Mass Spectrometry
in the analysis
of Persistent Organic Pollutants

Anton Kočan, Jana Klánová

Tools for determining chemical structure

- IR
- UV-VIS
- X-ray crystallography
- NMR
- **Mass spectrometry (MS)**

Kurt Wuthrich a John B. Fenn (1/4) + Koichi Tanaka (1/4) shared the Nobel Price in chemistry for 2002.

The latter two for the development of soft desorption ionization methods for mass spectrometric analyses of biological macromolecules

Altogether, 5 scientists have been awarded the Nobel Price in connection with mass spectrometry – Joseph Thompson in 1906, Francis Aston in 1922, Wolfgang Paul in 1989, and J. Fenn and K. Tanaka in 2002

Advantages of Mass Spectrometry

- A small amount of substance is sufficient
- Mixtures can be analyzed

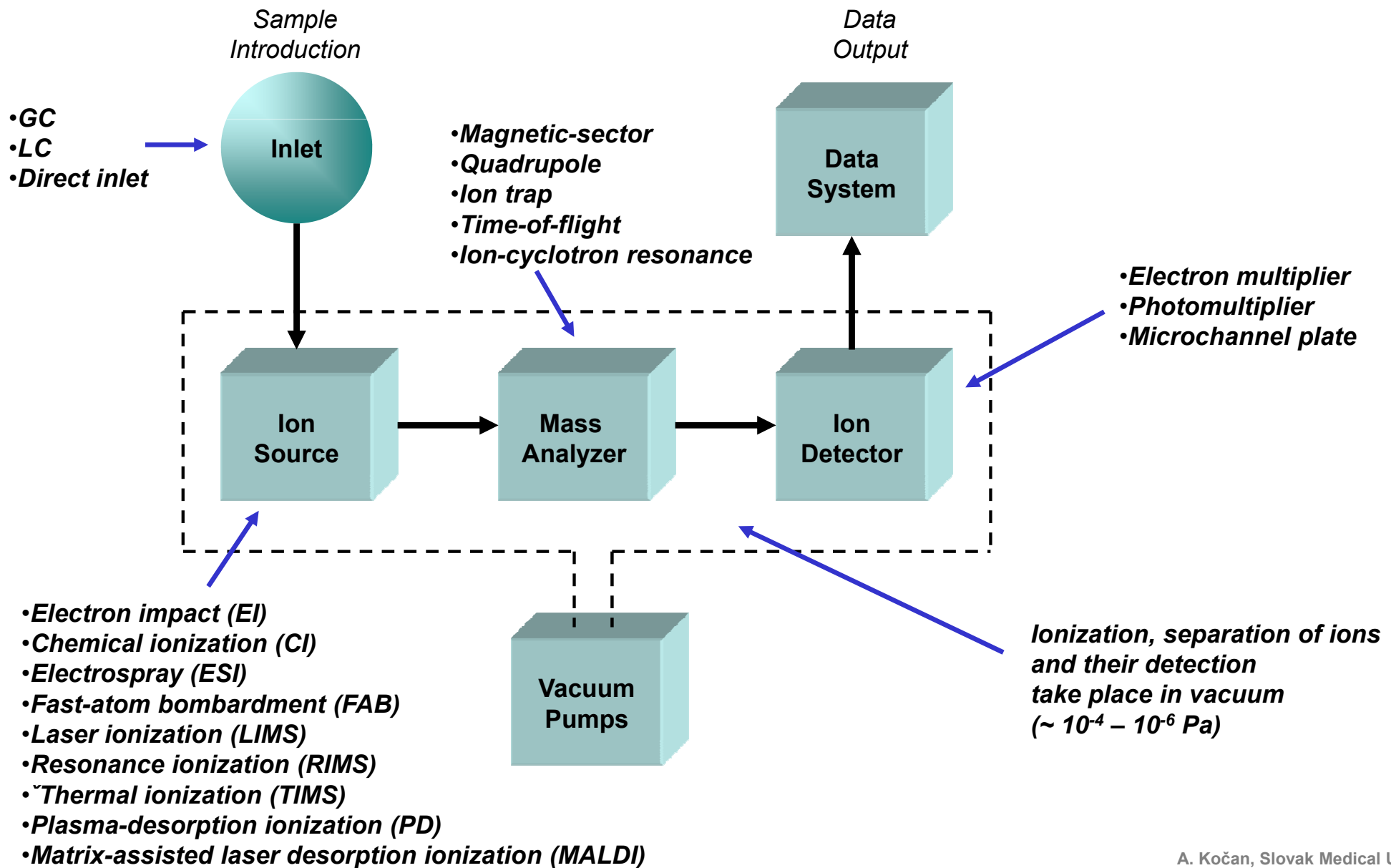# Disadvantages of Mass Spectrometry

- It is a destructive method
- The evaluation of mass spectra is demanding
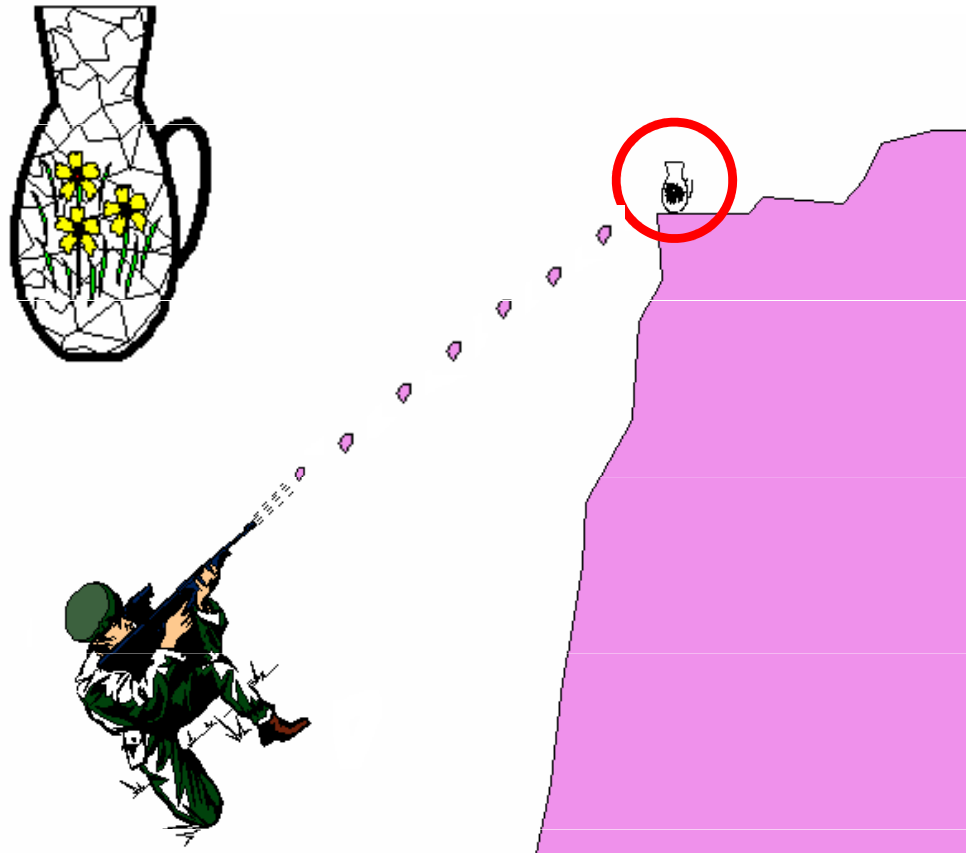
Mass Spectrometer

- **All the MS systems compose of the following parts:**

Sample Introduction

Data Output

- *GC*
- *LC*
- *Direct inlet*

Inlet

- *Magnetic-sector*
- *Quadrupole*
- *Ion trap*
- *Time-of-flight*
- *Ion-cyclotron resonance*

Data System

- *Electron multiplier*
- *Photomultiplier*
- *Microchannel plate*

Ion Source

Mass Analyzer

Ion Detector

- *Electron impact (EI)*
- *Chemical ionization (CI)*
- *Electrospray (ESI)*
- *Fast-atom bombardment (FAB)*
- *Laser ionization (LIMS)*
- *Resonance ionization (RIMS)*
- *˘Thermal ionization (TIMS)*
- *Plasma-desorption ionization (PD)*
- *Matrix-assisted laser desorption ionization (MALDI)*

Vacuum Pumps

Ionization, separation of ions and their detection take place in vacuum ($\sim 10^{-4} - 10^{-6}$ Pa)

A. Kočan, Slovak Medical University

Sample Introduction

- ## Direct inlet to the ion source
  - Introduction of the gaseous sample
  - Introduction of the liquid or solid sample after their vaporization

- ## Outlet from the chromatographic column
  - Gas chromatography – packed column (a carrier gas separator is needed)
    - capillary column (directly to the ion source)
  - Liquid chromatography (the separation of the mobile phase is essential)
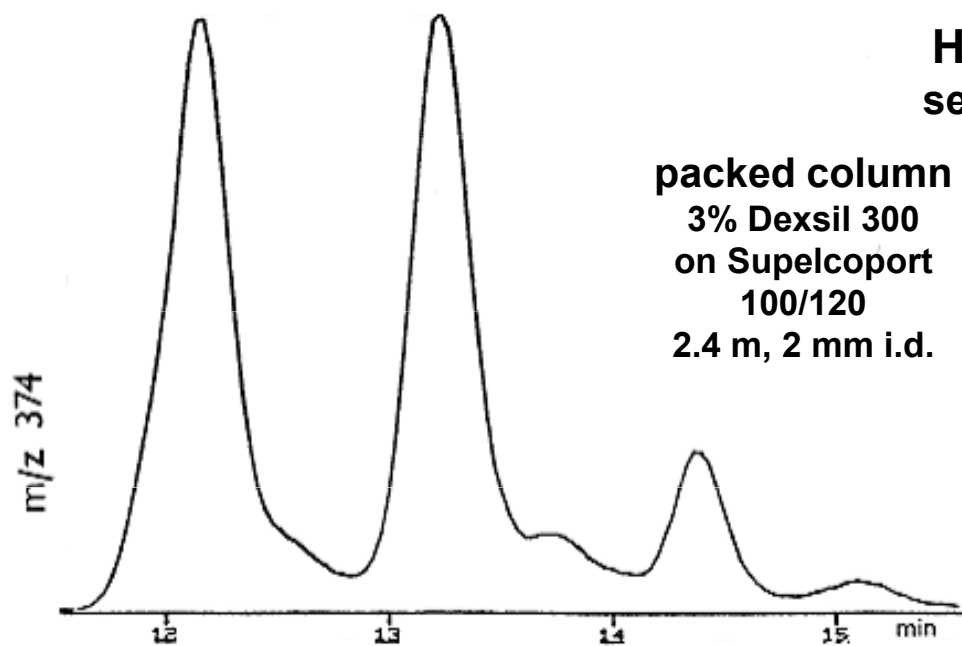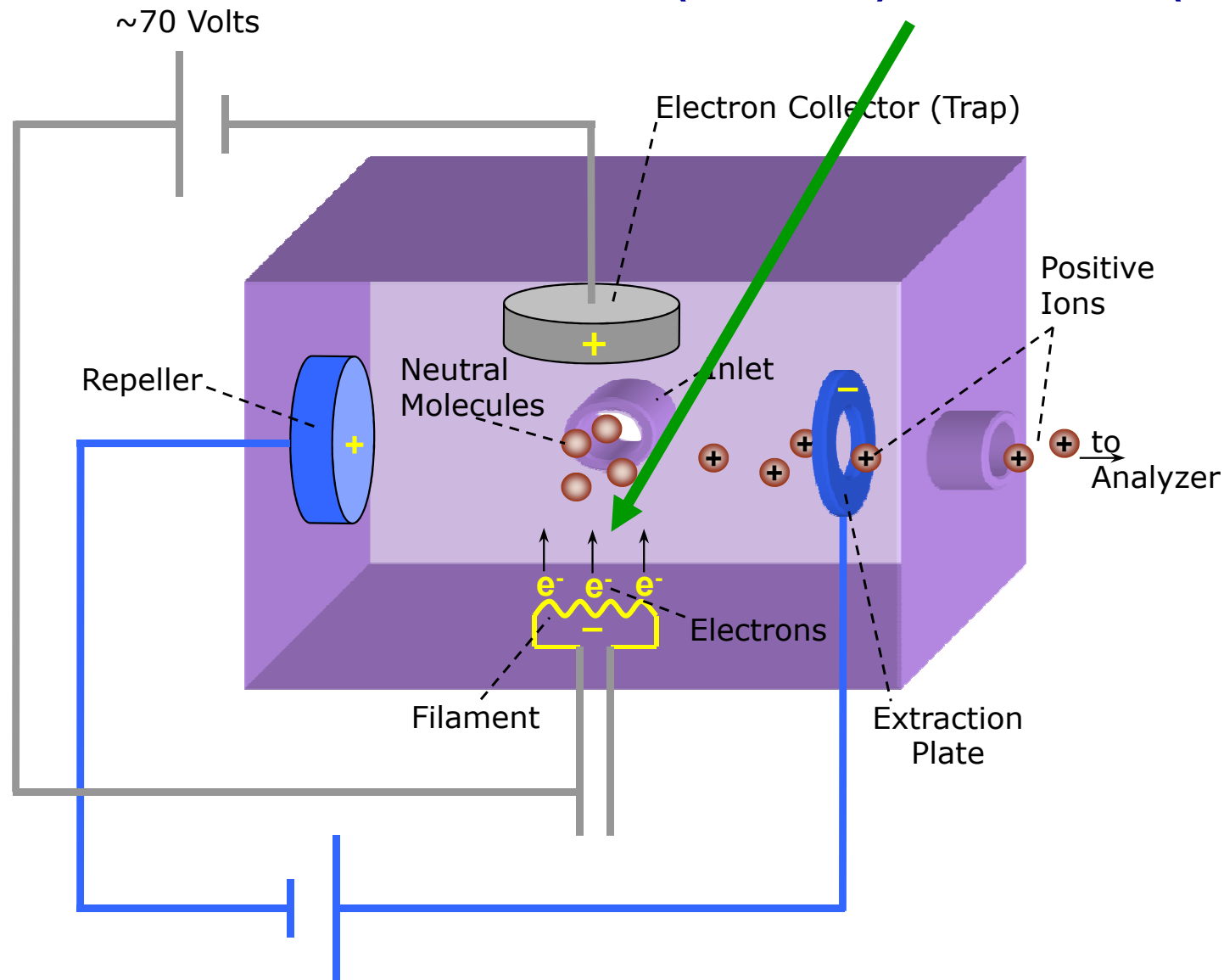
HexaCDFs separated by

packed column
3% Dexsil 300
on Supelcoport
100/120
2.4 m, 2 mm i.d.

