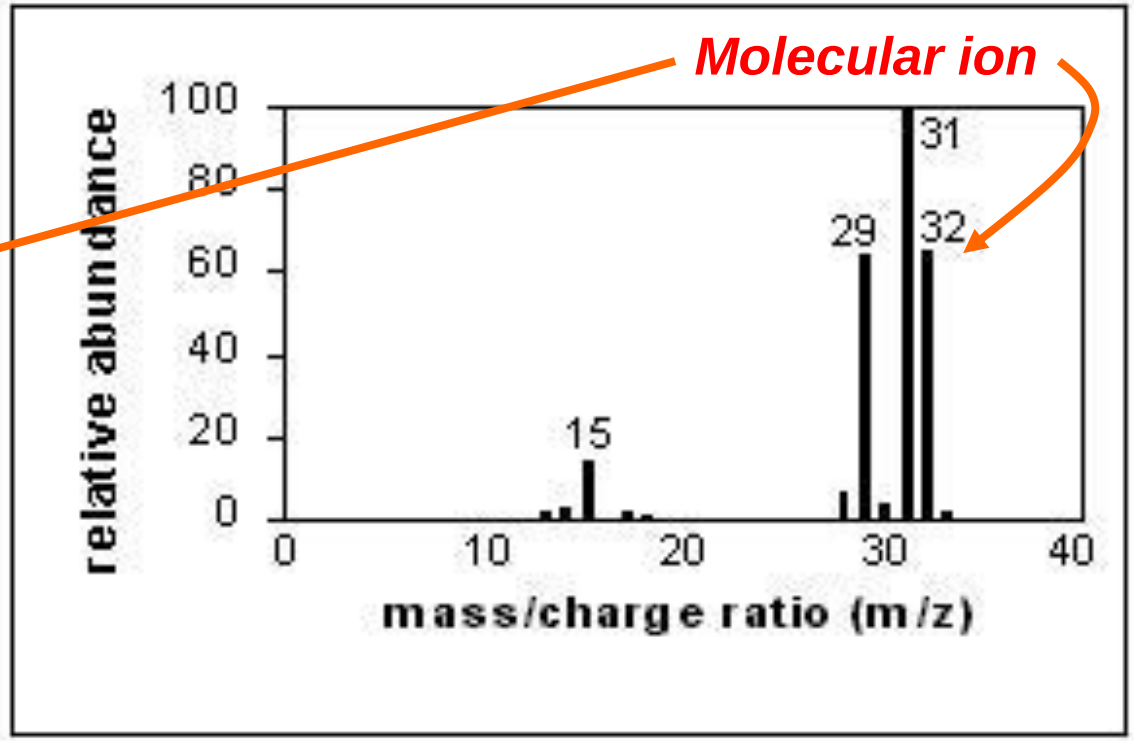
$$CH_3OH \longrightarrow CH_2O{=}H^+ + H$$

$$CH_3OH \longrightarrow {}^+CH_3 + OH$$

$$CH_2O{=}H^+ \longrightarrow CHO{\equiv}H^+ + H$$

Electron Impact MS of CH_3OH

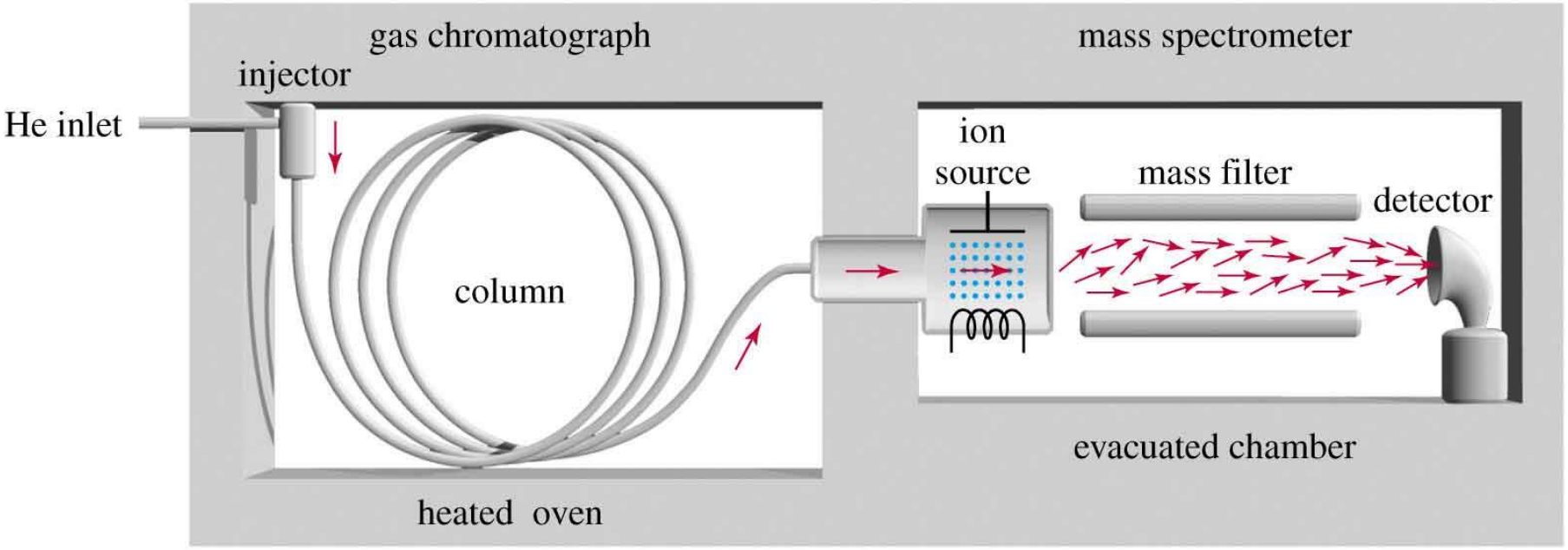
ions	m/z
$CH_3OH^{+\bullet}$	32
$H_2C{=}OH^{+}$	31
$HC{\equiv}O^{+}$	29
H_3C^{+}	15



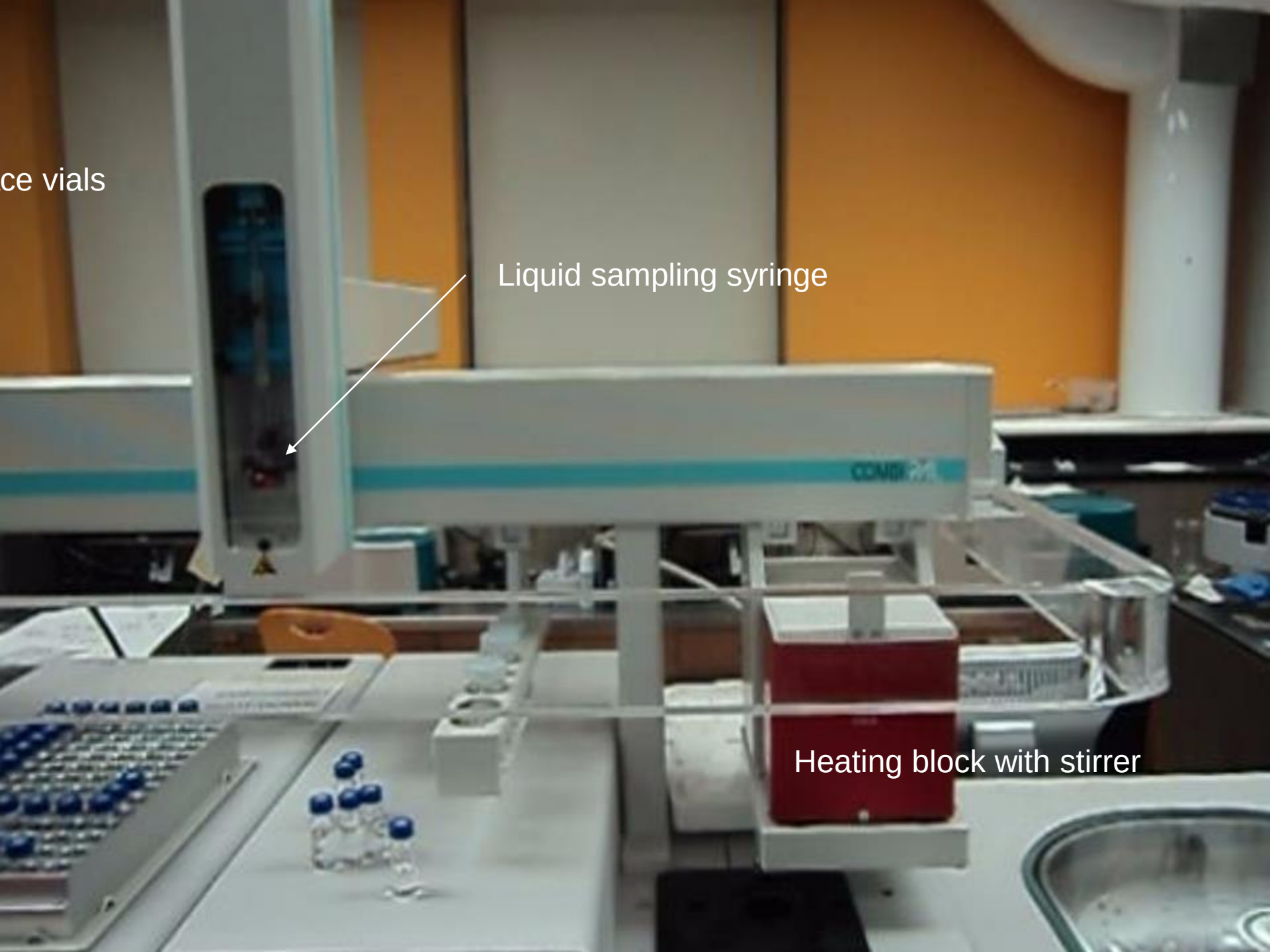
EI Breaks up Molecules in Predictable Ways