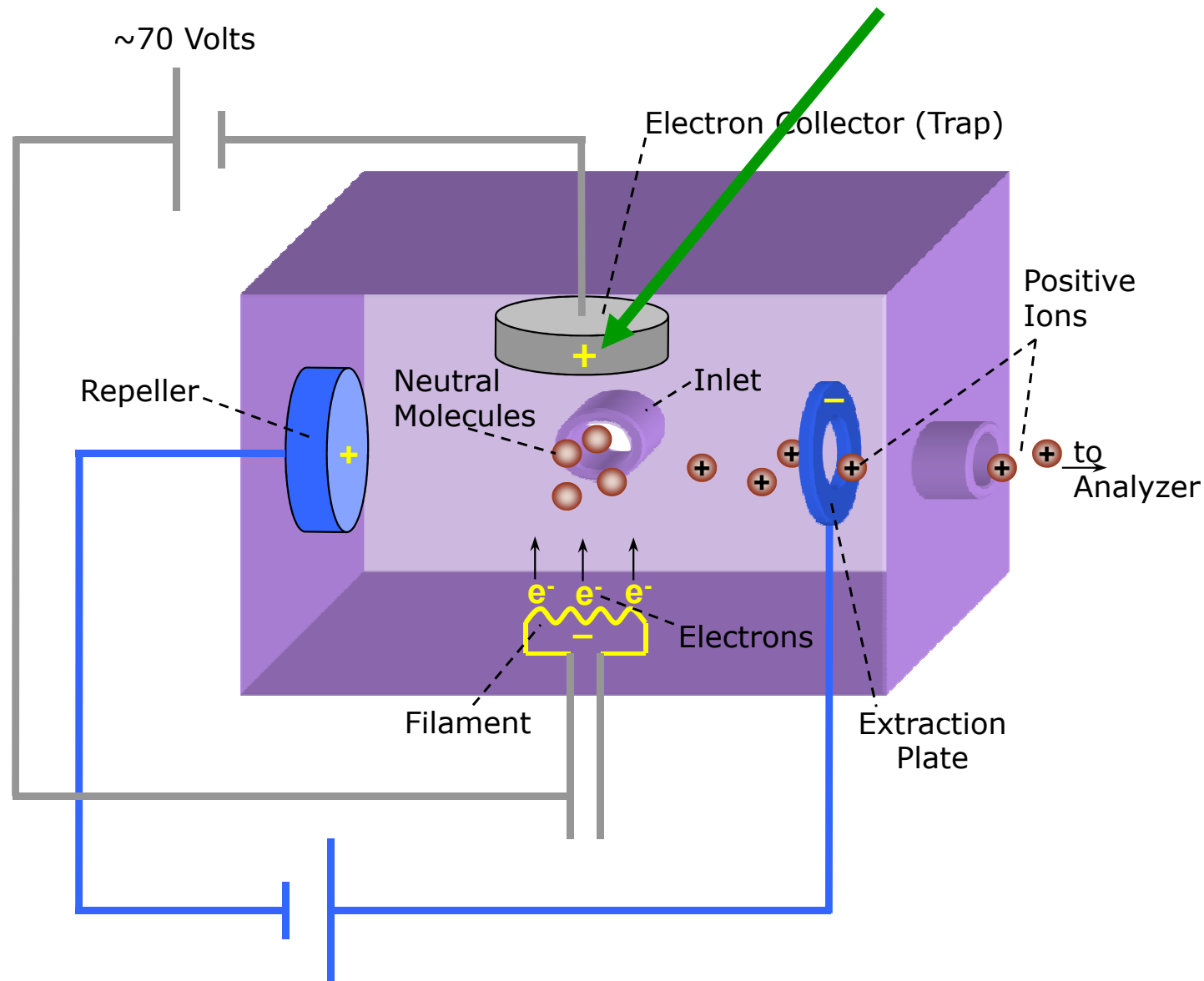**capillary column**
SP-2331
60 m
0.25 mm i.d.
0.2 μm
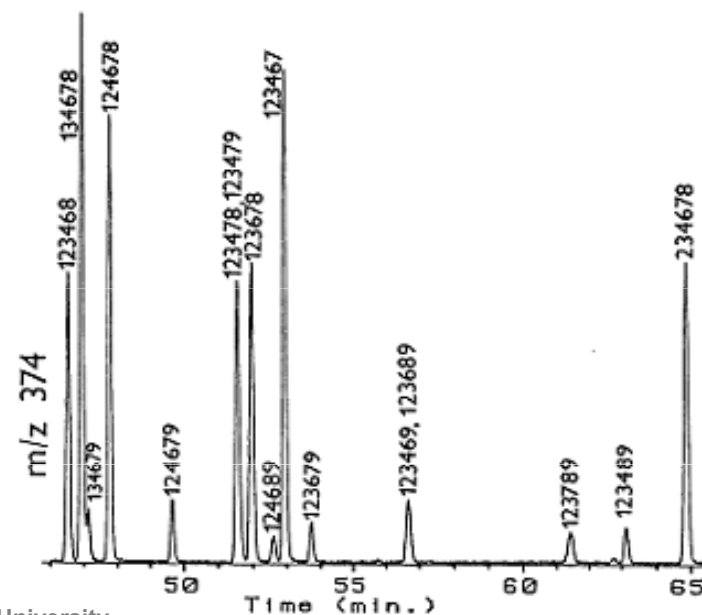
Electron Impact Ionization Source

The molecules are bombarded by electrons (e⁻) emitted by a heated filament (W or Re) in vacuum (~ $10^{-5} - 10^{-6}$ mbar)

~70 Volts

Electron Collector (Trap)

Positive Ions

Neutral Molecules

Inlet

Repeller

to Analyzer

e⁻ e⁻ e⁻

Electrons

Filament

Extraction Plate

Electron Impact Ionization Source

The electrons are accelerated towards an anode (electron trap)

~70 Volts

Electron Collector (Trap)

Positive Ions

Repeller

Neutral Molecules

Inlet

to Analyzer

Electrons

e⁻ e⁻ e⁻

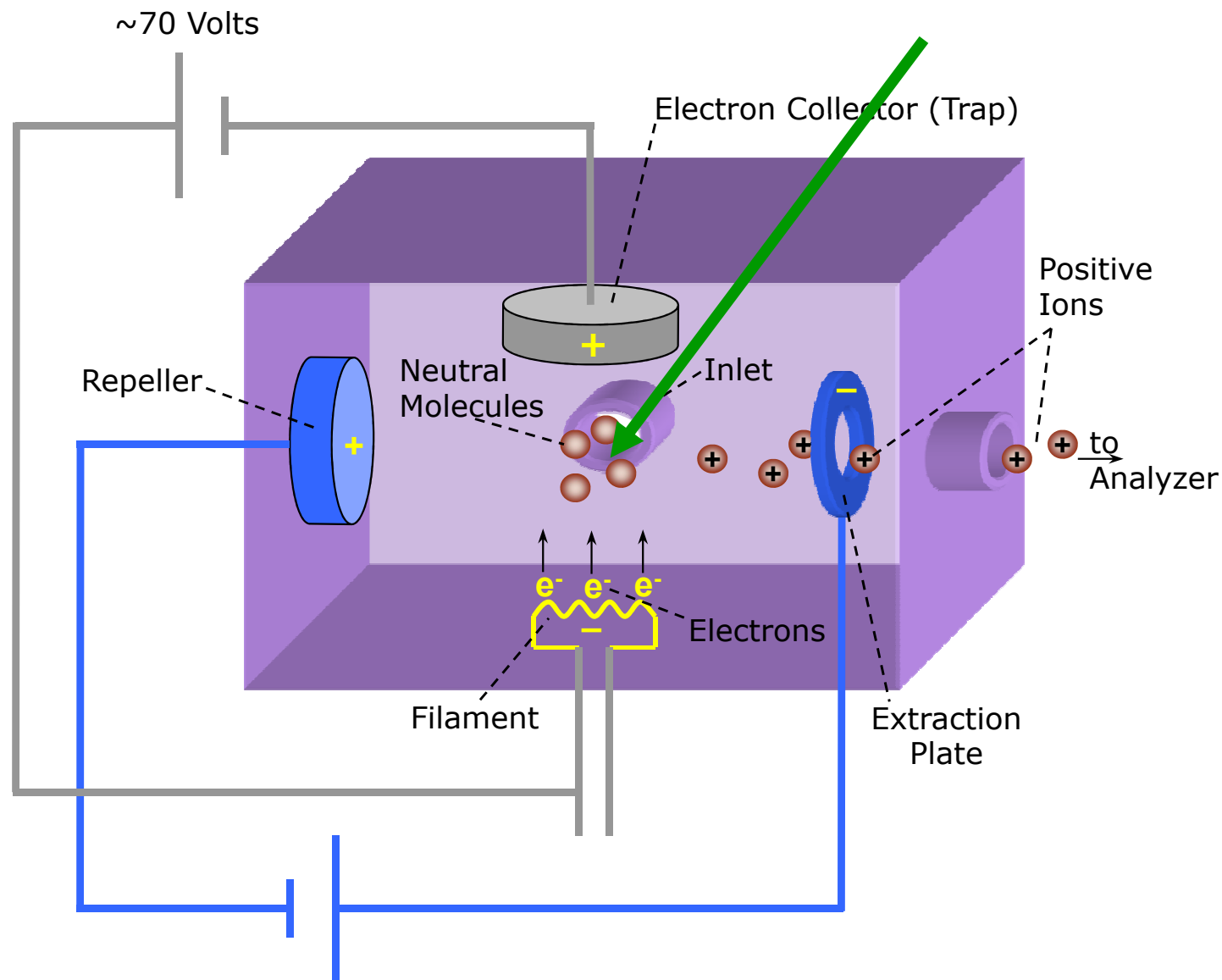Filament

Extraction Plate

Electron Impact Ionization Source

**Energy of e⁻ : 15 to 100 eV;
70 eV is usually used for scaning
mass spectra**
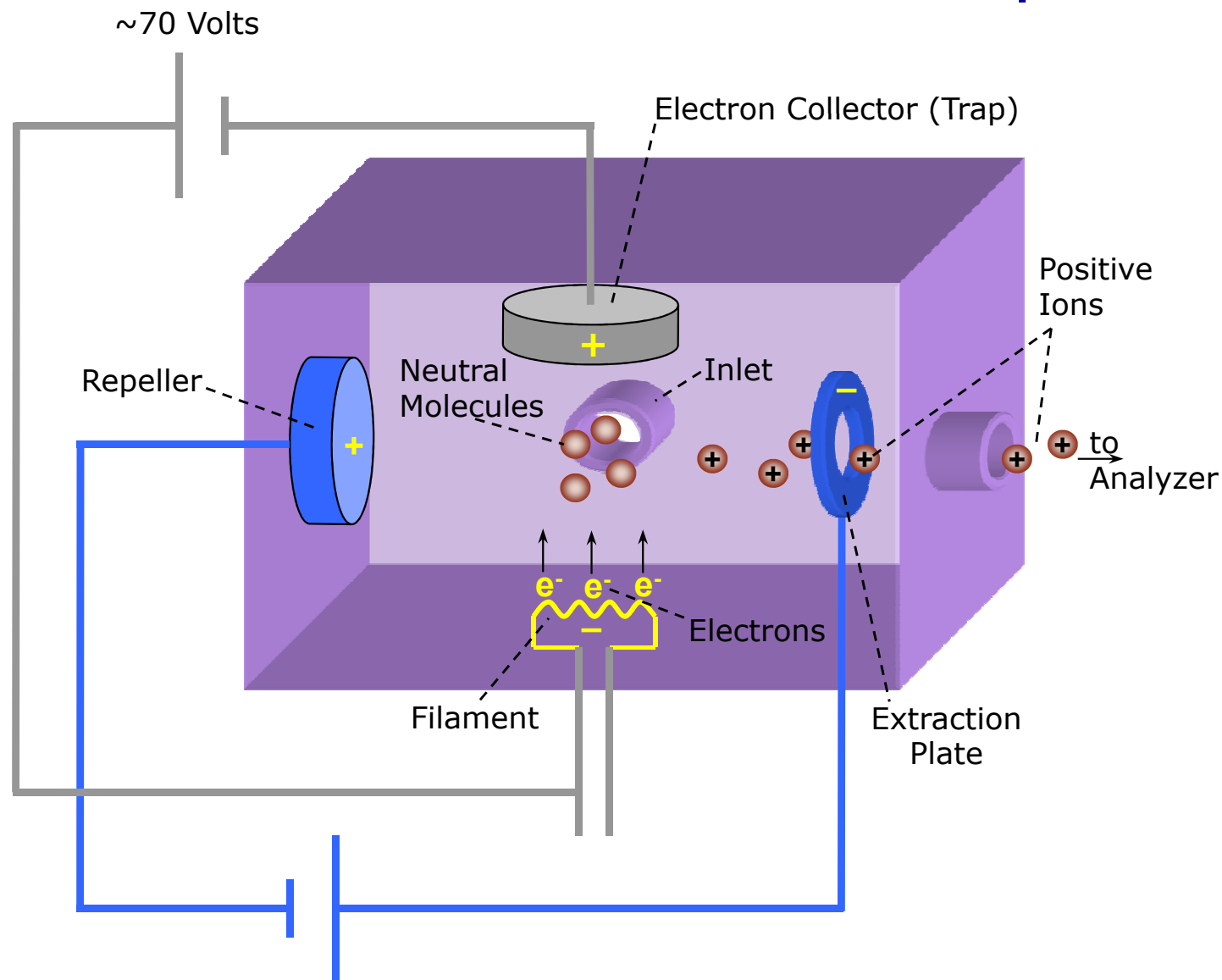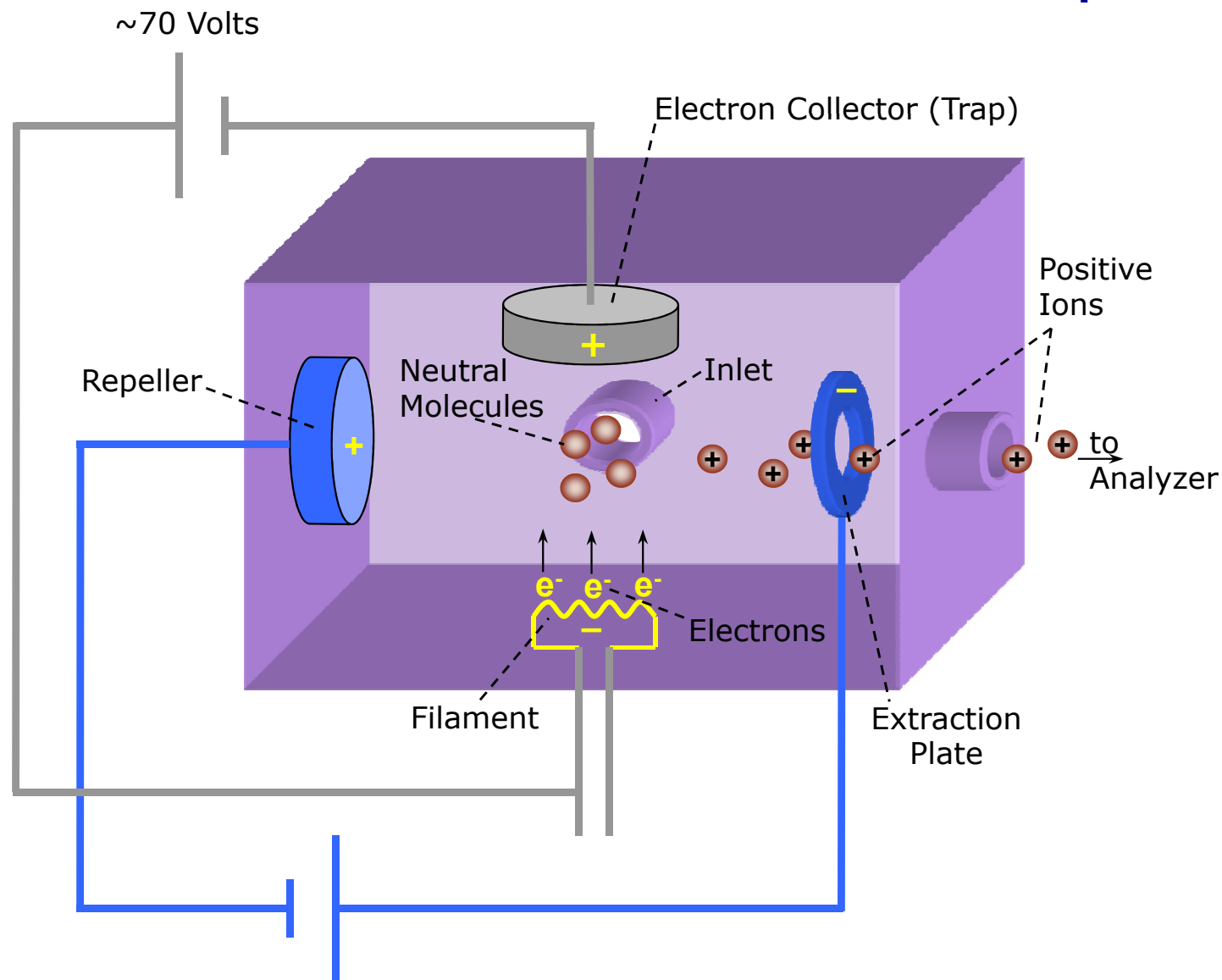
Electron Impact Ionization Source