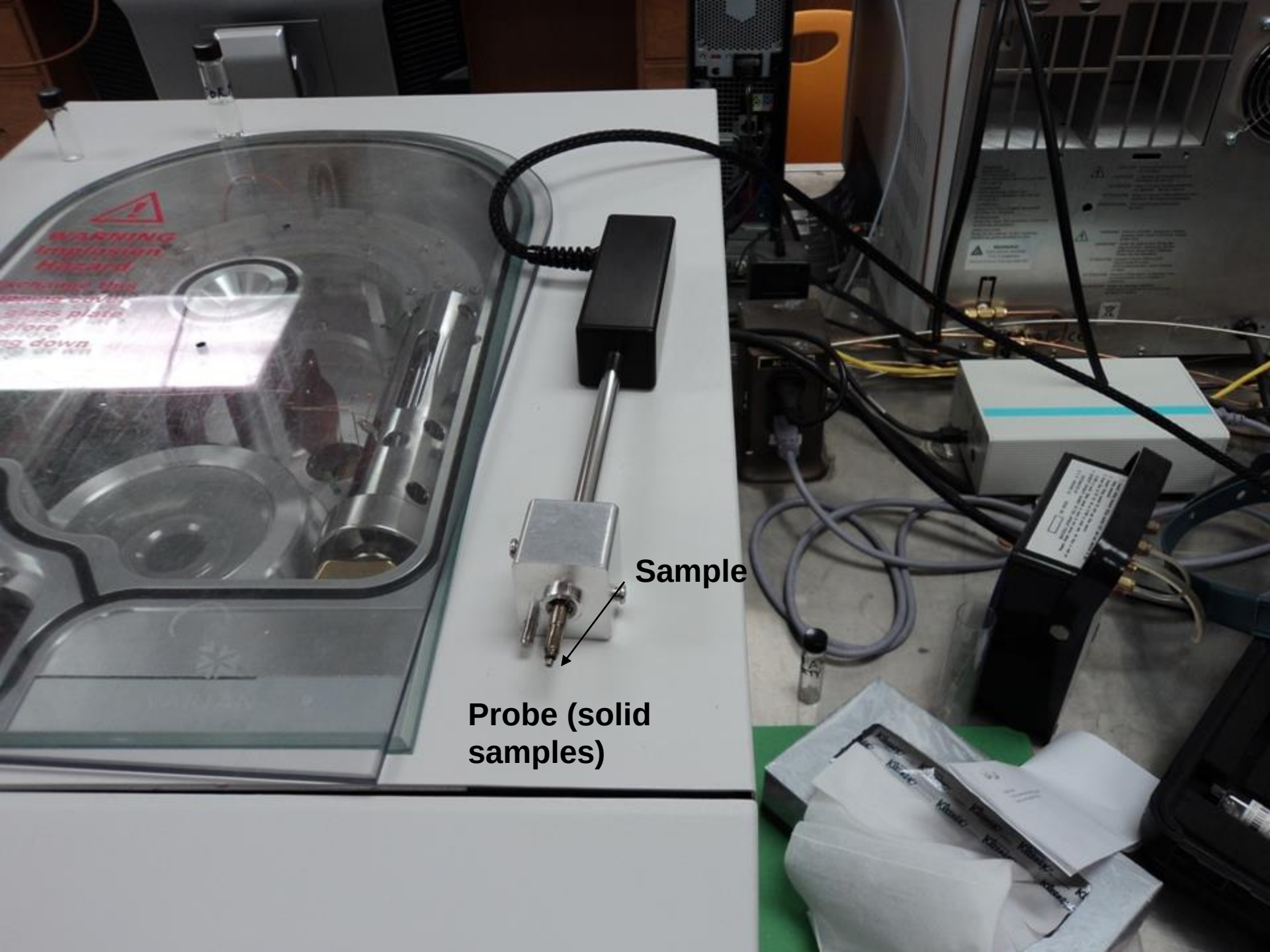
The GC-MS

A mixture of compounds is separated by gas chromatography, then identified by mass spectrometry.



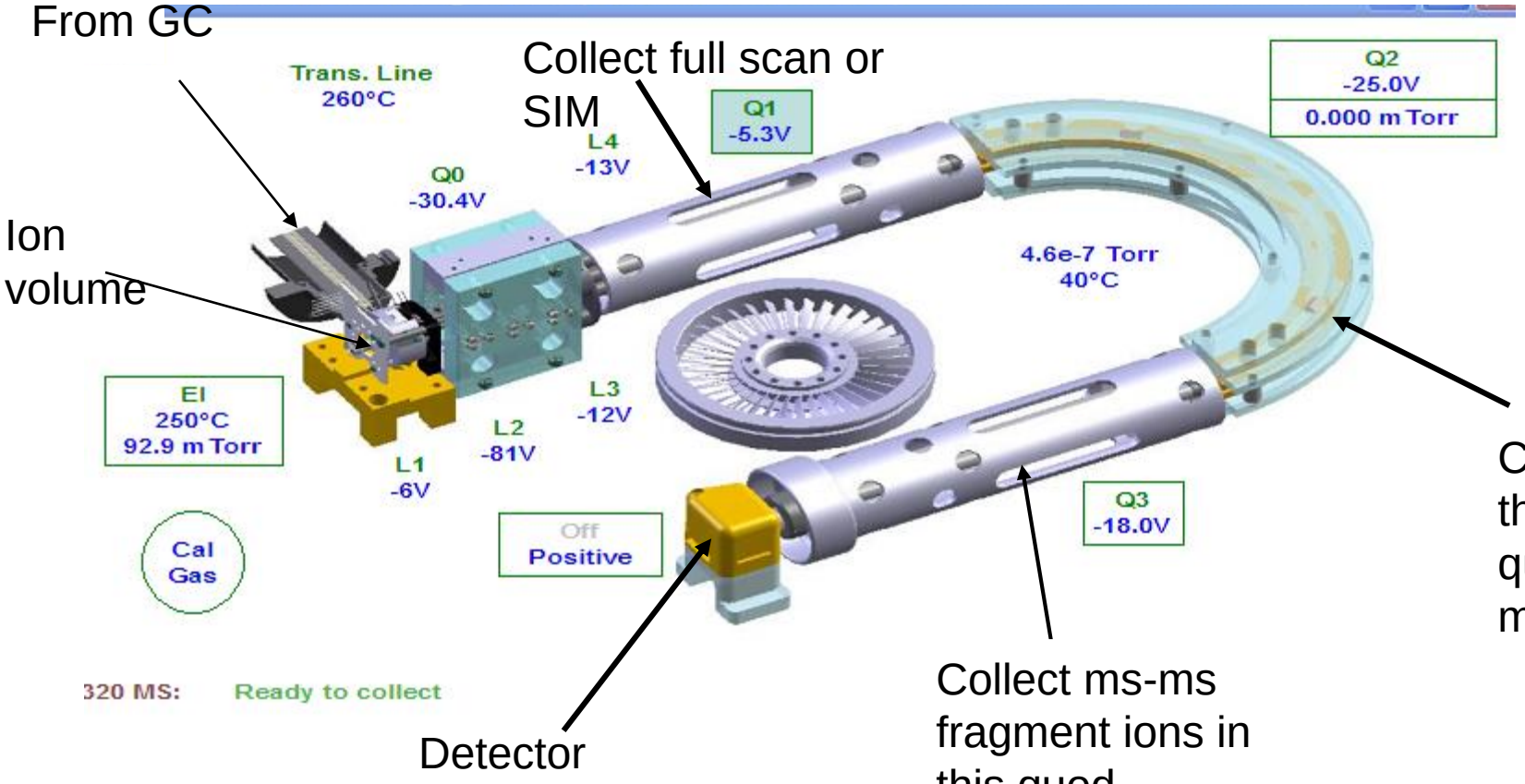
=>


ce vials
Liquid sampling syringe
Heating block with stirrer


Sample
Probe (solid samples)


Source On
Scanning
Power
Probe

Triple Quodrapole



Mass Spectrometer (Q3)

GC-MS

- The MS is equipped with a triple quadrupole analyzer and allows several types of mass detection to be performed.

- Full scan (direct MS) mode is used.
- Sim can be used
- MS-MS can be used

Key Components of MS

- Ionization – gas phase ions created in source
- High vacum – creates free path
- Mass Analyser- sorts ions by M/Z ratio
  - electric field
- Detector – creates signal multiplication

GC Settings

Column Oven Coolant: ○ On ◉ Off

Enable Coolant at (C): 50

Coolant Timeout (min): 20.00

Stabilization Time (min): 0.10

	Temp (C)	Rate (C/min)	Hold (min)	Total (min)
1	75		5.00	5.00
2	265	10.0	40.00	64.00
3				
4				
5				
6				
7				
8				

Add

Insert

Delete

Varian (Agilent) FactorFour Capillary GC Columns.

- Capillary column: thin fused-silica capillary.
- 50 m in length and 250 µm inner diameter.
- The stationary phase is CP-sil 8, with 5% phenyl 95% dimethyl polysiloxane coated on the inner surface.

Electron Ionization

- It uses a heated filament to produce electrons. The filament is usually made of rhenium or tungsten.

- Once the electrons are produced they are accelerated through a potential difference of around 70V this gives electrons with 70eV of energy.

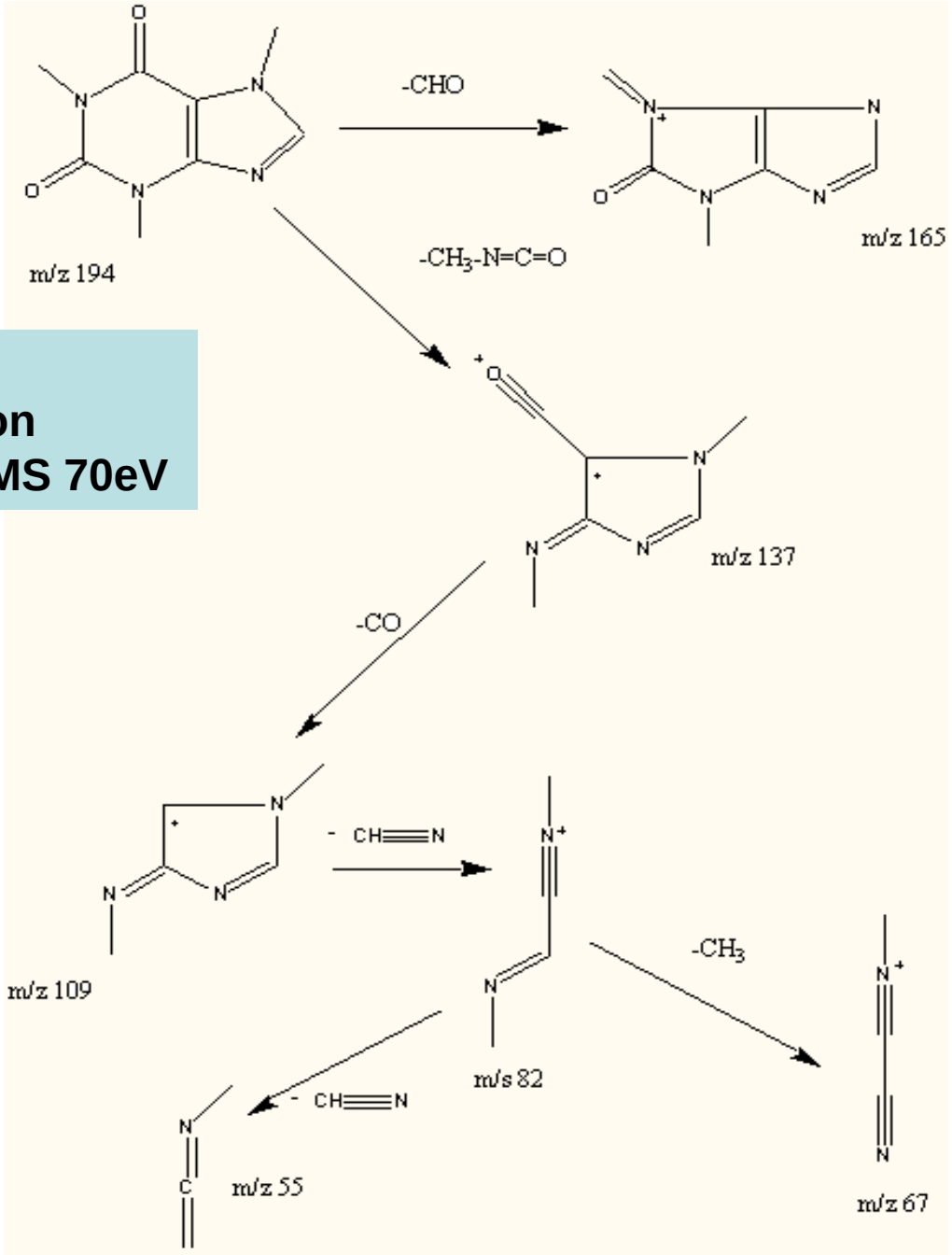
Electron Impact

- Electrons produced by the source will then collide with the sample and remove an electron to give ions

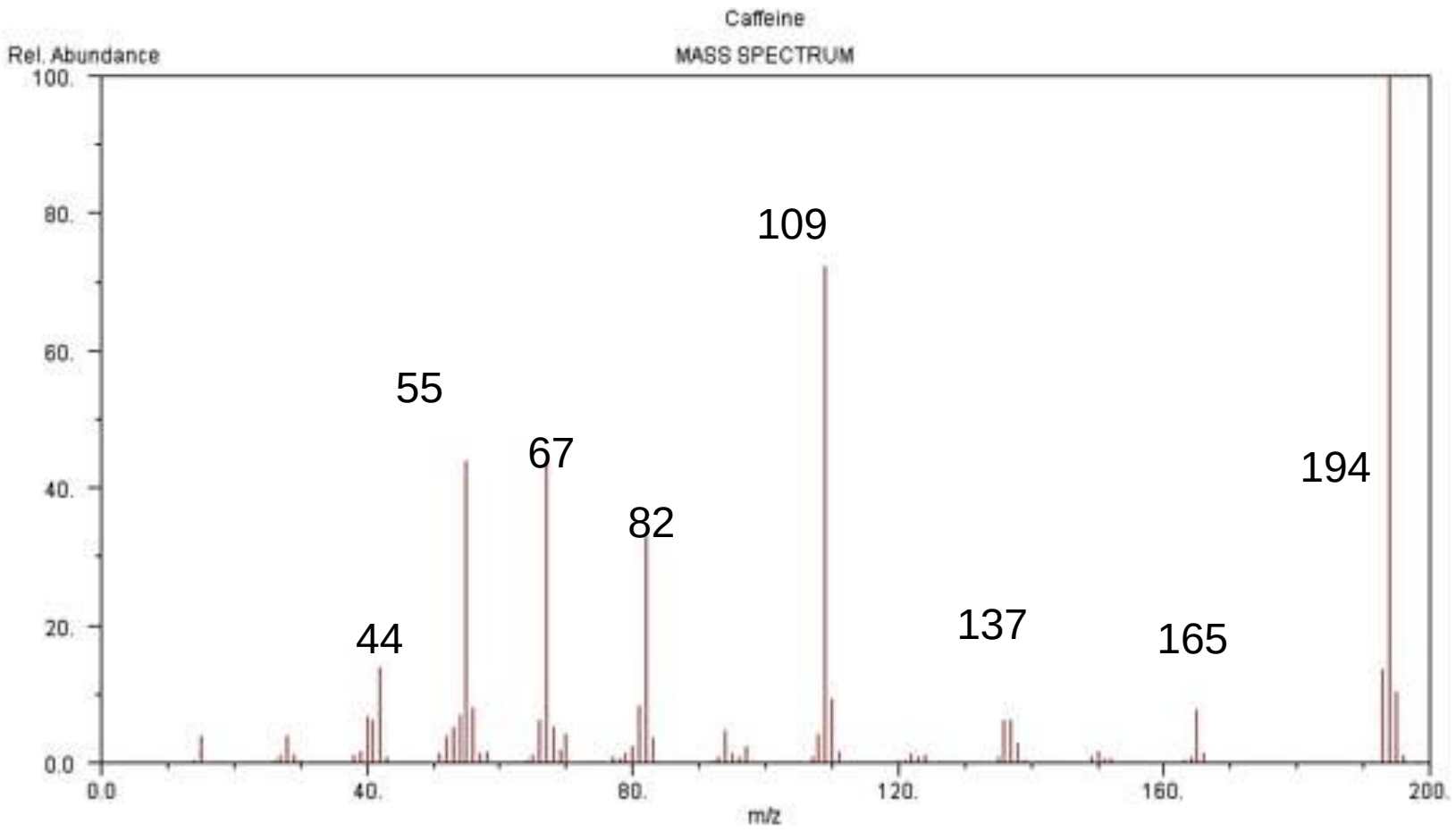
  $e^- + M \longrightarrow M^{+\cdot} + 2e^-$

- Fragmentation is a result of an excited molecular ion, which in attempting to gain stability
- Fragmentation is not random and occurs through real chemical reactions.

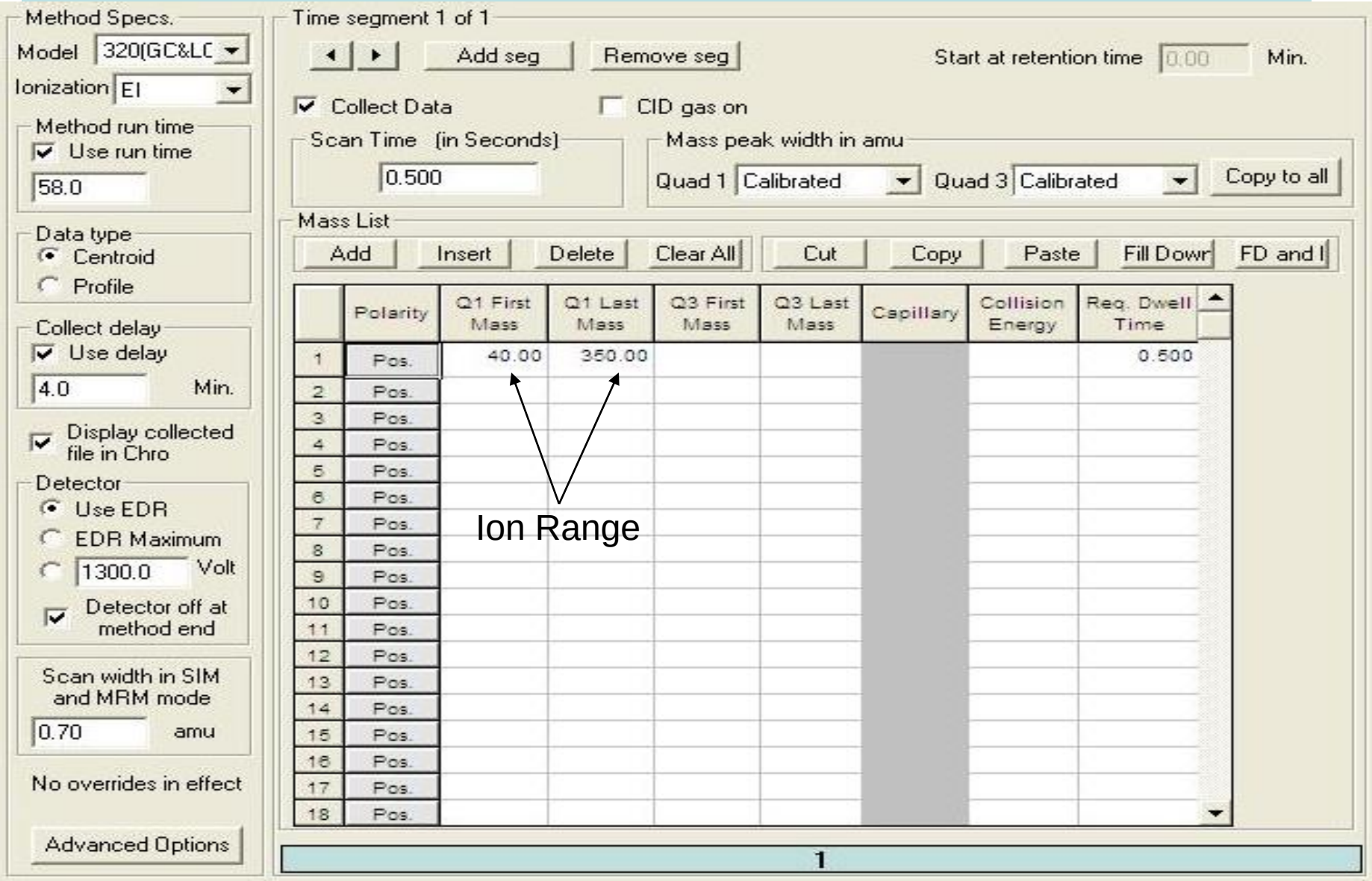
Caffeine fragmentation pattern GC-MS 70eV