Electron Impact Ionization Source

10 to 20 eV out of those 70 eV are transferred to the molecules during the ionization process;

Since ~ 10 eV are enough to ionize most organic molecules the excess energy leads to extensive fragmentation;
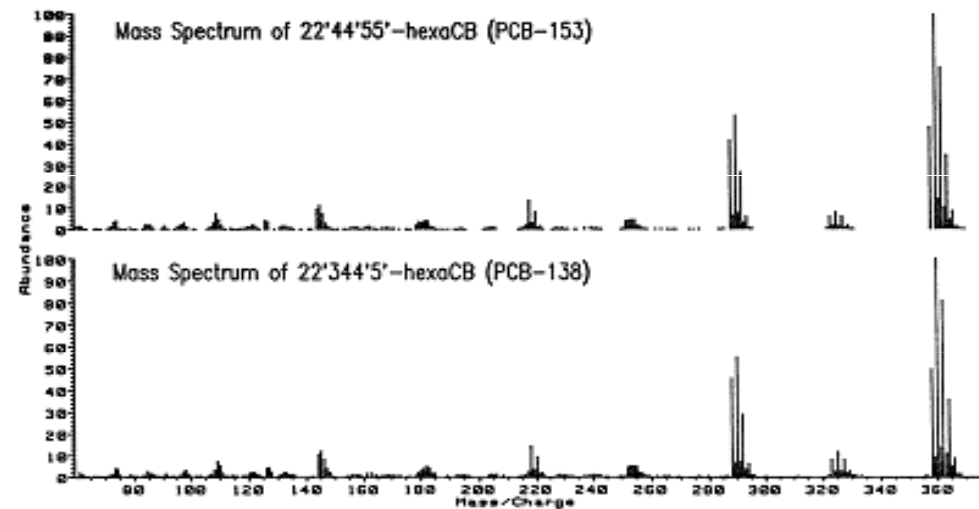
Hence EI is classified as a „hard" ionization technique

The fragmentation gives structural information

~70 Volts

Electron Collector (Trap)

Positive Ions

Repeller

Neutral Molecules

Inlet

+

to Analyzer

e⁻ e⁻ e⁻

Electrons

Filament

Extraction Plate
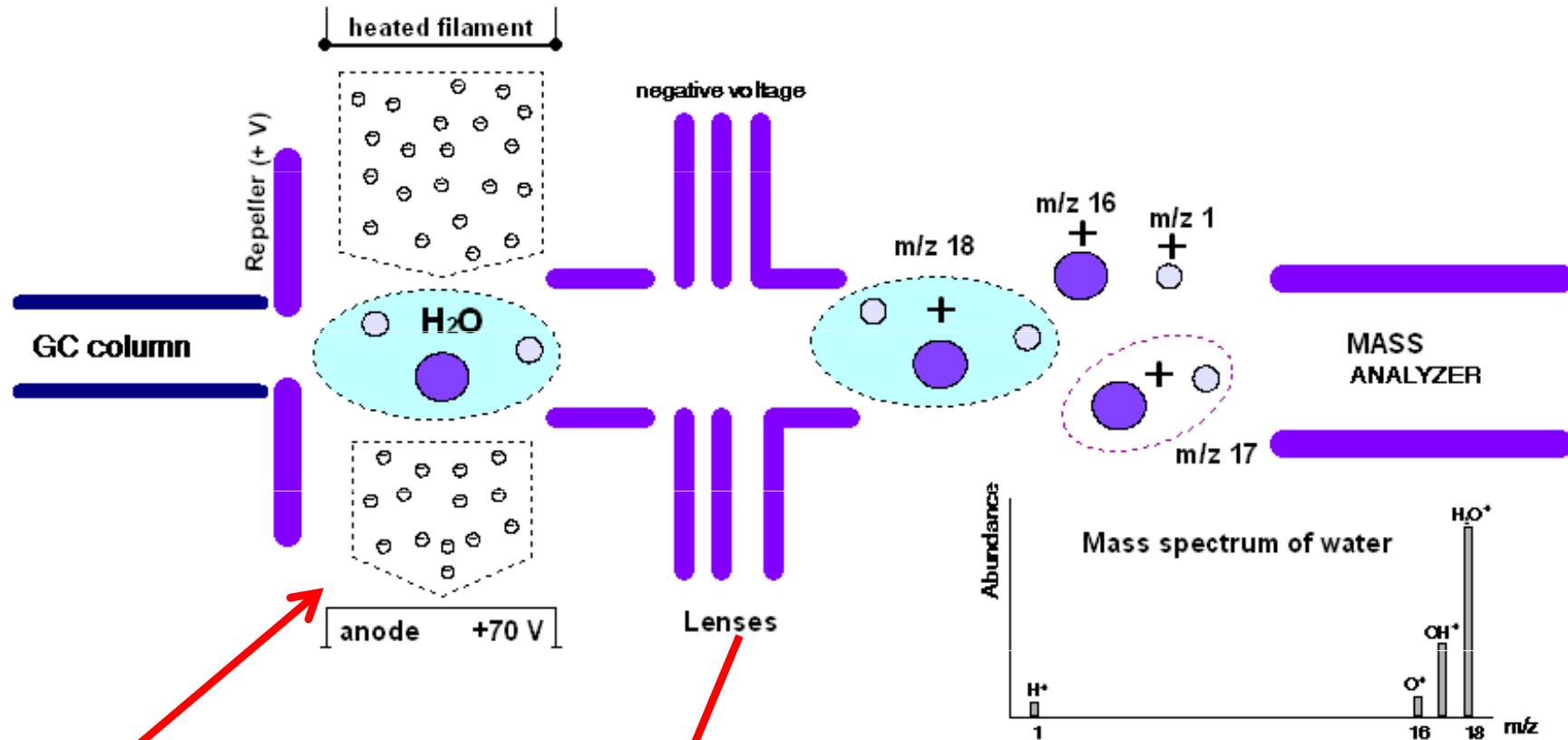
A. Kočan, Slovak Medical University
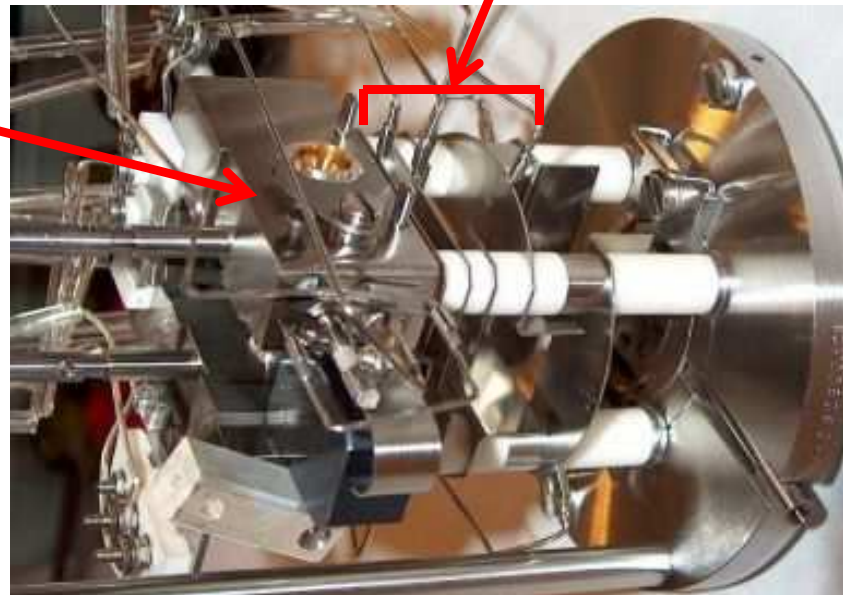
Electron Ionization

- **One of the oldest and simplest methods (1930 – 1950)**
- **Applicable for vaporizable samples only, e.g. compounds with low molecular weight (< 1000 Da), less polar organic molecules**
- **Advantages:**
  - **Unimolecular fragmentation (ions spend 1 μs in EI source)**
  - **Reproducible technique**

- **Disadvantages:**
  - **Sometimes $M^{+\cdot}$ is not observed due to fragmentation**
  - **Almost impossible distinguish between isomers (GC separation needed)**
  - **Some compounds can undergo thermal decomposition before ionization because of high temperatures used to vaporize the sample**
  - **Inappropriate to too involatile compounds**

Mass Spectrum of 22'44'55'−hexaCB (PCB−153)

Mass Spectrum of 22'344'5'−hexaCB (PCB−138)

Schematic of Electron Ionization

heated filament

Repeller (+ V)

negative voltage

GC column

H_2O

m/z 18

m/z 16

m/z 1

m/z 17

MASS ANALYZER

anode +70 V

Lenses

Abundance

Mass spectrum of water

H^+

O^+

OH^+

H_2O^+

1

16

18

m/z

Ionization chamber

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Positive Chemical Ionization

- Developed in the 1960s.
- Gaseous ions such as CH_5^+, $C_2H_5^+$, $C_4H_9^+$, NH_4^+, H_3^+ generated by electron impact from a large excess of a reagent gas, such as CH_4, NH_3, H_2, or $i\text{-}C_4H_{10}$, interact with neutral molecules that may ionize.
- Generally, the amount of fragments is much less than in EI since little internal energy is imparted on the ionized molecule; Thus, the important molecular ion can be determined.
- Hence, CI is termed as „soft" ionization technique.
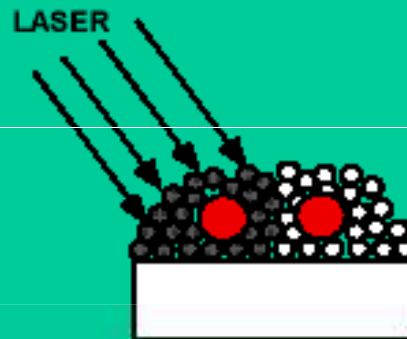
Electrospray
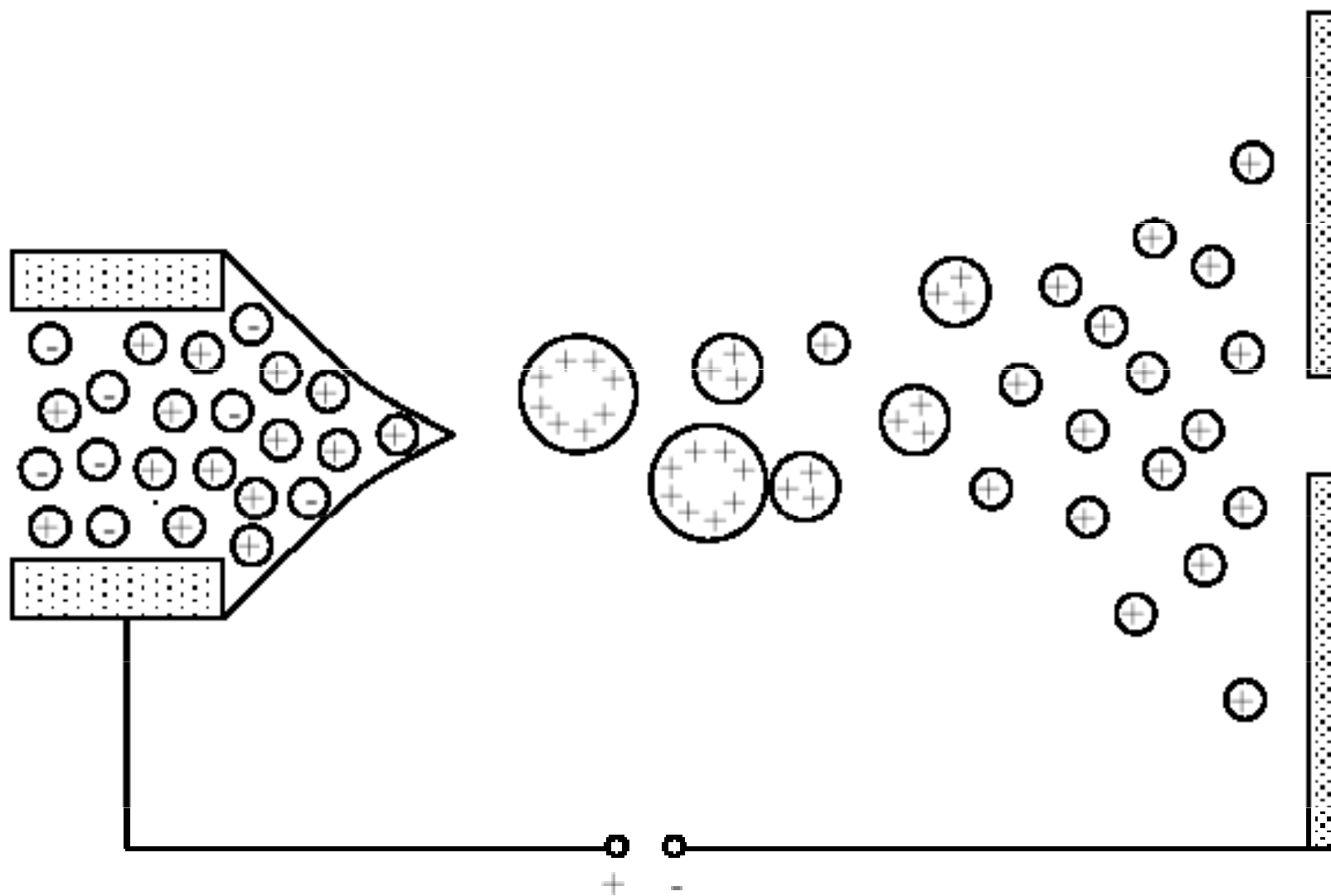
MALDI