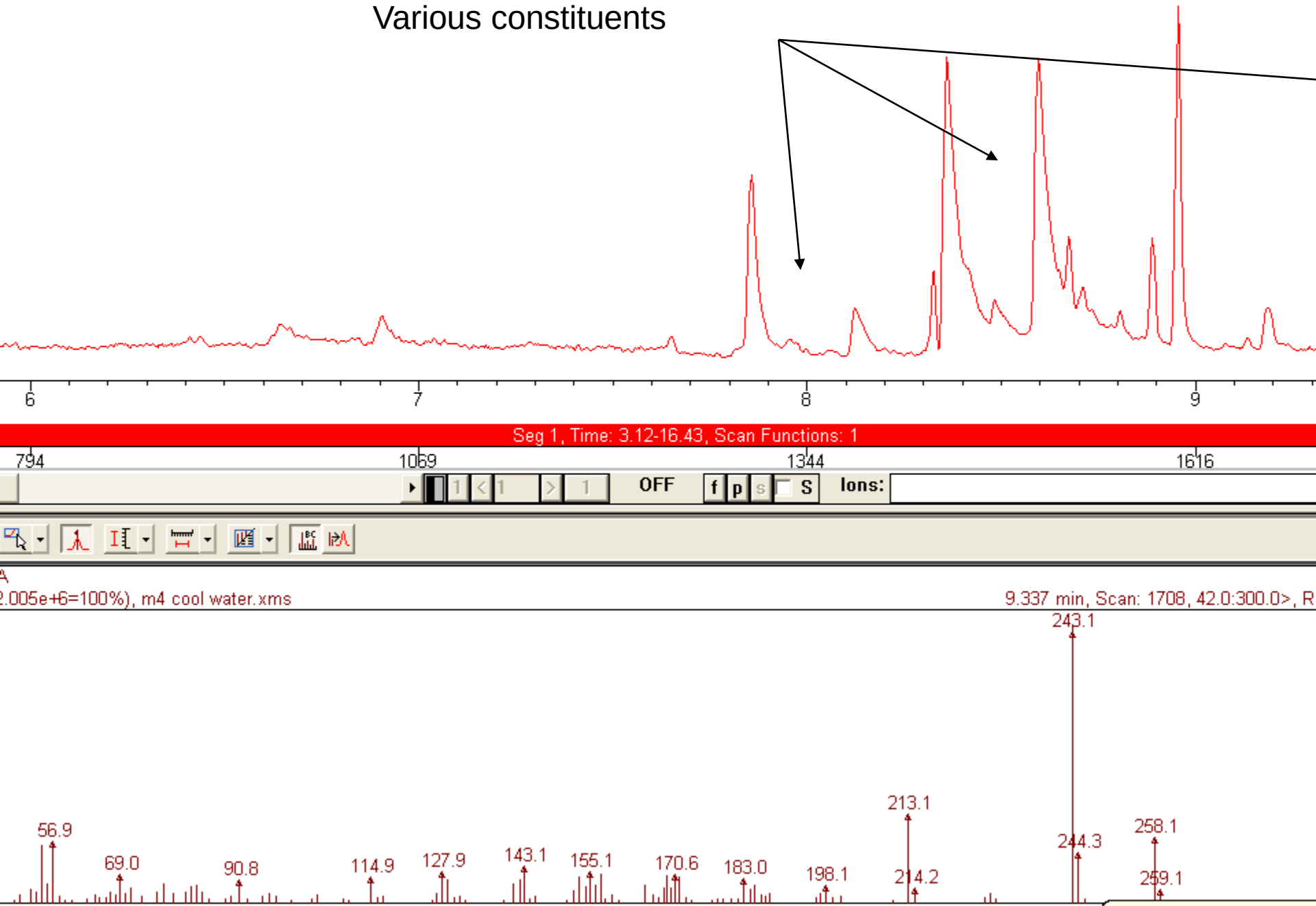
Caffeine Fragmentation Pattern

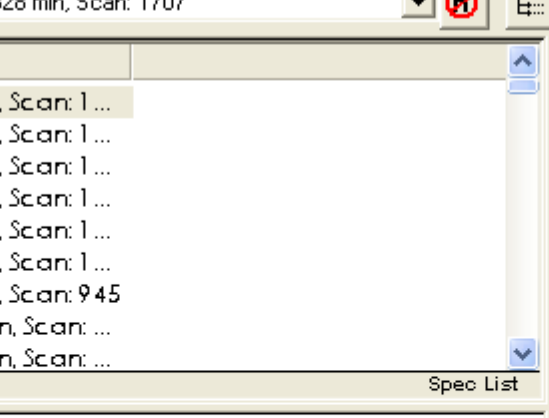


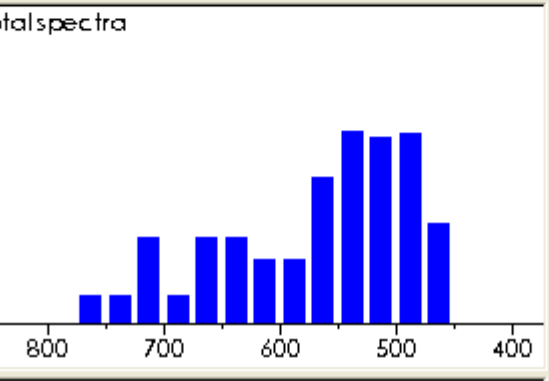
Q1 Window


Method Specs.
Model 320(GC&LC
Ionization EI
Method run time
Use run time
58.0
Data type
Centroid
Profile
Collect delay
Use delay
4.0 Min.
Display collected file in Chro
Detector
Use EDR
EDR Maximum
1300.0 Volt
Detector off at method end
Scan width in SIM and MRM mode
0.70 amu
No overrides in effect
Advanced Options
Time segment 1 of 1
Add seg
Remove seg
Start at retention time 0.00 Min.
Collect Data
CID gas on
Scan Time (in Seconds)
0.500
Mass peak width in amu
Quad 1 Calibrated
Quad 3 Calibrated
Copy to all
Mass List
Add
Insert
Delete
Clear All
Cut
Copy
Paste
Fill Dowr
FD and I
Polarity
Q1 First Mass
Q1 Last Mass
Q3 First Mass
Q3 Last Mass
Capillary
Collision Energy
Req. Dwell Time
1 Pos. 40.00 350.00 0.500
2 Pos.
3 Pos.
4 Pos.
5 Pos.
6 Pos.
7 Pos.
8 Pos.
9 Pos.
10 Pos.
11 Pos.
12 Pos.
13 Pos.
14 Pos.
15 Pos.
16 Pos.
17 Pos.
18 Pos.
Ion Range
1

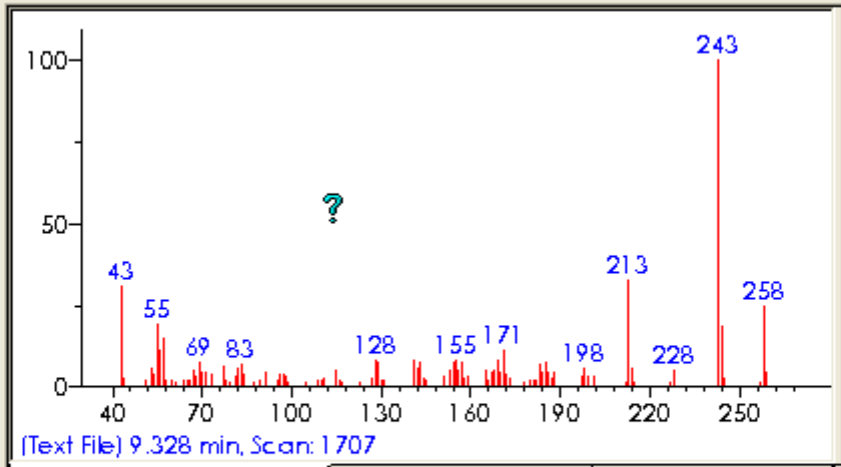
Various constituents



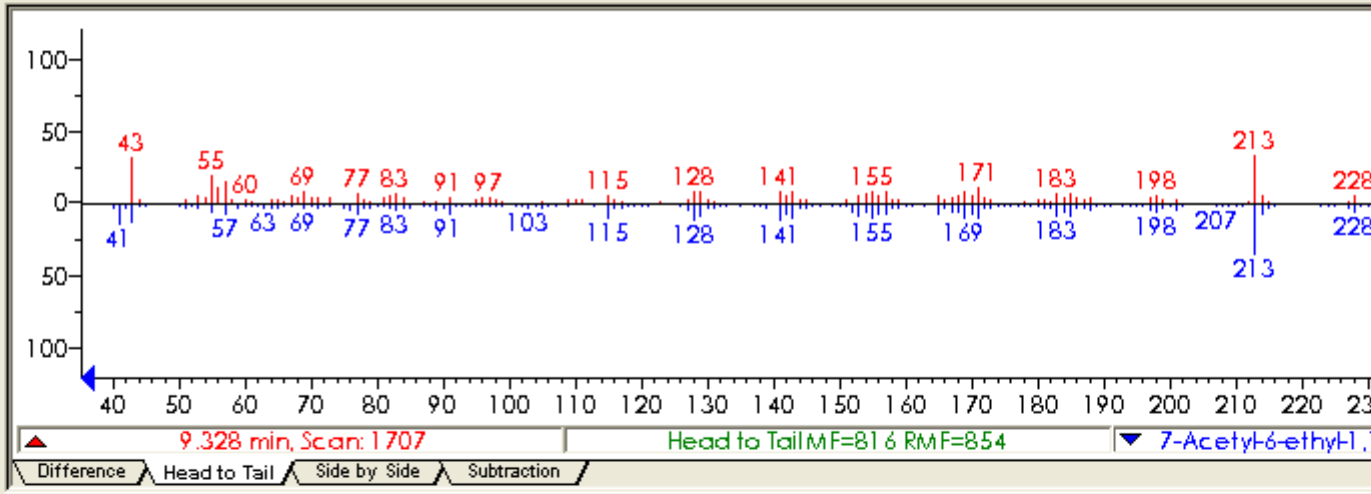


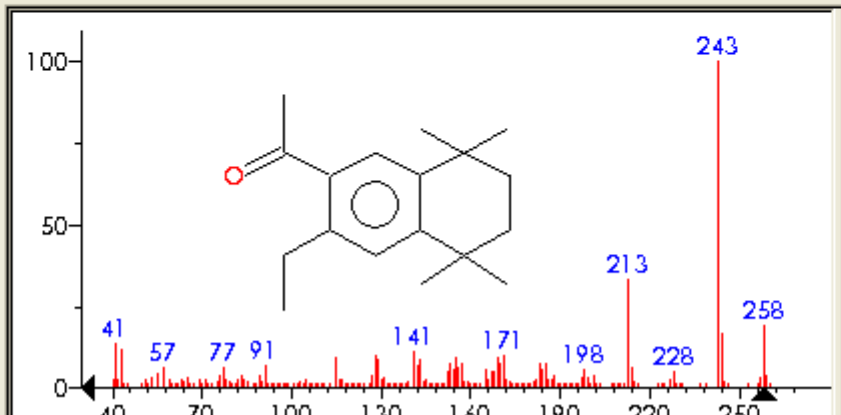


R.Match	Prob.	Name
885	67.5	7-Acetyl-6-ethyl-1,1,4,4
868	28.7	Cyclopenta[g]-2-benz
786	67.5	7-Acetyl-6-ethyl-1,1,4,4
766	1.88	Epistephamiersine
872	0.74	Galaxolide 1
739	67.5	7-Acetyl-6-ethyl-1,1,4,4
854	0.48	Galaxolide 2
793	0.26	Ethanone, 1-(5,6,7,8-tet
731	0.14	Stephamiersine
673	0.09	Isoxazole, 4,5-dihydro-3
668	0.06	Furo[2,3-H]coumarine,
731	0.03	1-Phenanthrenecarbox
690	0.03	6-Acetyl-5-methoxy-2,7
682	0.03	1-Phenanthrenecarbox
705	0.03	1-Phenanthrenecarbox
648	0.01	9-Amino-1-phenyl-3,6-c
596	0.00	6H-7-Oxa-11-thia-2,4,10
579	0.00	1-Allyl-4-(4-methoxyphe
619	0.00	Styrene, 2,3,5,6-tetraet



Name: 9.328 min, Scan: 1707
MW: N/A ID#: 3141 DB: Text File
Comment: M4 X.xms
10 largest peaks:
243 999 | 213 322 | 43 306 | 258 241
244 180 | 57 145 | 171 110 | 56 106
Synonyms:
no synonyms.