Matrix-assisted Laser Desorption/Ionization (MALDI)

Sample embedded in light-absorbing matrix
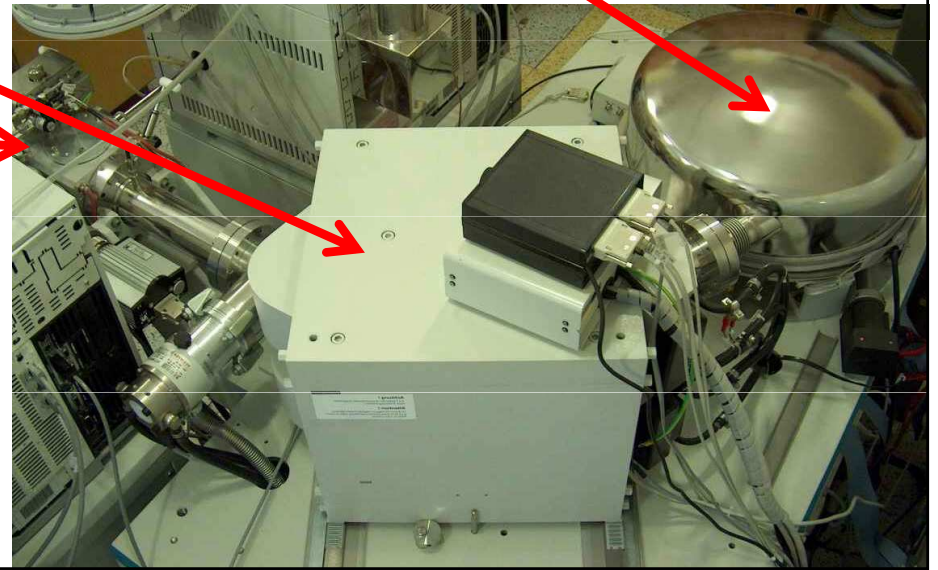
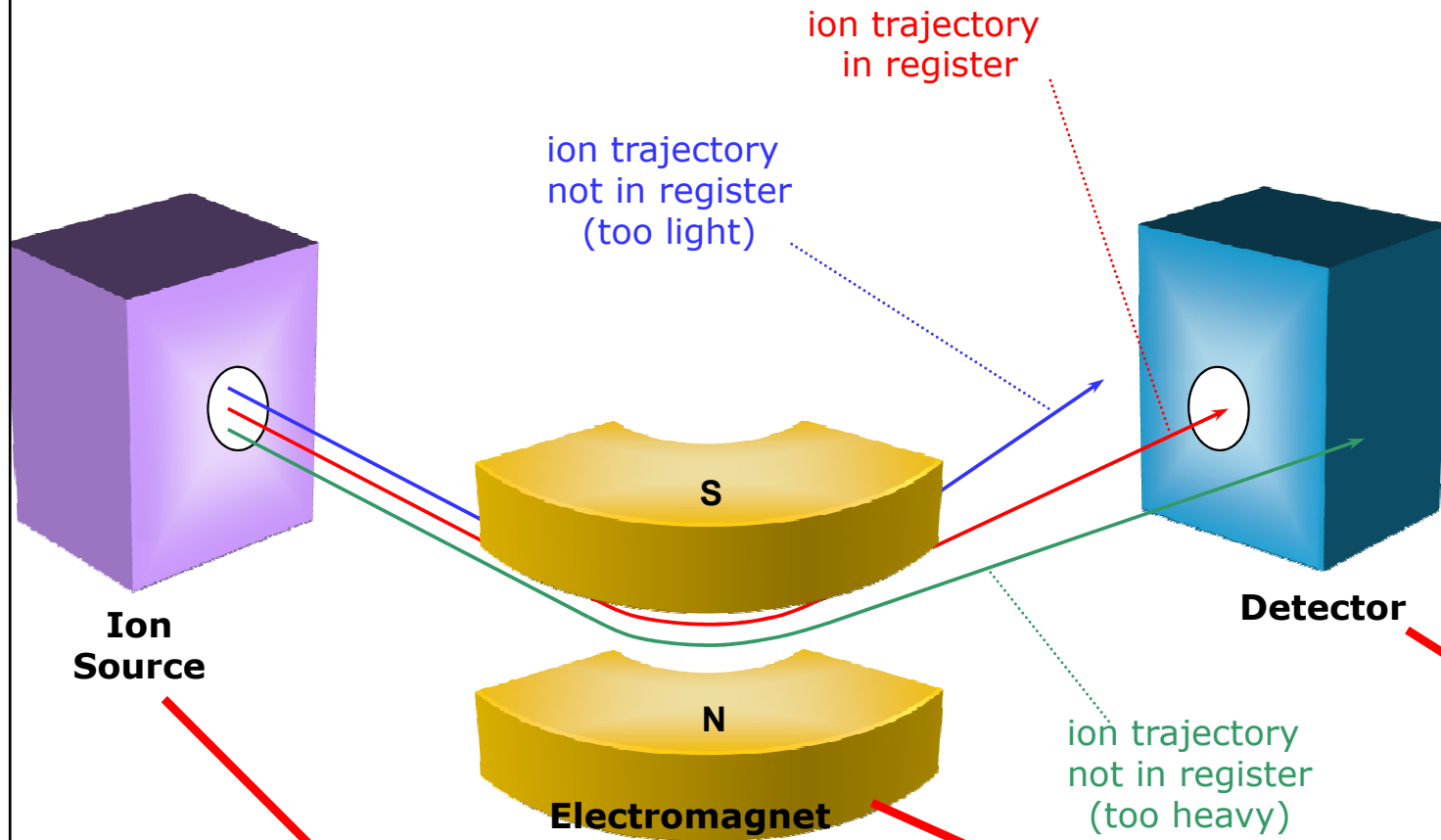LASER-excitation of matrix molecules

Sample desorption and protonation

LASER

BRUKER DALTONICS®

Enabling Life Science Tools Based on Mass Spectrometry™

Magnetic Sector Mass Analyzer

ion trajectory
in register

ion trajectory
not in register
(too light)

ion trajectory
not in register
(too heavy)

Ion Source

S

N

Electromagnet

Detector

A. Kočan, Slovak Medical University

High Resolutionn MS Autospec-Ultima

- **3-sector instrument (2 electrostatic sectors, 1 magnetic sector)**
- **Mattauch-Herzog geometry**
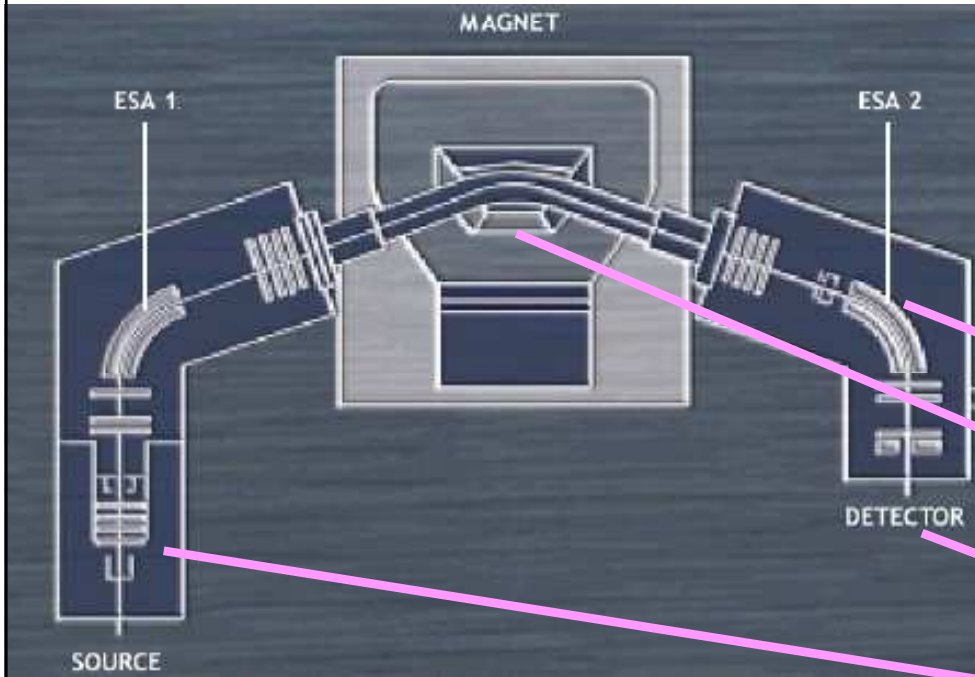
Dual HRGC / HRMS System

Rtx-2330

DB5-ms

MAT 95 XL
ThermoQuest

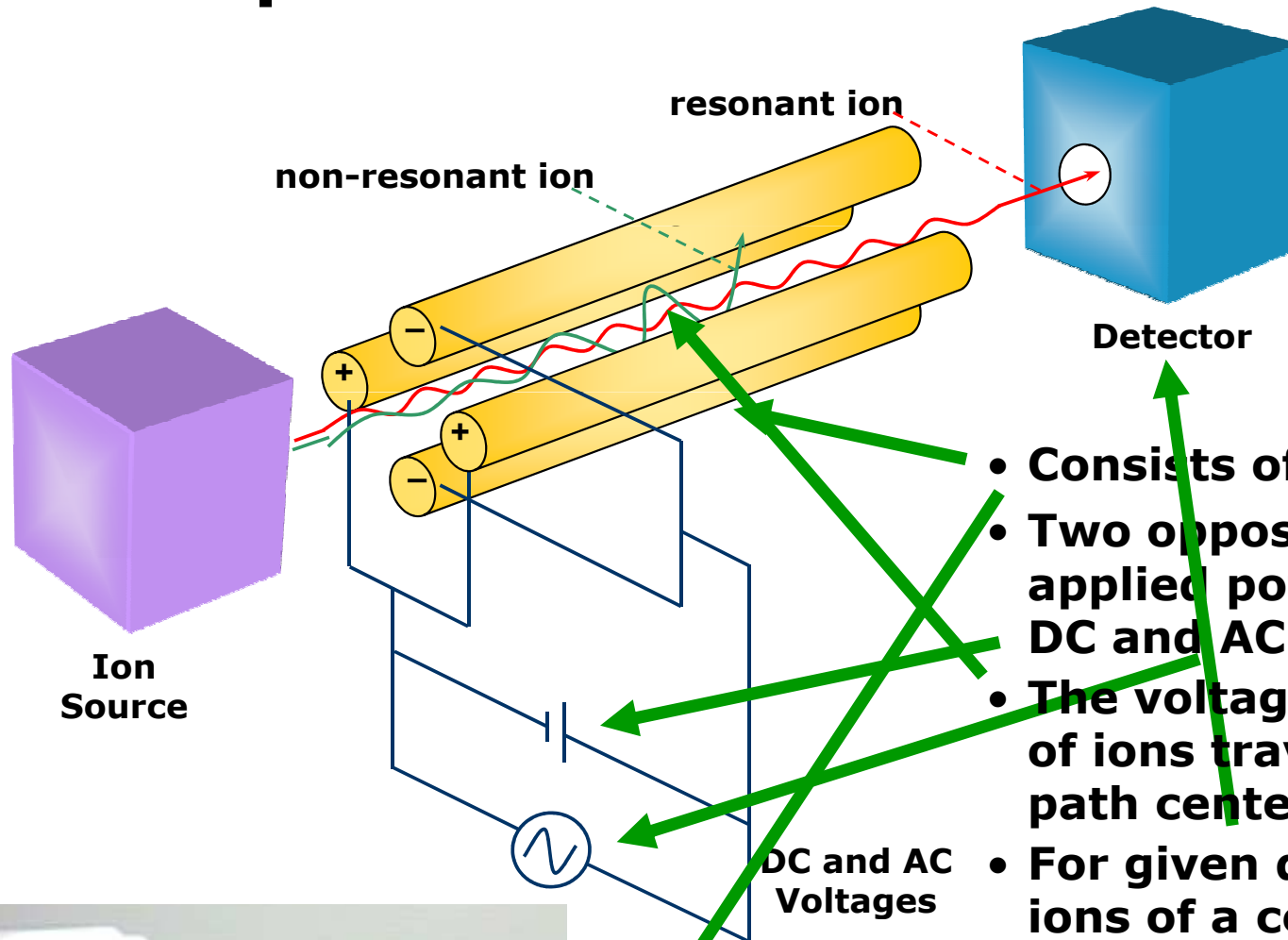A. Kočan, Slovak Medical University

Quadrupole Mass Filter

resonant ion

non-resonant ion
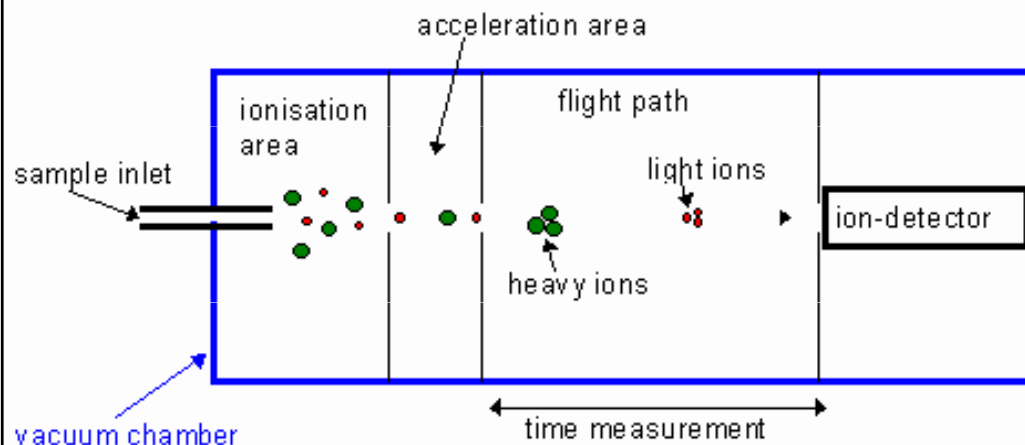
Detector

Ion Source

DC and AC Voltages

- **Consists of 4 parallel metal rods.**
- **Two opposite rods have an applied potential combined from DC and AC voltages.**
- **The voltages affect the trajectory of ions traveling down the flight path centered between the rods.**
- **For given dc and ac voltages, only ions of a certain mass-to-charge ratio pass through the filter and all other ions are thrown out of their original path.**
- **A mass spectrum is obtained by monitoring the ions passing through the quadrupole filter as the voltages or frequency on the rods are varied.**

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Time-Of-Flight Mass Spectrometry (TOFMS)

- **It uses differences in transit time through a drift region to separate ions of different masses**
- **An electric field accelerates all ions into a field-free drift region with the same initial kinetic energy for all the ions produced**
- **It operates in a pulsed mode so ions must be produced or extracted in pulses**
- **Since the ion kinetic energy is $0.5mv^2$, lighter ions have a higher velocity than heavier ions and reach the detector sooner (e.g., ions of m/z 500 arrive in ~ 15 μs and m/z 50 in ~ 4.6 μs**
- **By TOF-MS, up to 50 000 full spectra can be measured in a second**
- **Since full spectra are available, peak deconvolution software enabling to differentiate non-separated GC peaks may be applied**
- **The TOF ultra-fast scanning is suitable for fast GC where peak widths can be much less then a second**

Reflectron Flight Tube Liner Detector Pre Amplifier Deflectors Source Pulser

Ion Formation

acceleration area

ionisation area flight path

sample inlet

light ions

ion-detector

heavy ions

vacuum chamber

time measurement
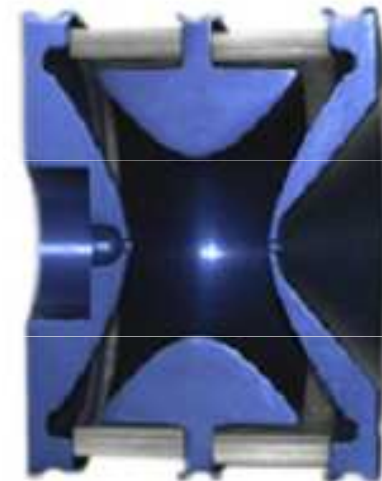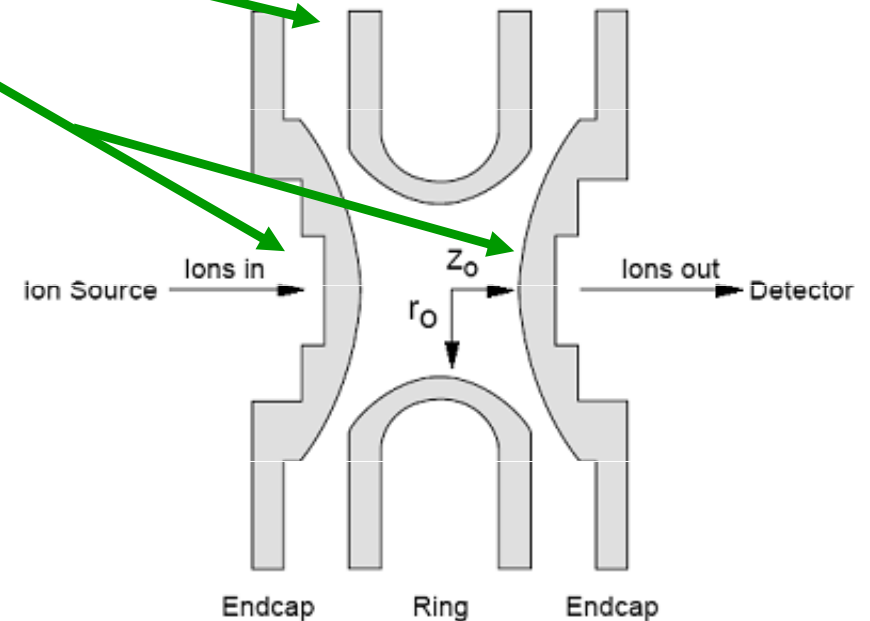
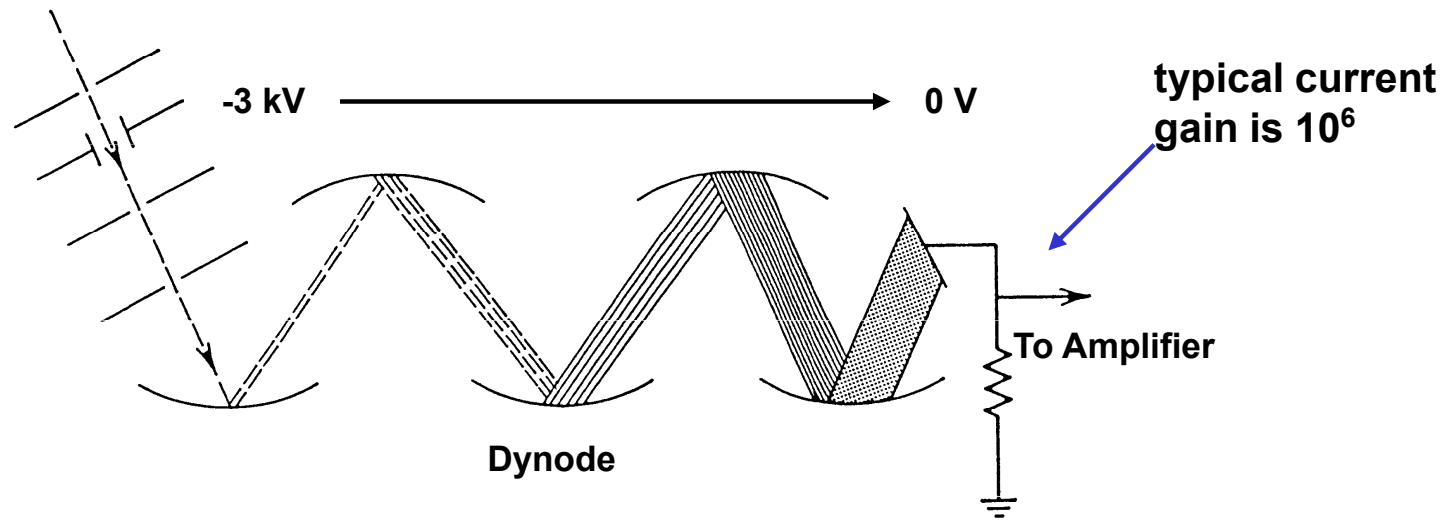A. Kočan, Slovak Medical University
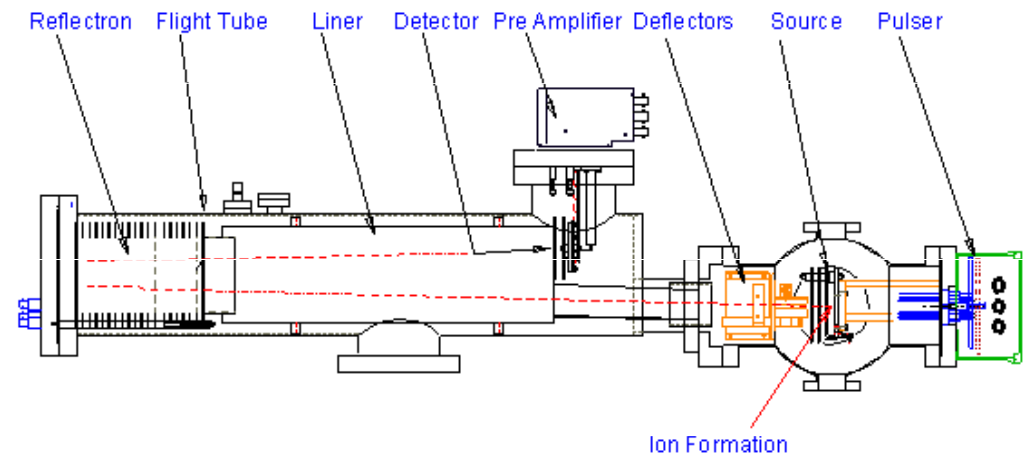
Ion Trap Mass Spectrometry

- **The ion-trap analyzer consists of 3 electrodes with hyperbolic surfaces to trap ions in a small volume – the central ring electrode and 2 adjacent endcap electrodes. A mass spectrum is obtained by changing the electrode voltages to eject the ions from the trap.**

- **The advantages of the ion-trap mass spectrometer include compact size, and the ability to trap and accumulate ions to increase the signal-to-noise ratio of a measurement.**

- **This technique can be used easily in the MS/MS (MS^n) mode**

ion Source   Ions in Z_o Ions out   Detector
r_O

Endcap   Ring   Endcap

Electron Multiplier

-3 kV → 0 V

typical current gain is 10^6

Dynode

To Amplifier

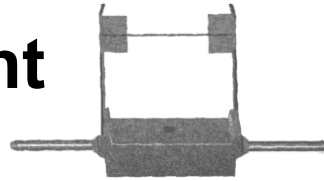Discrete-dynode electron multiplier

Continuous-dynode electron multiplier

Vacuum System

Why needed?

- **Mean free path of molecules**
- **Ion-molecular reactions**
- **Interferences in mass spectra**
- **Contamination of the ion source**
- **Glowing/sparking in the high-voltage area**
- **Burning of the filament**

✓ **Rough pumps**

✓ **Diffusion pumps**

✓ **Turbomolecular pumps**

Mass Spectrum

Relative abundance (%)

100

Fragment ion

[M-Y]$^{+\cdot}$

Base peak
[M-X]$^{+\cdot}$

Molecular ion
M$^{+\cdot}$

m/z
(mass-to-charge ratio)
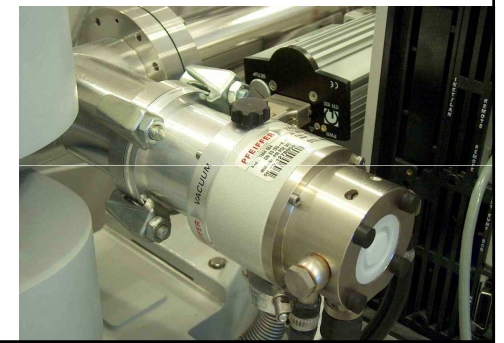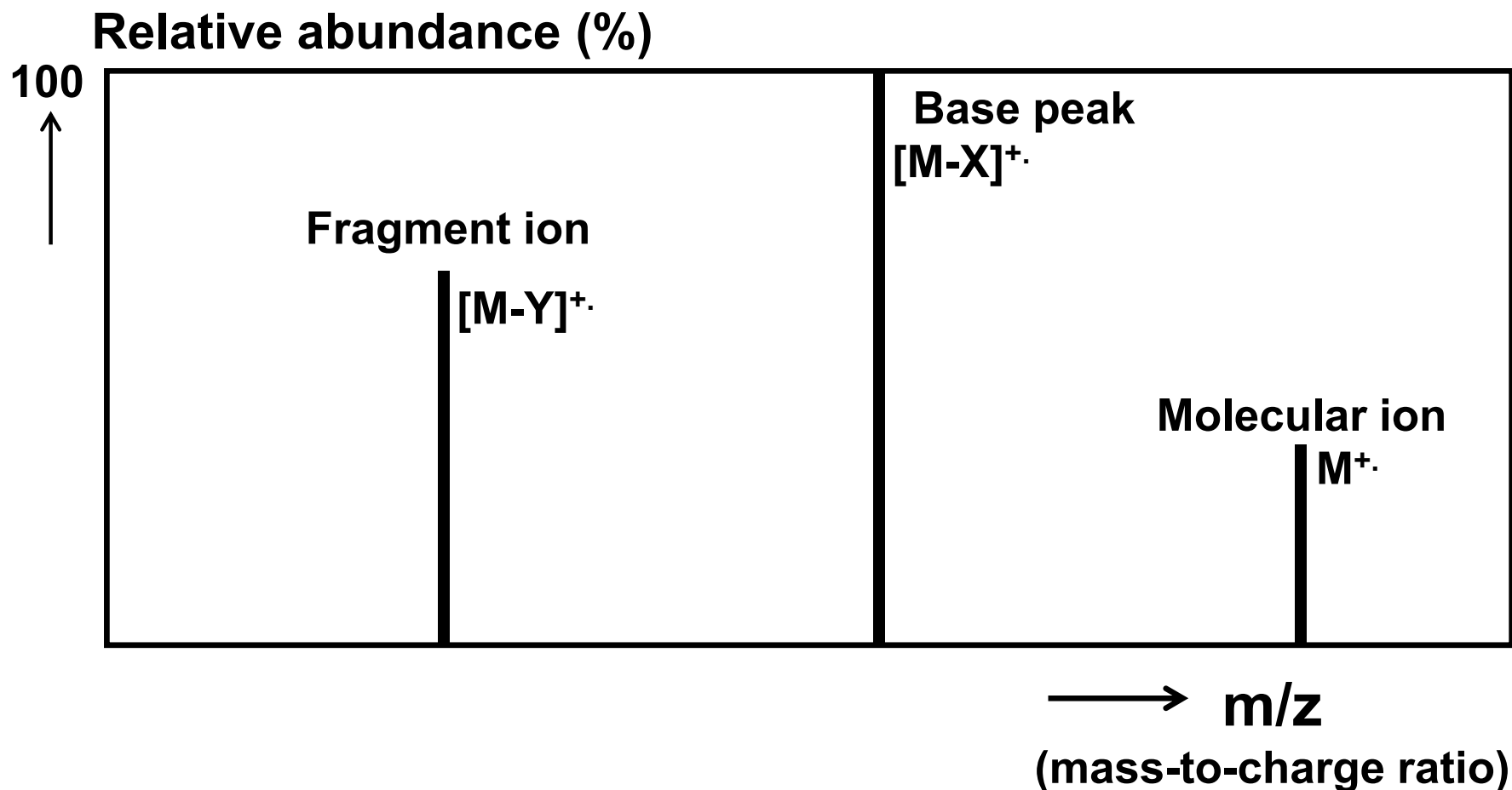
There are now Internet-accessible databases containing mass spectra of thousands of compounds, e.g.:
http://www.aist.go.jp/RIODB/SDBS/menu-e.html
http://webbook.nist.gov