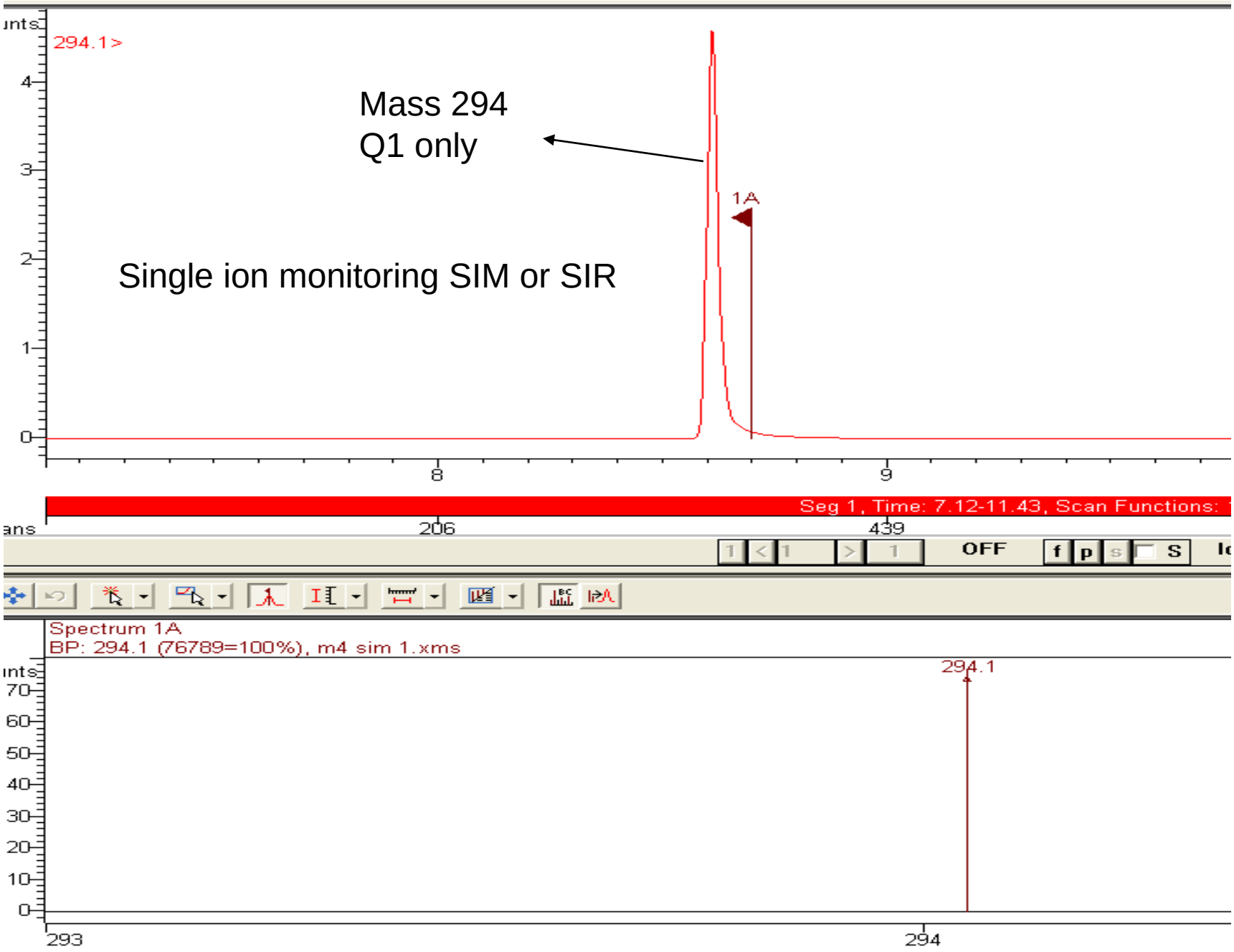


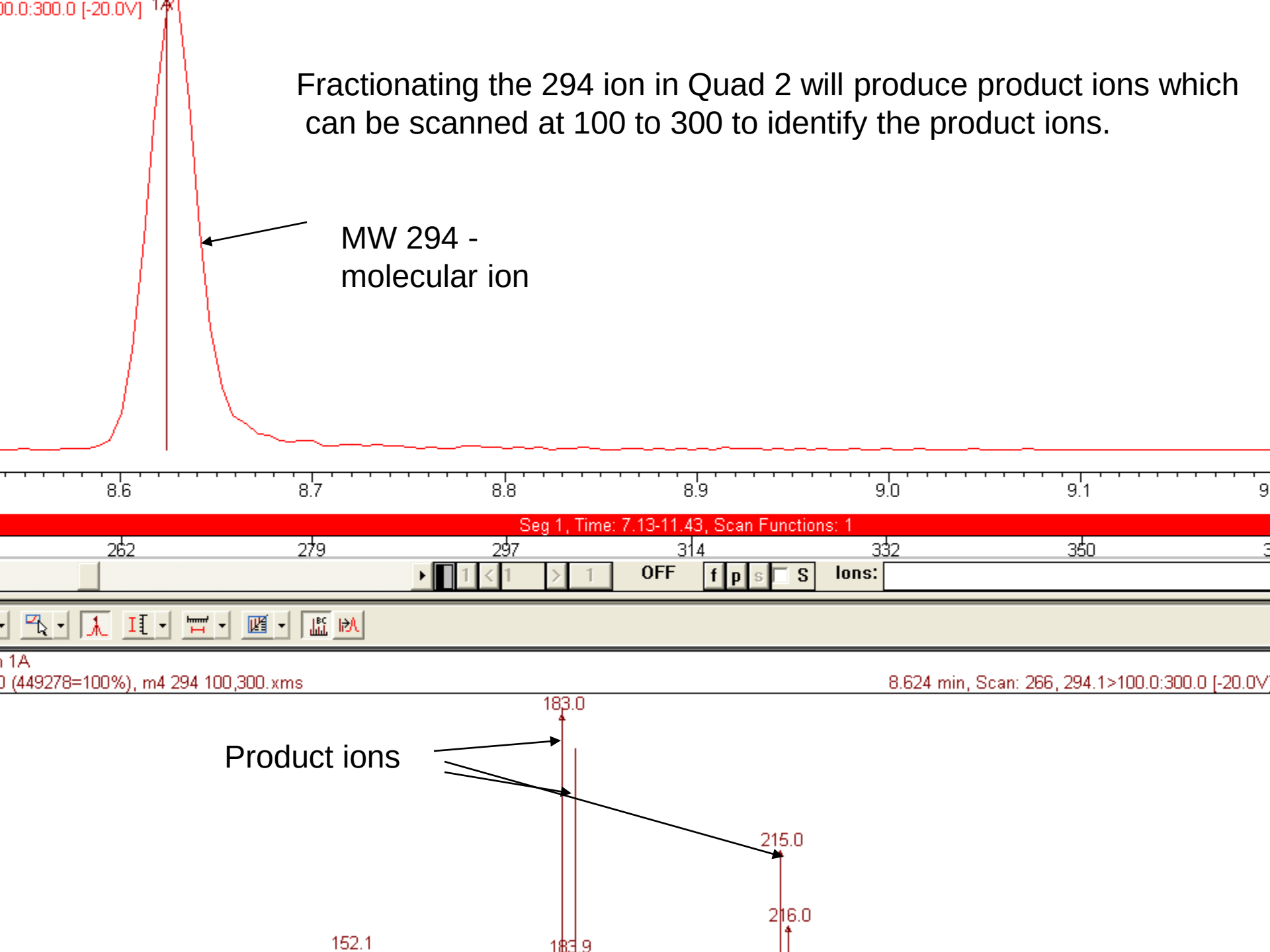
Name: 7-Acetyl-6-ethyl-1,1,4,4-tetramethylte
Formula: $C_{18}H_{26}O$
MW: 258 CAS#: 88-29-9 NIST#: 261786 ID#: 2
Other DBs: TSCA, RTECS, NIH, EINECS
Contributor: A.A.Kutin, Moscow, Russia
10 largest peaks:
243 999 | 213 330 | 258 187 | 244 165
43 116 | 141 105 | 128 97 | 171 95
Synonyms:
1.Ethanone, 1-(3-ethyl-5,6,7,8-tetrahydro-5,5,
2.Musk 36A
3.Polycyclic musk
4.Versalide

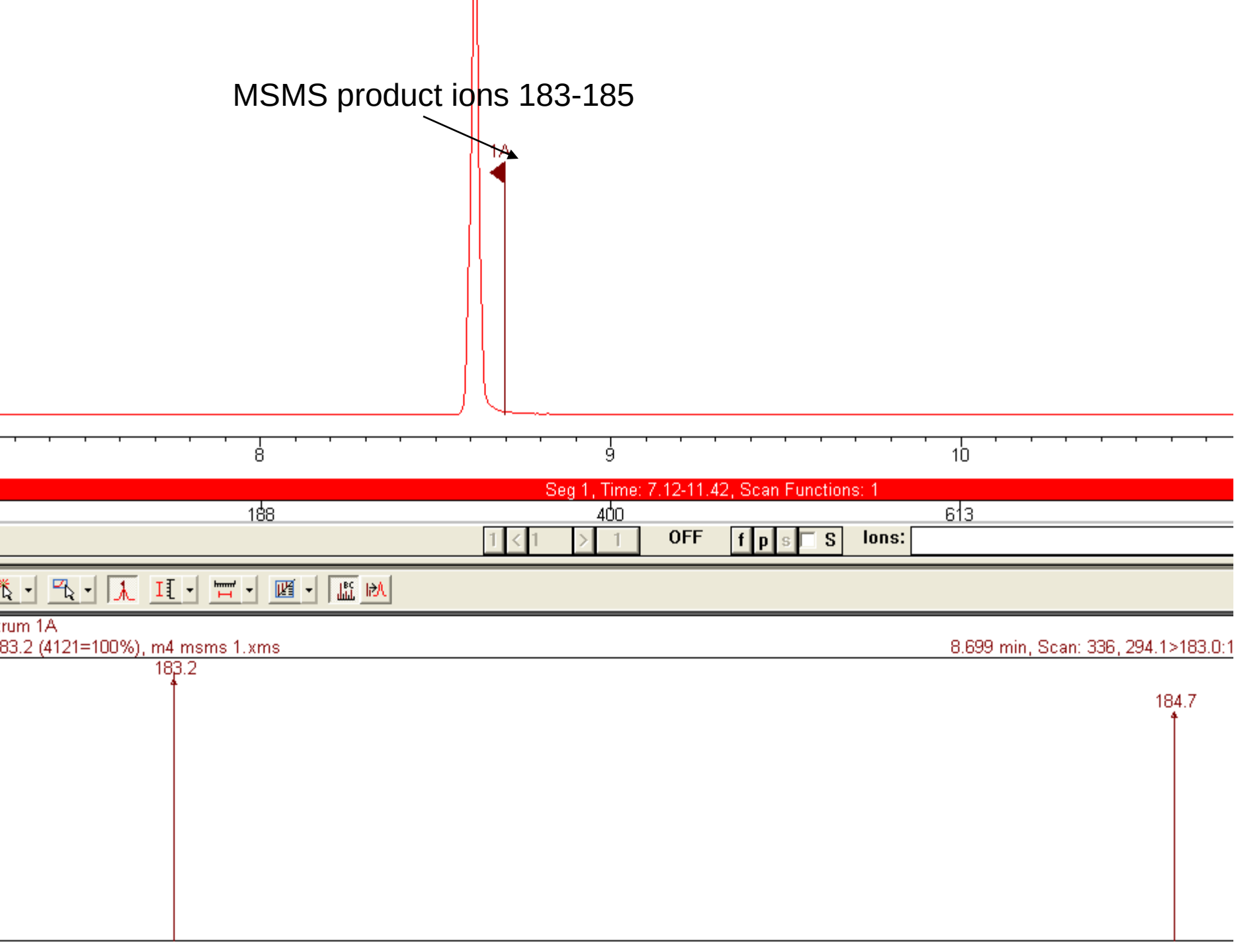
Quantitation of Ingredients

- You usually quantitate the ingredient that you are interested in by:
  - SIM which measures one of the ions in your sample

    or
  - MSMS which measures one of the ions produced in the collission cell (Quad 2)


294.1>
Mass 294
Q1 only
1A
Single ion monitoring SIM or SIR
8
9
Seg 1, Time: 7.12-11.43, Scan Functions:
206
439
OFF
Spectrum 1A
BP: 294.1 (76789=100%), m4 sim 1.xms
294.1
293
294


00.0:300.0 [-20.0V]
1A
Fractionating the 294 ion in Quad 2 will produce product ions which can be scanned at 100 to 300 to identify the product ions.
MW 294 - molecular ion
8.6
8.7
8.8
8.9
9.0
9.1
Seg 1, Time: 7.13-11.43, Scan Functions: 1
262
279
297
314
332
350
OFF
f
p
s
S
Ions:
1A
0 (449278=100%), m4 294 100,300.xms
8.624 min, Scan: 266, 294.1>100.0:300.0 [-20.0V]
Product ions
183.0
215.0
216.0
152.1
183.9


MSMS product ions 183-185
8
9
10
Seg 1, Time: 7.12-11.42, Scan Functions: 1
188
400
613
OFF
Ions:
rum 1A
83.2 (4121=100%), m4 msms 1.xms
8.699 min, Scan: 336, 294.1>183.0:1
183.2
184.7

Mass Spectrometry in MCAL

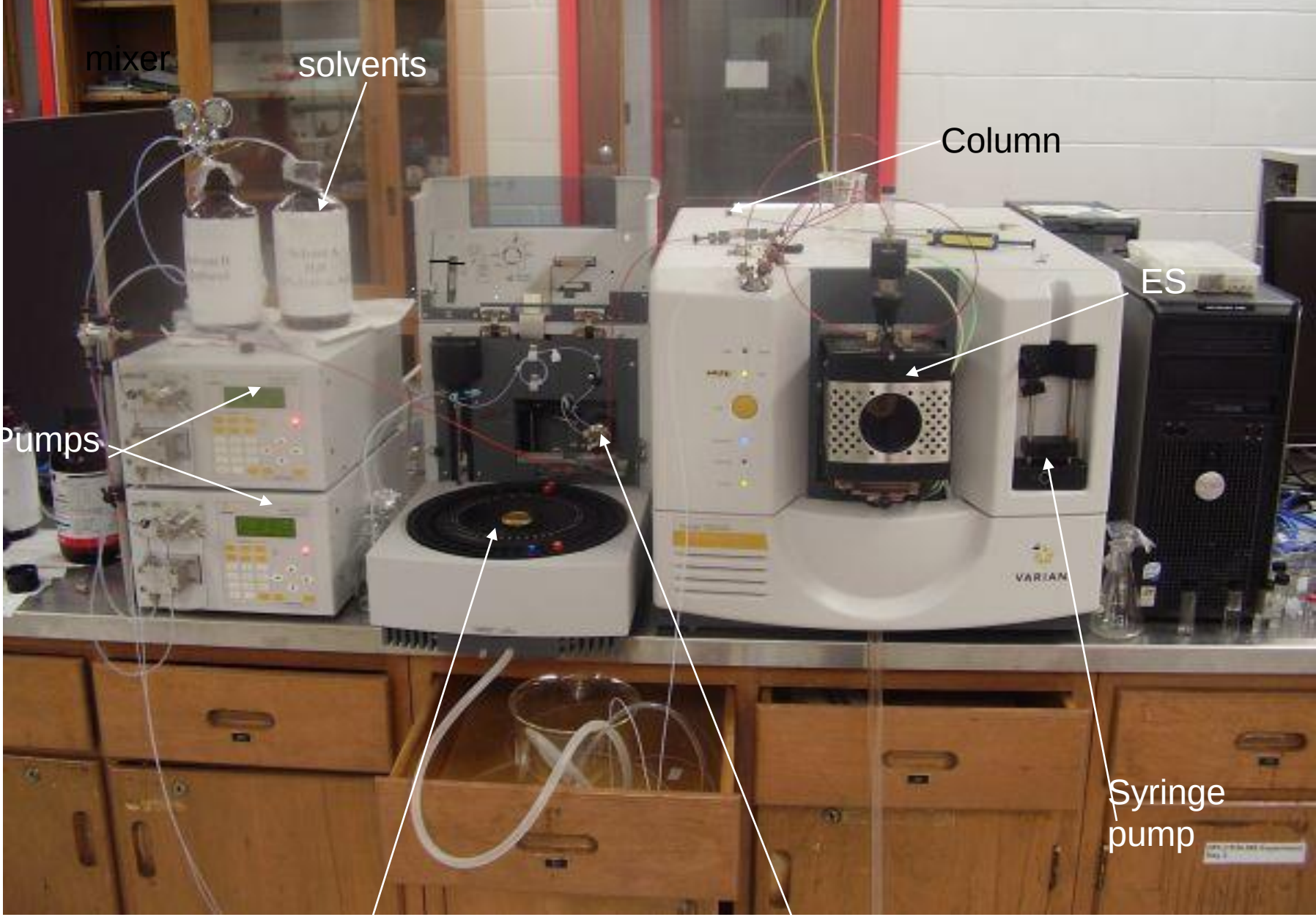
- Two systems: GC-MS, LC-MS
  - LC seperates small, volatile, polar and non-polar material
  - MS is detection devise (Agilent 500-MS IonTrap (IT) Mass Spectrometer
    - Full scan monitoring
    - SIM single ion monitoring
    - MSMS monitoring
- Liquid samples are analysed

Agilent LCMS

- The LCMS can be used either with the LC seperating the sample on the HPLC column before introduction into MS

or

- Stand alone MS where sample is introduced via the syringe pump.


mixer
solvents
Column
ES
Pumps
VARIAN
Syringe pump
Sample rack (autosampler)
Injection valve

From HPLC or Syringe pump

Inner Needle Adjustment

ES unit

Adjustment of the inner, liquid capillary needle to maximize nebulization efficiency

Hexapo

Ion guid

Source

N_2 drying gas @150-300^{O}C

Scimmer cone

Electrospray Ionization

- During standard electrospray ionisation the sample is dissolved in a polar, volatile solvent and pumped through a narrow, **stainless steel capillary (needle)**.

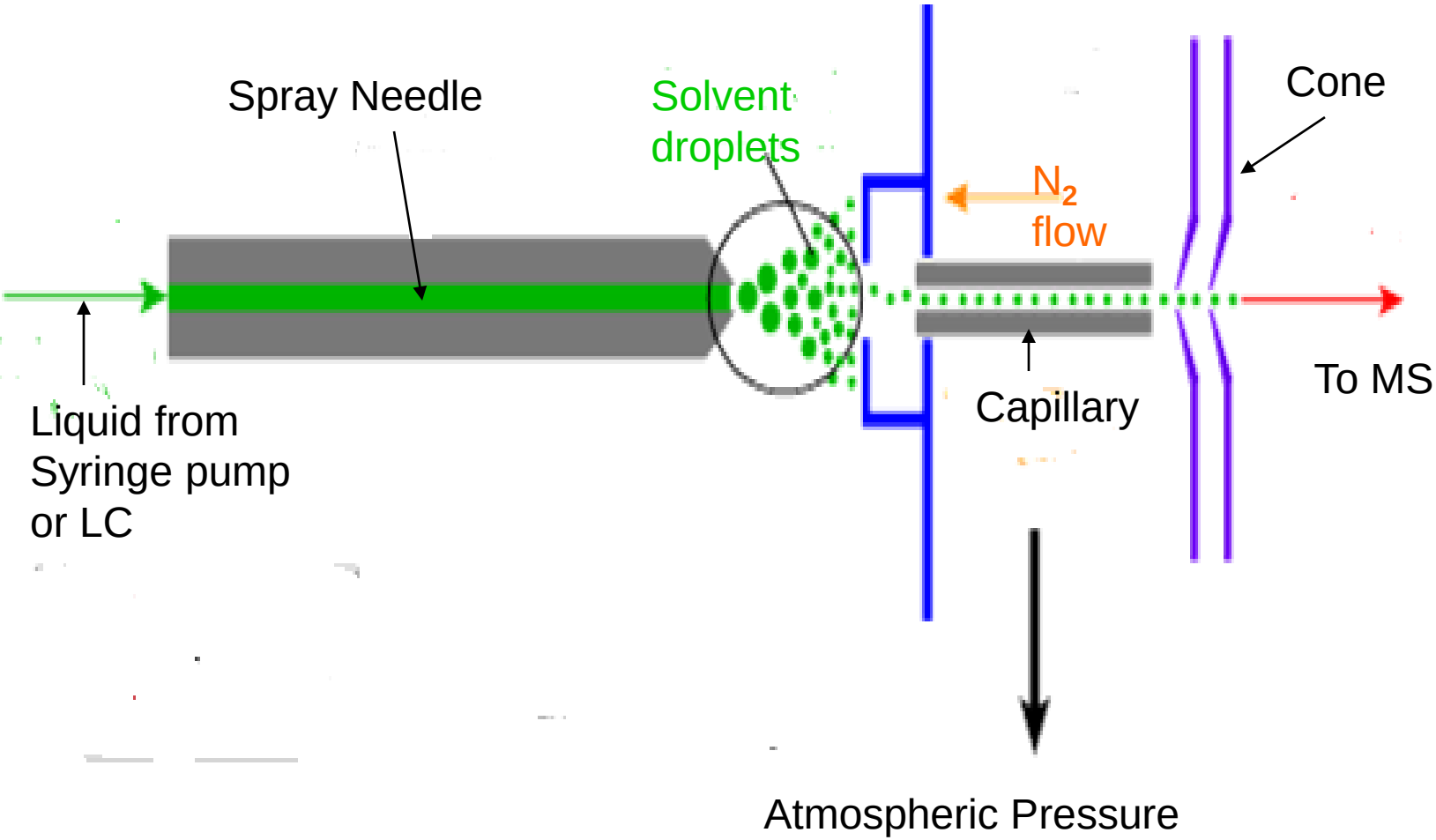
- A **high voltage** of 3 to 6 kV is applied to the tip of the needle, which is situated within the ionisation source of the mass spectrometer

- As a consequence of this strong electric field, the sample emerging from the tip is dispersed into an **aerosol of highly charged droplets**,