Segment of the Real Signal (not computer-processed) of a Mass Spectrum
(n-cethyl amine – $C_{16}H_{33}NH_2$)
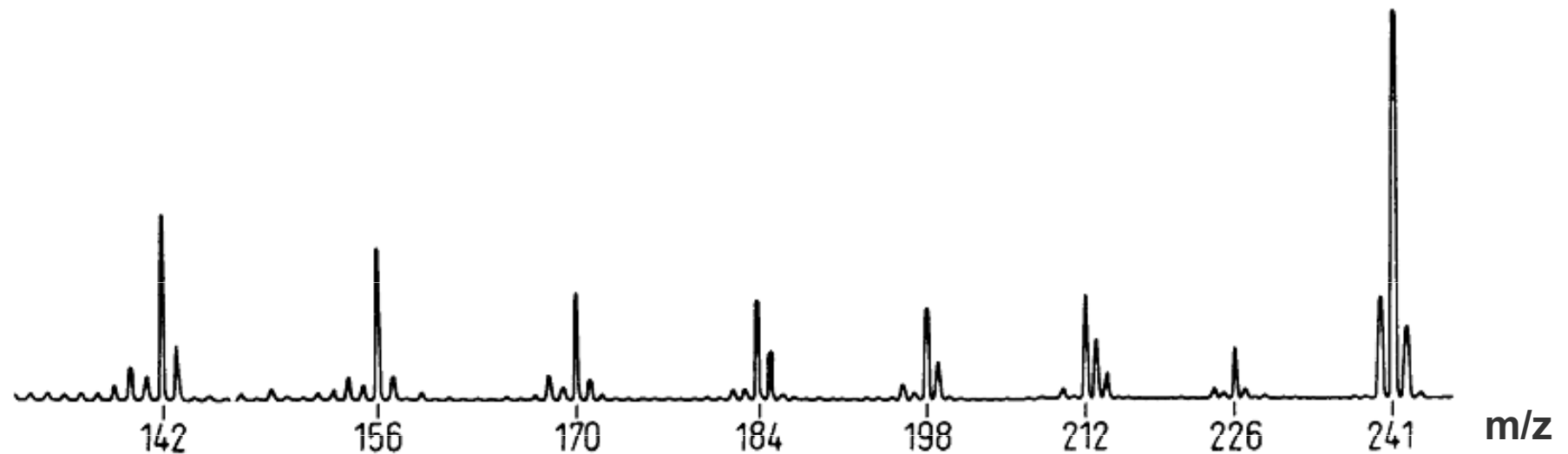
- **Such a Gauss-shaped peaks are processed by a computer program to be converted to the line spectrum**

GC-MS Uses

- **Identification and quantification of volatile and semivolatile organic compounds in complex mixtures**
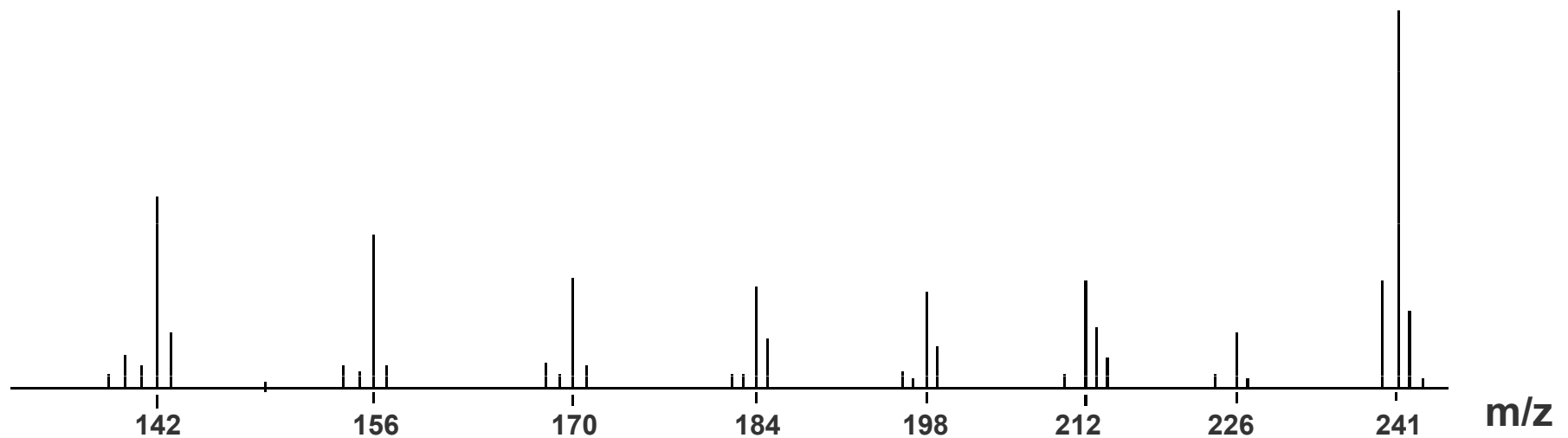
- **Determination of molecular weights and (sometimes) elemental composition of unknown organic compounds in complex mixtures**

- **Structural determination of unknown organic compounds in complex mixtures both by matching their mass spectra with reference spectra and by a priori spectral interpretation**

When to Use HRMS ?

- **For the determination of the exact masses of ions in spectra (peak matching) – suitable for the determination of elemental composition**

- **Decreasing interferences caused by the ions of co-extracted compounds or by GC column bleeding and thus, to improve considerably the signal-to-noise ratio**

Fragmentation schemes under EI conditions

Polychlorinated dibenzo-p-dioxins

$[M-2CO]^{+\cdot}$ $\xleftarrow{\;*\;-2CO\;}$ ... $M^{+\cdot}$ $\xrightarrow{\;*\;-HCO\cdot\;}$ $[M-HCO]^{+}$

$M^{+\cdot}$ $\xrightarrow{\;-Cl\cdot\;}$ $[M-Cl]^{+}$ $\xrightarrow{\;-Cl\cdot\;}$ $[M-Cl_2]^{+\cdot}$

$[M-(CO)_2HCl]^{+\cdot}$ $\xleftarrow{\;*\;-HCO\cdot\;}$ $[M-COCl]^{+}$ $\xleftarrow{\;-CO\;}$
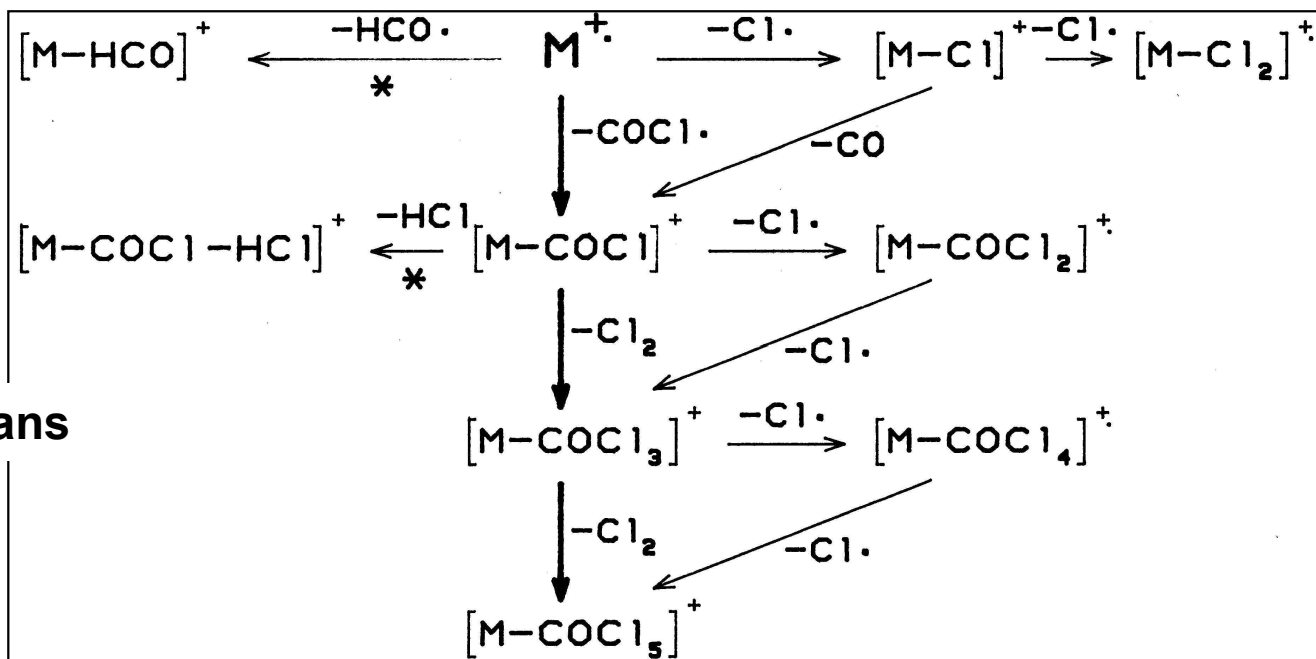
$M^{+\cdot}$ $\xrightarrow{\;-COCl\cdot\;}$ $[M-COCl]^{+}$

$[M-COCl_2]^{+\cdot}$ $\xleftarrow{\;-Cl\cdot\;}$ $[M-COCl]^{+}$ $\xrightarrow{\;-CO\;}$ $[M-(CO)_2Cl]^{+}$

$[M-COCl_3]^{+}$ $\xleftarrow{\;-Cl\cdot\;}$ $[M-COCl_2]^{+\cdot}$ $\xrightarrow{\;-CO\;}$ $[M-2COCl]^{+\cdot}$

$[M-COCl]^{+}$ $\xrightarrow{\;-COCl\cdot\;}$ $[M-2COCl]^{+\cdot}$ $\xleftarrow{\;-Cl\cdot\;}$ $[M-(CO)_2Cl]^{+}$

$[M-2COCl]^{+\cdot}$ $\xrightarrow{\;-Cl\cdot\;}$ $[M-(CO)_2Cl_3]^{+}$

$[M-2COCl]^{+\cdot}$ $\xrightarrow{\;-Cl_2\;}$ $[M-(CO)_2Cl_4]^{+\cdot}$ $\xleftarrow{\;-Cl\cdot\;}$ $[M-(CO)_2Cl_3]^{+}$
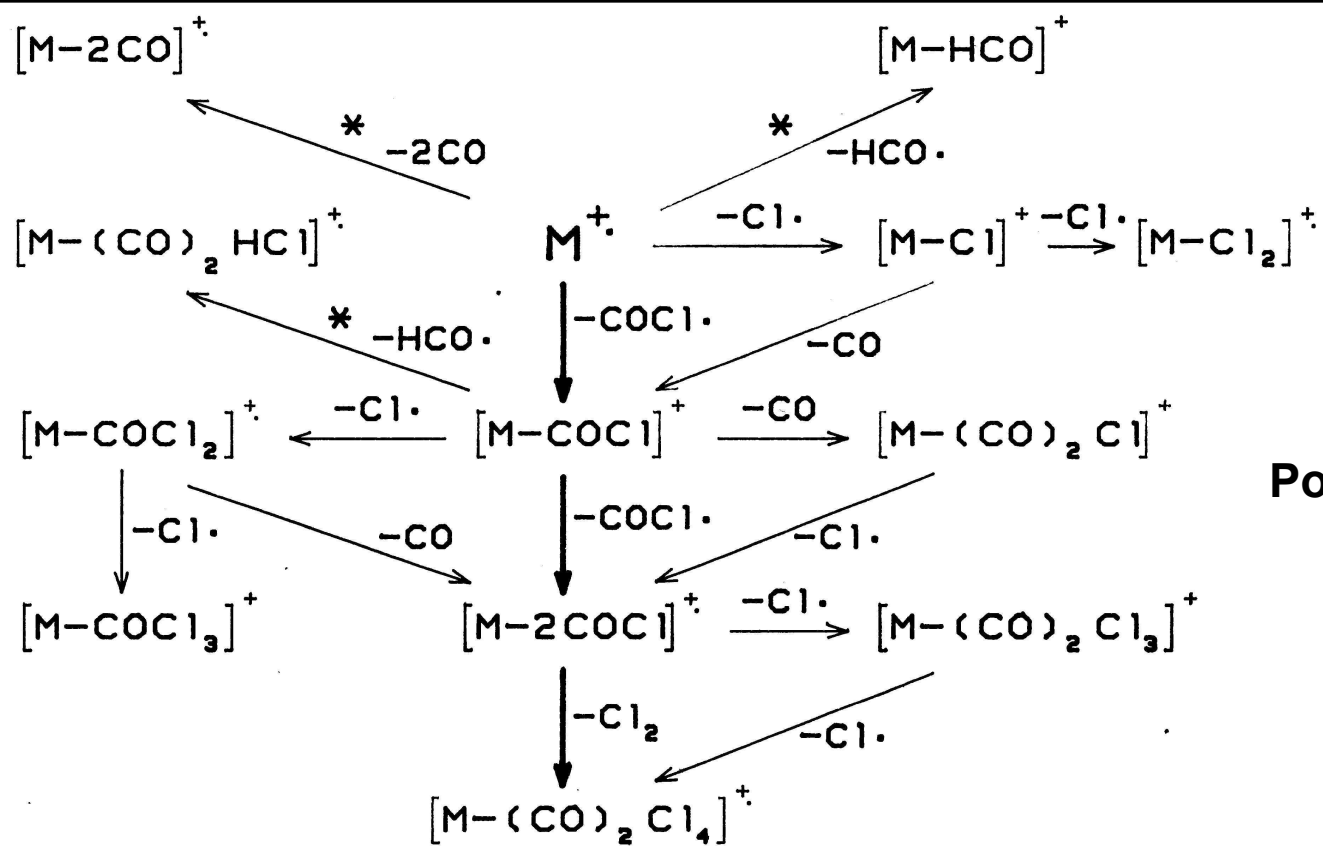
* **fragmentation for lower chlorinated congeners**

Polychlorinated dibenzofurans

$[M-HCO]^{+}$ $\xleftarrow{\;-HCO\cdot\;*\;}$ $M^{+\cdot}$ $\xrightarrow{\;-Cl\cdot\;}$ $[M-Cl]^{+}$ $\xrightarrow{\;-Cl\cdot\;}$ $[M-Cl_2]^{+\cdot}$

$M^{+\cdot}$ $\xrightarrow{\;-COCl\cdot\;}$ $[M-COCl]^{+}$ $\xleftarrow{\;-CO\;}$

$[M-COCl-HCl]^{+}$ $\xleftarrow{\;-HCl\;*\;}$ $[M-COCl]^{+}$ $\xrightarrow{\;-Cl\cdot\;}$ $[M-COCl_2]^{+\cdot}$

$[M-COCl]^{+}$ $\xrightarrow{\;-Cl_2\;}$ $[M-COCl_3]^{+}$ $\xleftarrow{\;-Cl\cdot\;}$ $[M-COCl_2]^{+\cdot}$