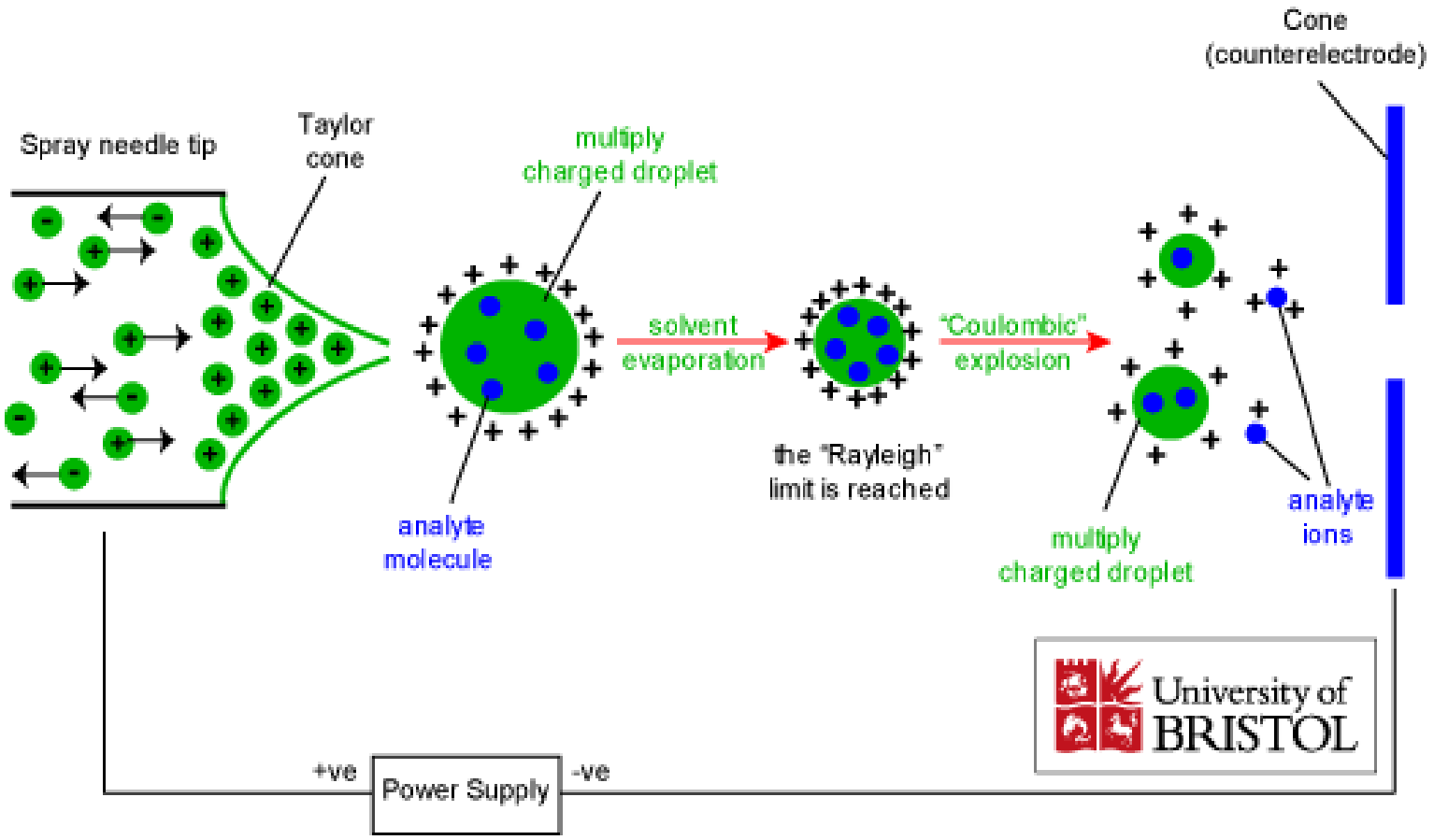
Electrospray Ionization

- The charged droplets diminish in size by **solvent evaporation**, **(N_2 drying gas)**
- Charged **sample ions**, free from solvent, are released from the droplets,
- Some pass through an orifice in the cone into an **intermediate vacuum** region,
- Then into the analyser of the mass spectrometer, which is held under **high vacuum**.

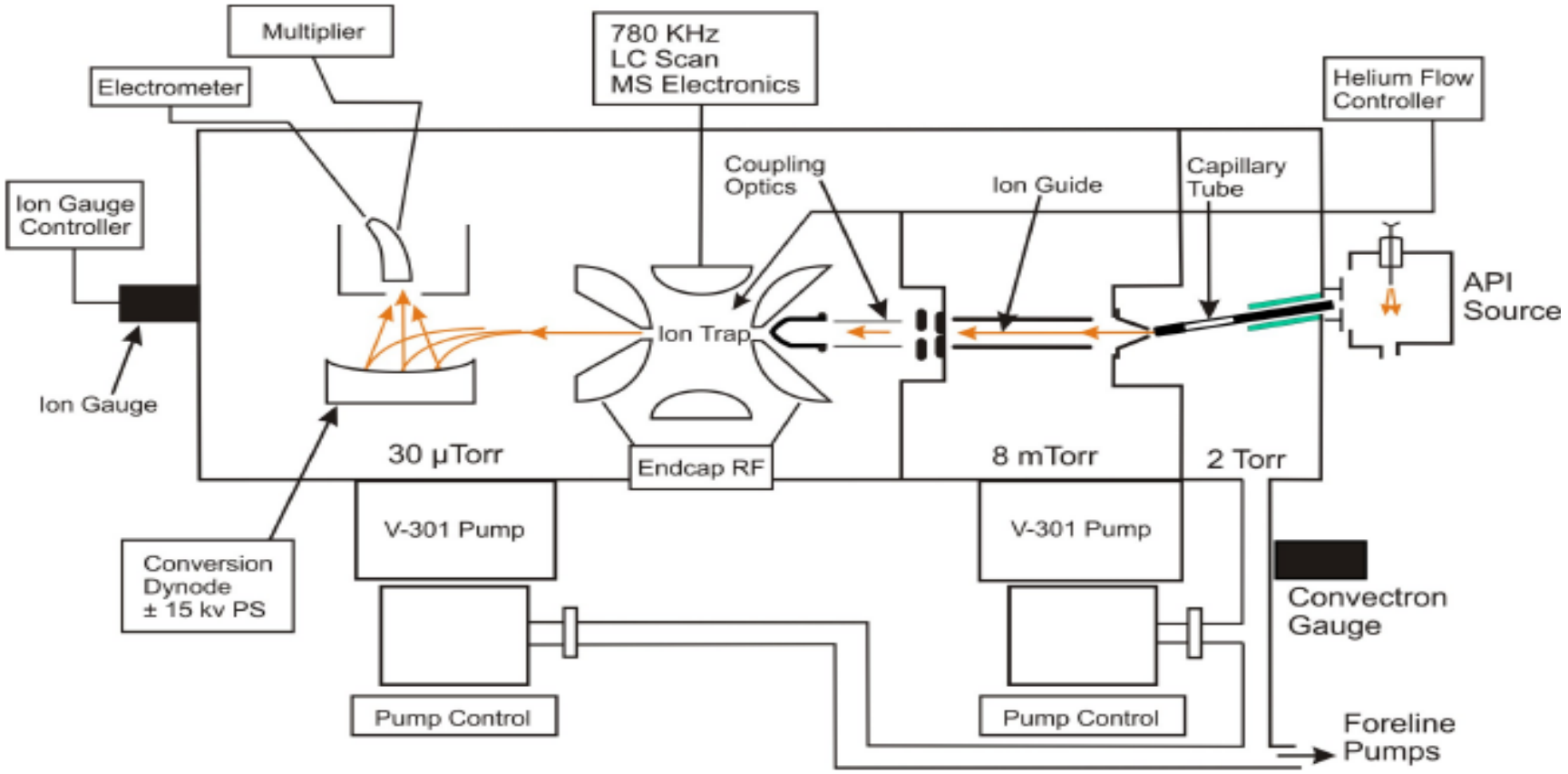
Electrospray Ionization



Electrospray Ionization (ESI)



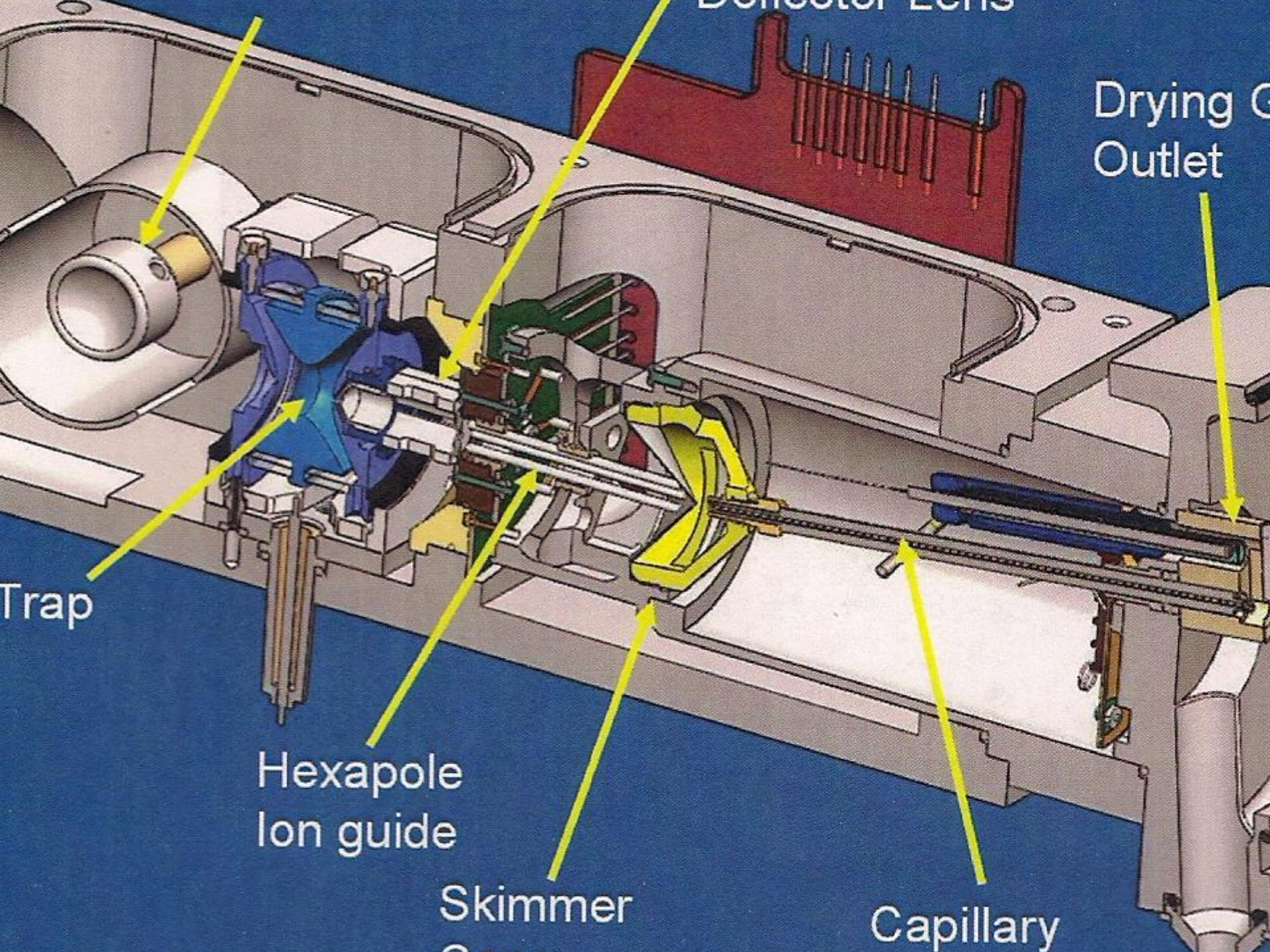
500-MS Operation



Varian 500-MS System Block Diagram

500 –MS Operation

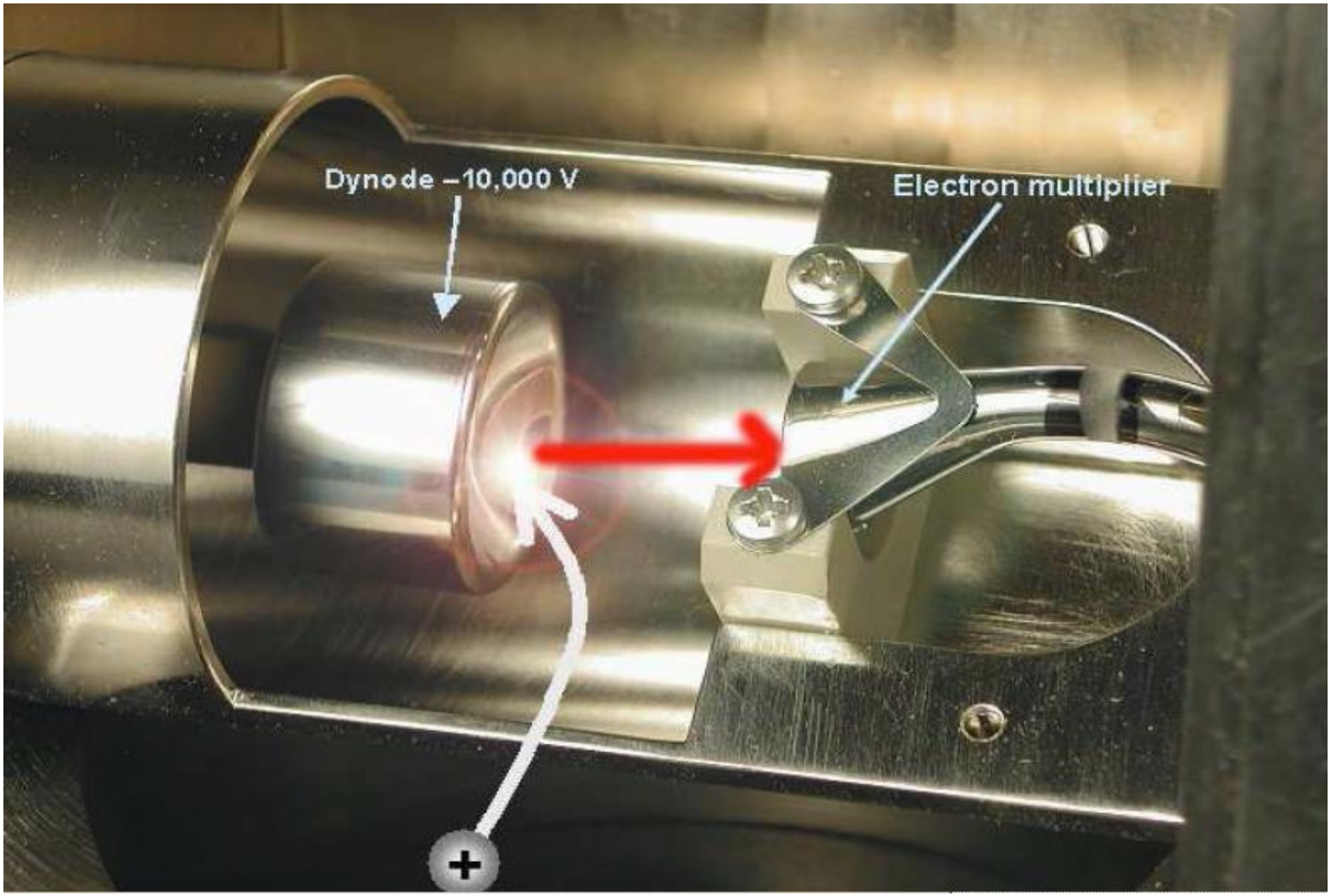
- Gas phase ions are generated from solution at atmospheric pressure using either Electrospray Ionization (ESI) or Atmospheric Pressure Chemical Ionization (APCI).
- The ions are transported through two vacuum interfaces via a metal capillary tube.


Drying G
Outlet
Trap
Hexapole
Ion guide
Skimmer
Capillary

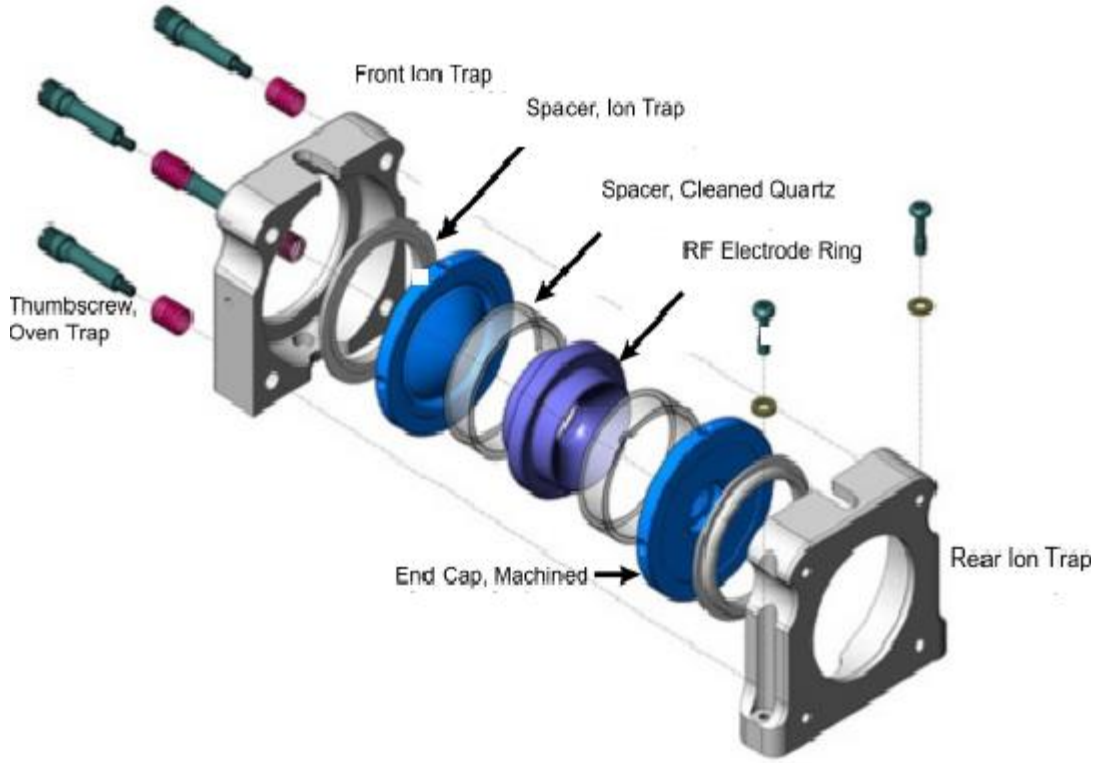
500-MS Operation Continued

- After exiting the capillary, the ion stream expands into a supersonic beam.
- The sample passes through a skimmer cone (which is at ground potential). If the potential difference between the capillary and skimmer cone is high enough, some molecules may fragment (Capillary Induced Dissociation (CID)).
- After passing through the skimmer cone, ions enter an 8 m Torr vacuum chamber containing ion optics.
- The ions enter a hexapole ion guide. The ion guide uses an oscillating potential across six cylindrical rods to confine the ions and a direct current (dc) potential to facilitate ion transport. Collisions with neutral gas in the ion guide reduce the kinetic energy of the ions to facilitate focusing.
- The ions pass through the ion guide exit and focus lenses,
- The ions then pass through a split cylinder that acts like a gate for the ion trap by either focusing the beam or deflecting it. The final end cap lens focuses the ions through the entrance hole of the trap.

Electron Multiplier



Ion Trap


Front Ion Trap
Spacer, Ion Trap
Spacer, Cleaned Quartz
RF Electrode Ring
Thumbscrew, Oven Trap
End Cap, Machined
Rear Ion Trap

Operation of Ion Trap

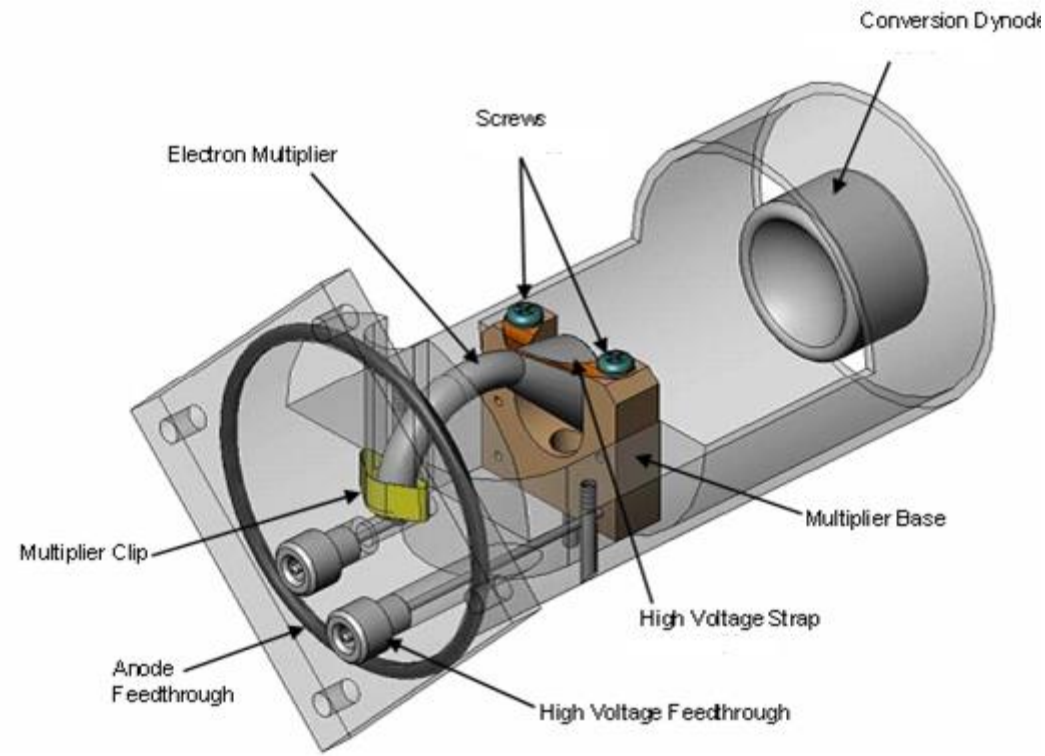
- There is a hole in the center of both the entrance and exit end cap electrodes. This hole allows the ions to enter the trap and exit to the detector. The holes in the edge of the end caps contain posts that make contact with pogo pins that carry supplemental waveform signals.

- An RF generator provides a high voltage, 781 kHz RF, that is applied to the ring electrode. With the proper RF voltage, the ion trap electrodes create a three-dimensional, hyperbolic electric field.

- This field traps the ions in stable orbits. In the presence of helium damping gas, the ions are cooled towards the center of the trap.

- As the RF voltage increases, the ion trajectories become unstable in increasing order of mass to charge ratio. The ion trap ejects the ions and sends them to the conversion dynode and then to the electron multiplier for detection.

500-MS Detector



500-MS Detector

Ion Detection

After exiting the trap, ions accelerate toward an off axis conversion dynode that gene a combination of positive ions and electrons through secondary electron emission.

For the detection of positive ions, the conversion dynode is set to a large negative vo (typically -15 kV). The secondary electrons are attracted to the relatively positive mul

Electrons or ions emitted from the conversion dynode strike the cathode with suffici velocity to dislodge additional electrons from the inner curving surface of the cathod increasingly positive potential gradient draws the ejected electrons into the electron multiplier, further accelerating them in the process., the ejected electrons strike the c inner surface of the multiplier, resulting in the emission of more electrons.

This configuration produces a cascade of electrons that accelerate toward ground potential at the exit end of the cathode.

The anode collects the electrons and passes the resulting ion signal to the ion ampli The ion current is proportional to the total number of ions the ion trap ejects.

Each electron or positive ion that enters the electron multiplier generates approxima 10^5 electrons.

Separation Of Analgesics

- Seperation is based on HPLC and mixture of Tylenol (acetaminophen, caffeine) and aspirin are separated.

- MS substance in mixture, can be optimized for each of RF, needle voltage, capillary voltage and CID.

Seperation Under Optimum Conditions

- Precursor (parent) ion produces breakdown ions (daughter ions) which can be used to quantitate the substances in a HPLC separated mixture.

Caffeine fragmentation pattern