$[M-COCl_3]^{+}$ $\xrightarrow{\;-Cl\cdot\;}$ $[M-COCl_4]^{+\cdot}$

$[M-COCl_3]^{+}$ $\xrightarrow{\;-Cl_2\;}$ $[M-COCl_5]^{+}$ $\xleftarrow{\;-Cl\cdot\;}$ $[M-COCl_4]^{+\cdot}$
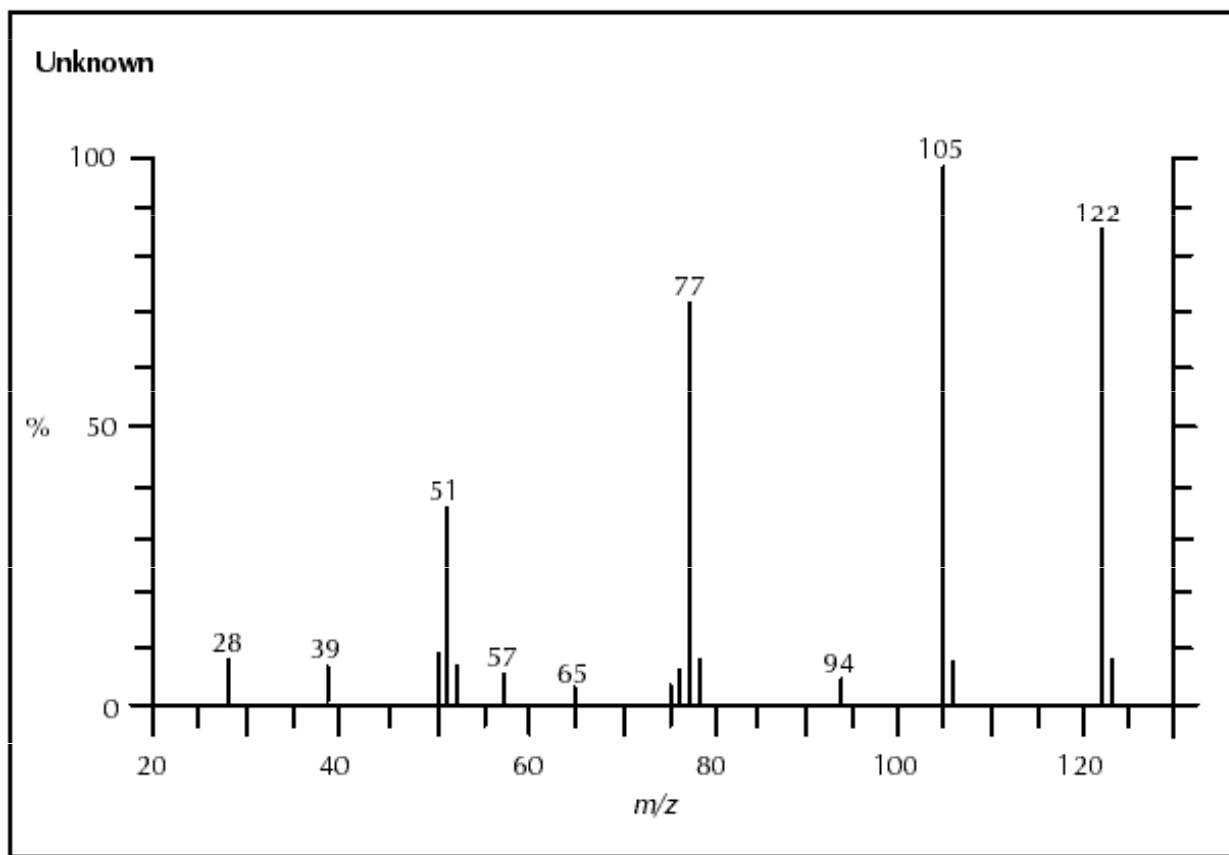
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Demonstration of Mass Spectrometric Resolution (10% Valley Definition)

$$R_{10\%} = m / \Delta m$$
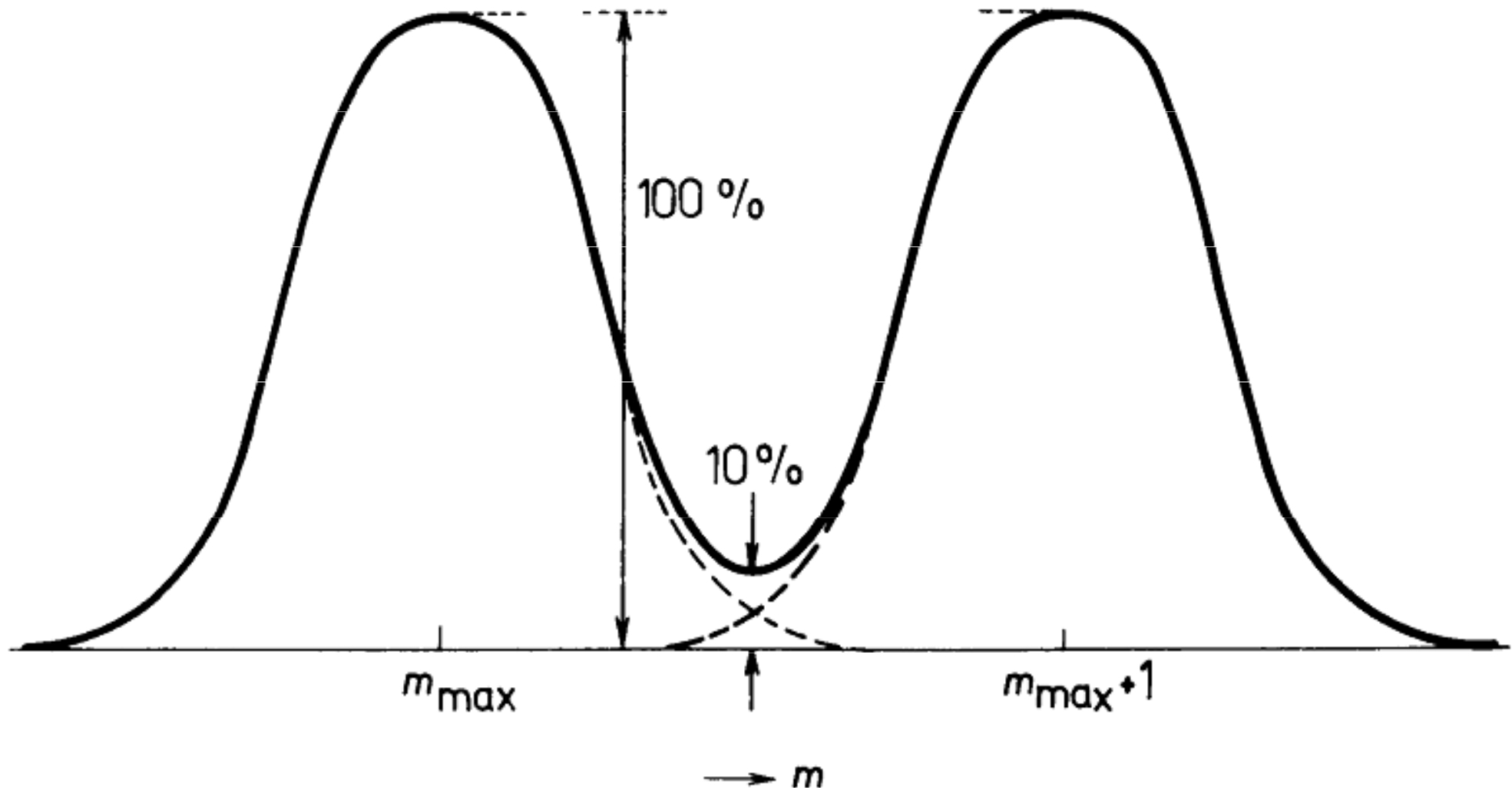
Some formulae corresponding to nominal m/z = 122

| Formulae	Actual mass
$C_4H_4N_5$	122.046668
$C_4H_{10}O_4$	122.057903
$C_6H_4NO_2$	122.024201
$C_6H_6N_2O$	122.048010
$C_6H_8N_3$	122.071819
$C_7H_6O_2$	122.036776
C_7H_8NO	122.060585
$C_7H_{10}N_2$	122.084394
$C_8H_{10}O$	122.073161
$C_8H_{12}N$	122.096970
C_9H_{14}	122.109545

These are based on the following relative atomic masses:

C = 12.0000000
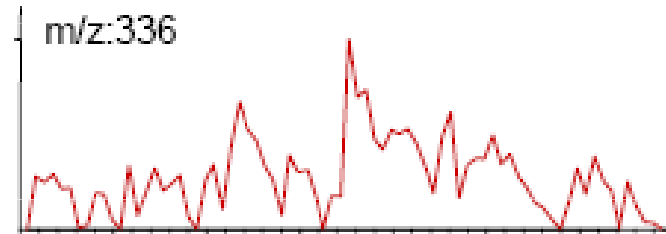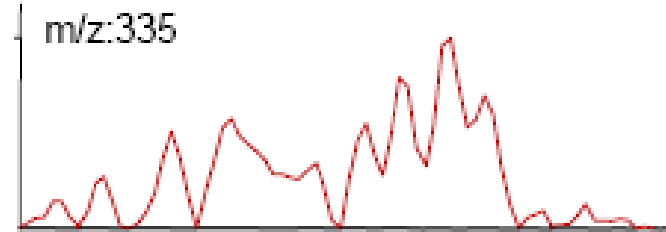H = 1.0078246
N = 14.0030738
O = 15.9949141

- **In general, more ions have the same nominal mass**
- **To distinguish between them certain MS resolution is needed**

- **For example, to separate these 2 ions we need a resolution of 5 124**

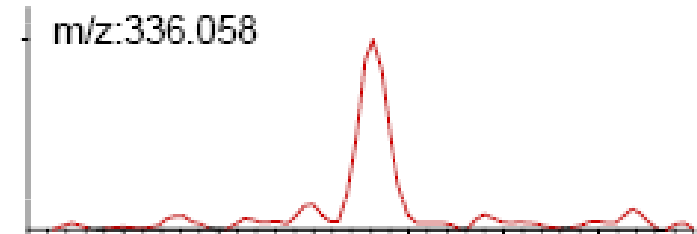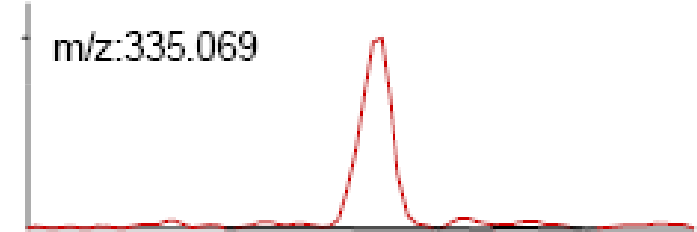**R = (122.060585 – 122.036776) / 122 = 5 124**
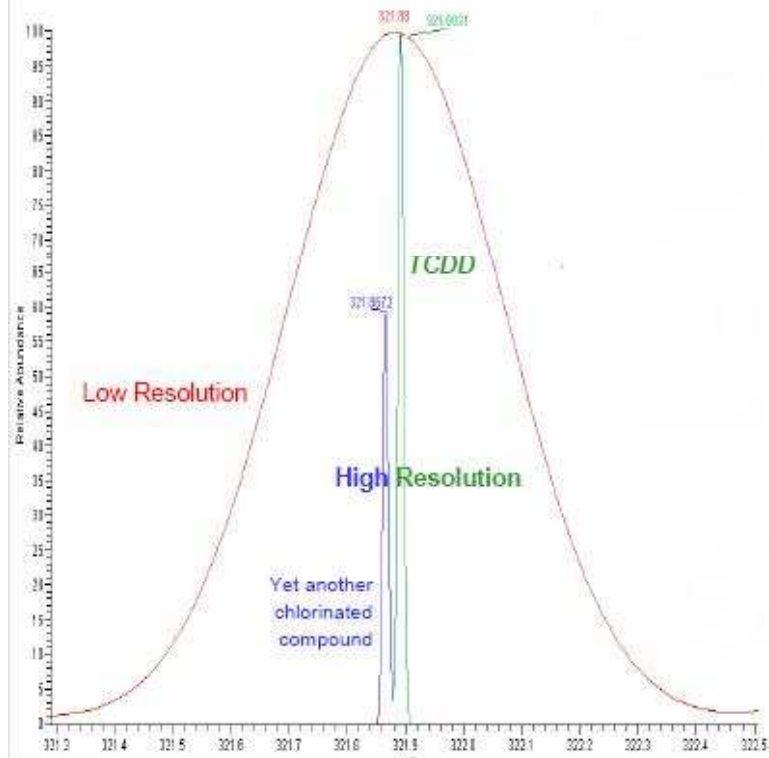
Prvek	"M"		"M+1"		"M+2"		Typ prvku
	m/z	%	m/z	%	m/z	%	
H	1	100	2	0.015	-	-	"M"
C	12	100	13	1.1	-	-	"M+1"
N	14	100	15	0.37	-	-	"M+1"
O	16	100	17	0.04	18	0.2	"M+2"
F	19	100	-	-	-	-	"M"
Si	28	100	29	5.1	30	3.4	"M+2"
P	31	100	-	-	-	-	"M"
S	32	100	33	0.79	34	4.4	"M+2"
Cl	35	100	-	-	37	32	"M+2"
Br	79	100	-	-	81	97.3	"M+2"
I	127	100	-	-	-	-	"M"

m/z	Int.
35	12.0
36	100.0
37	4.1
38	33.0

HCl
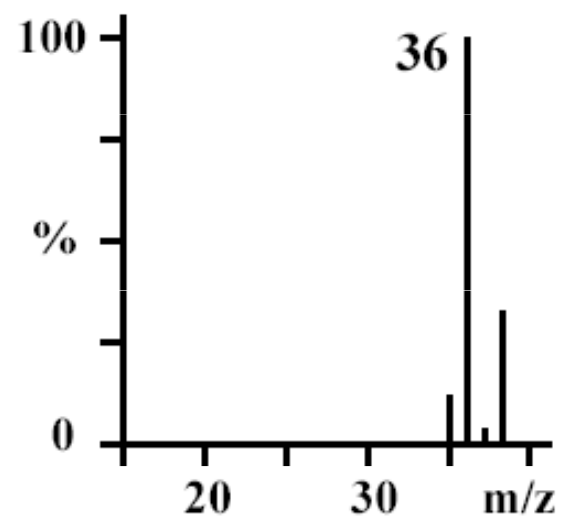
m/z	Int.
94	100.0
95	1.1
96	96.0
97	1.1

CH$_3$Br
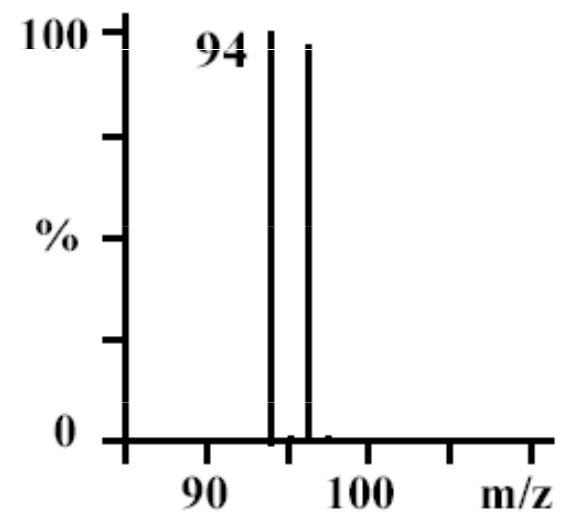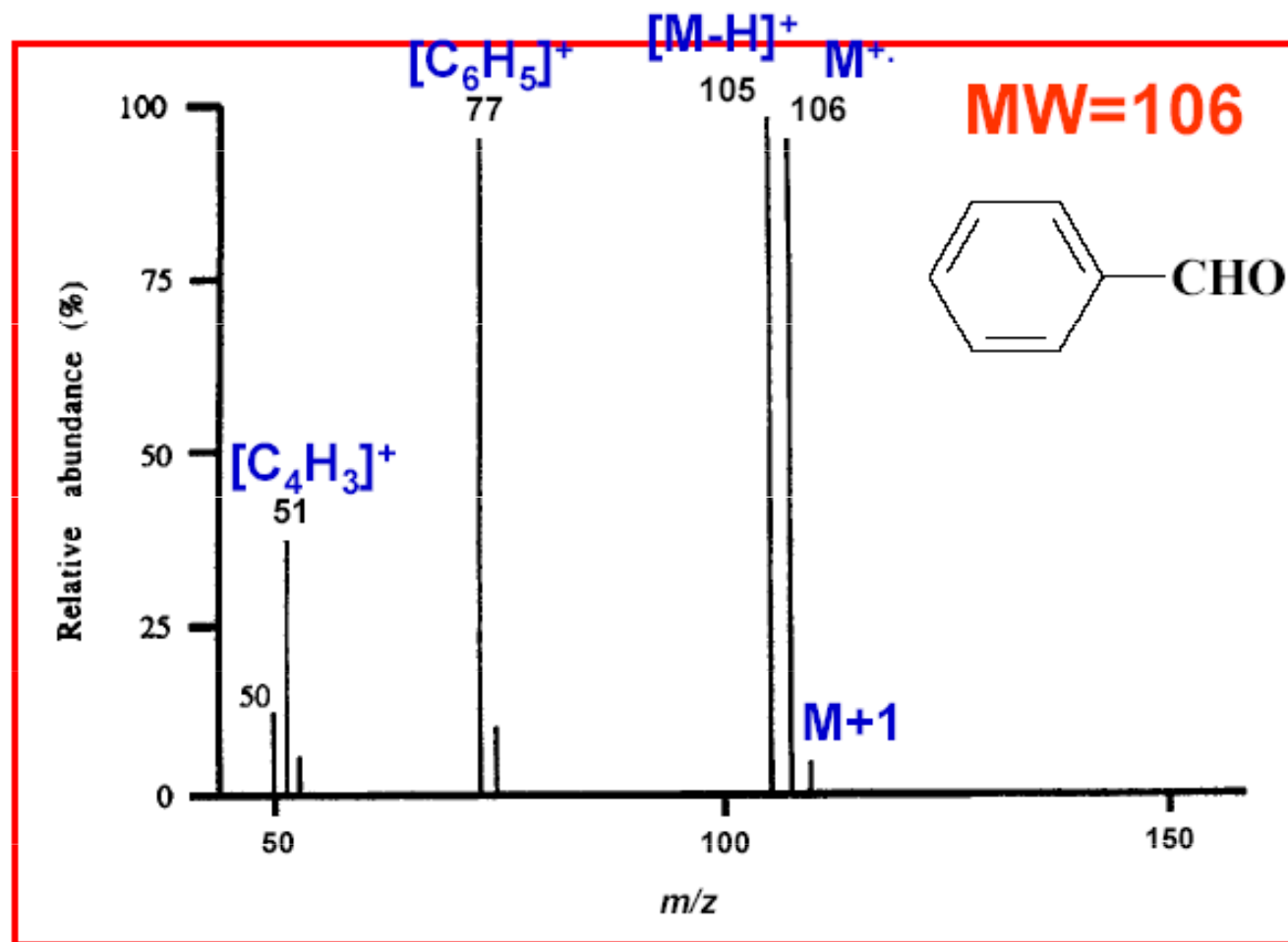
No. 2

M+1= 4.2 / 64.1*100 = 6.6% : 1.1 = 6*C

M+2= 63.7 / 64.1*100 = 99.4% = Br

m/z	% Relative abundance
50	17.2
51	38.9
74	3.2
75	5.7
76	5.7
77	100.0
78	7.8
156	64.1
157	4.2
158	63.7
159	3.8

MW=156

$[C_6H_5]^+$ 77

$[C_4H_3]^+$ 51

Br

M+· 156   M+2 158
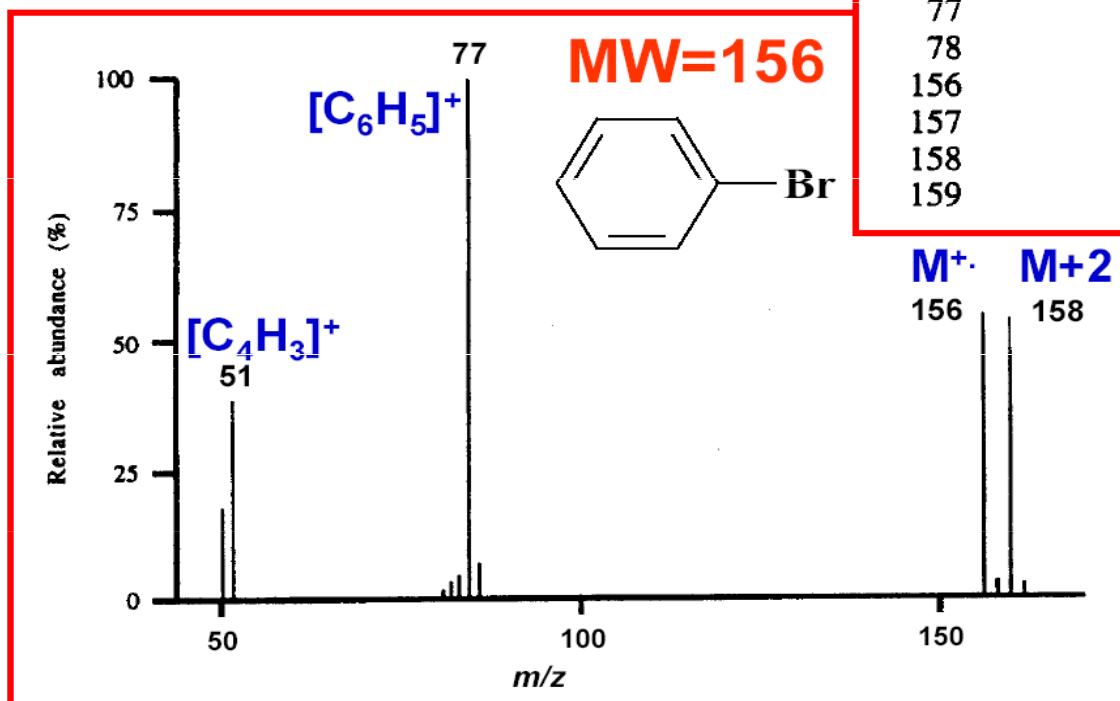
No. 3

m/z	%		m/z	%
14	1.4		44	2.6
15	10.2		50	1.3
26	6.4		51	1.8
27	56.9		53	2.5
28	16.1		55	8.0
29	61.2		56	44.8
30	1.3		57	100.0
39	27.3		58	4.5
40	4.2		71	5.2
42	38.8		86	14.0
43	78.0		87	0.9

$[H(CH_2)_x]^+$
$[H(CH_2)_x - 2H]^+$

MW=86

57

41   43

$CH_3 - [CH_2]_4 - CH_3$

27   29

39

$M^{+\cdot}$

86

15

71

% Intensity

100
80
60
40
20
0

10   30   50   70

m/z
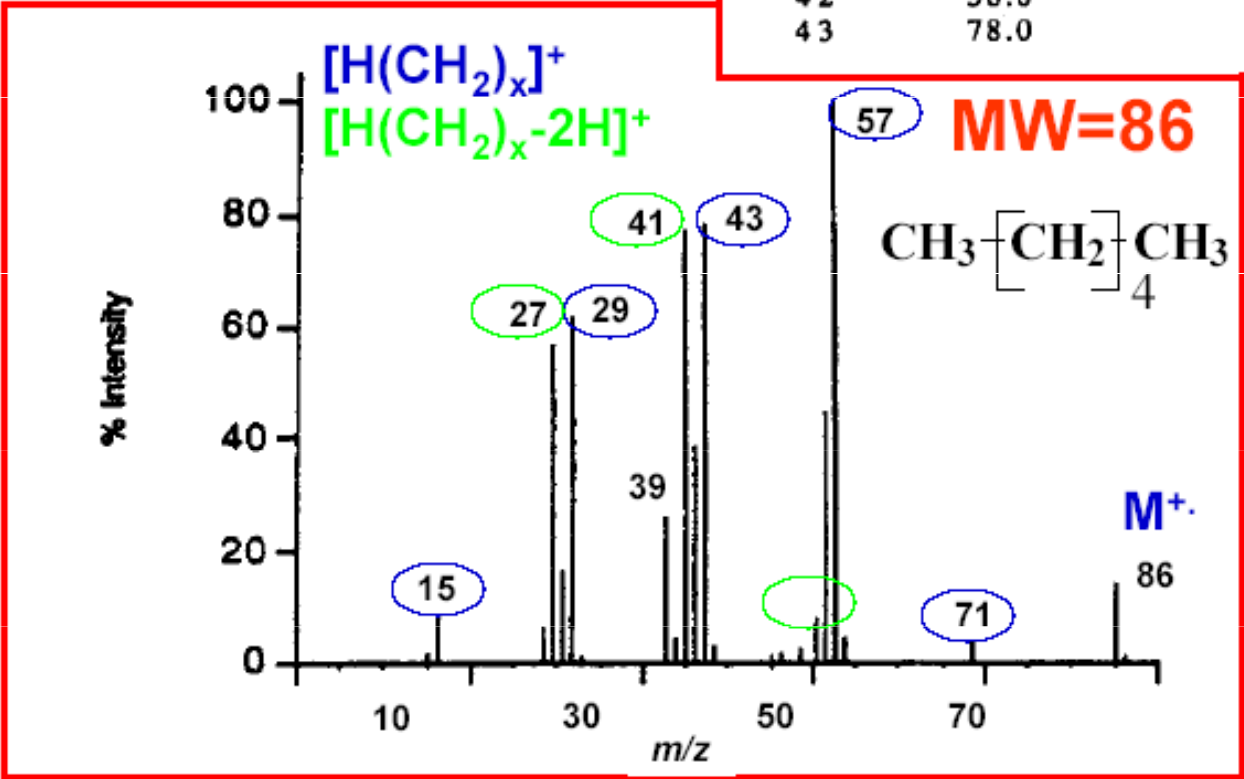
Relative Abundances of the Combinations of Halogen Isotopes

- **Fluorine and iodine are monoisotopic**

- **However, chlorine is present in the nature as ^{35}Cl (75.8%) and ^{37}Cl (24.2%), and bromine as ^{79}Br (50.5%) and ^{81}Br (49.5%)**

- **Therefore molecular ions (or fragment ions) containing various numbers of Cl and/or Br atoms give rise to typical patterns spaced 2 amu apart based on a combination of $(a+b)^n$; for Cl a is 75.8%, b 24.2% and n is the number of chlorine substituents in a molecule**

Molecular Ion Cluster
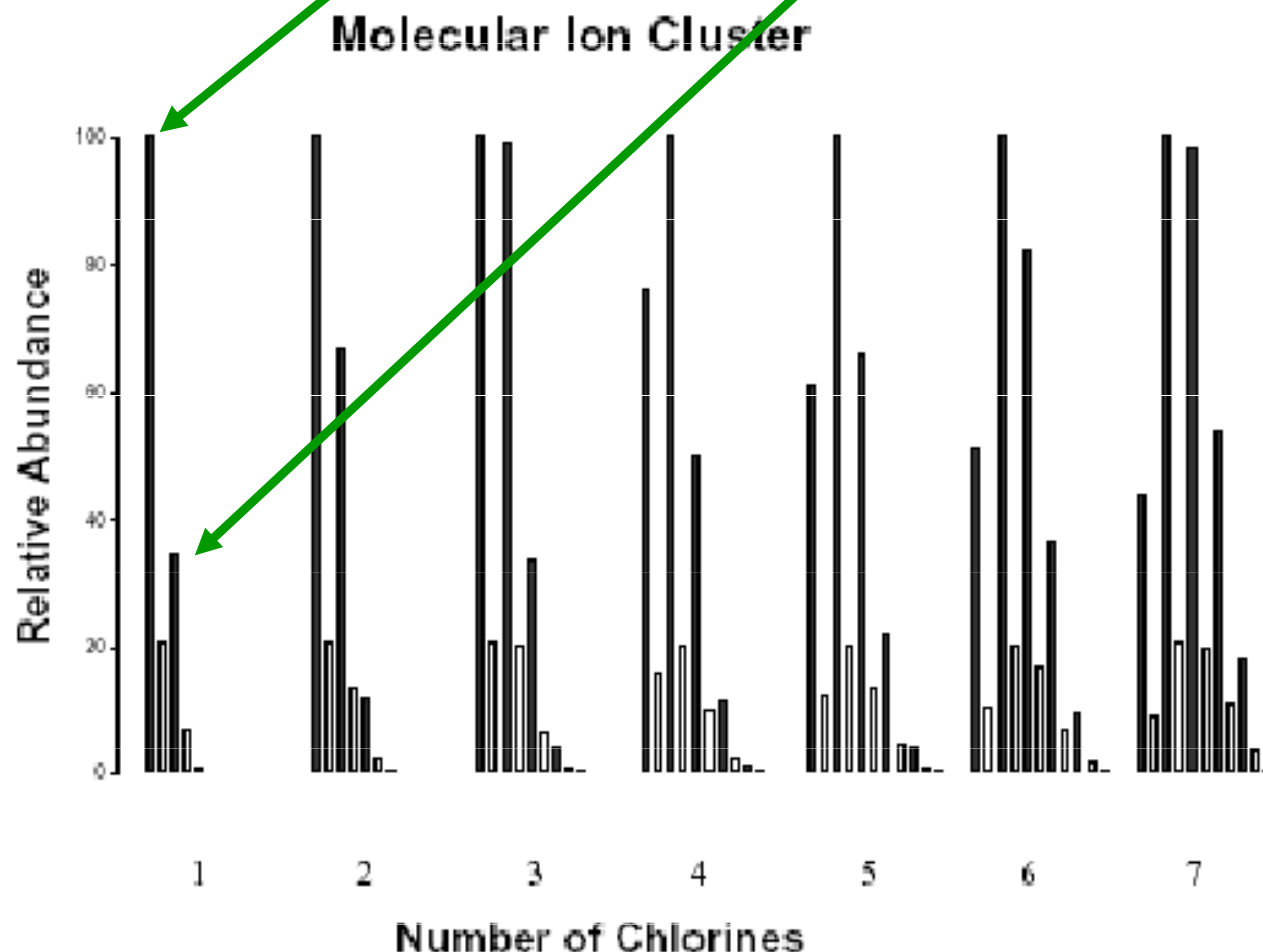
Upper Segment of 2378-TCDD Mass Spectrum

Mass Spectrum of 2378–TCDD (EI, 70 eV)

3×^{35}Cl
1×^{37}Cl

[M+2]$^{+.}$
322

4×^{35}Cl

A cluster created
by four Cl-
substituents

M$^{+.}$
320

2×^{35}Cl
2×^{37}Cl

324
[M+4]$^{+.}$

1×^{35}Cl
3×^{37}Cl

[M-COCl]$^{+.}$ **259**

4×^{37}Cl

[M+6]$^{+.}$

[M-Cl]$^{+.}$ **287**

252

315
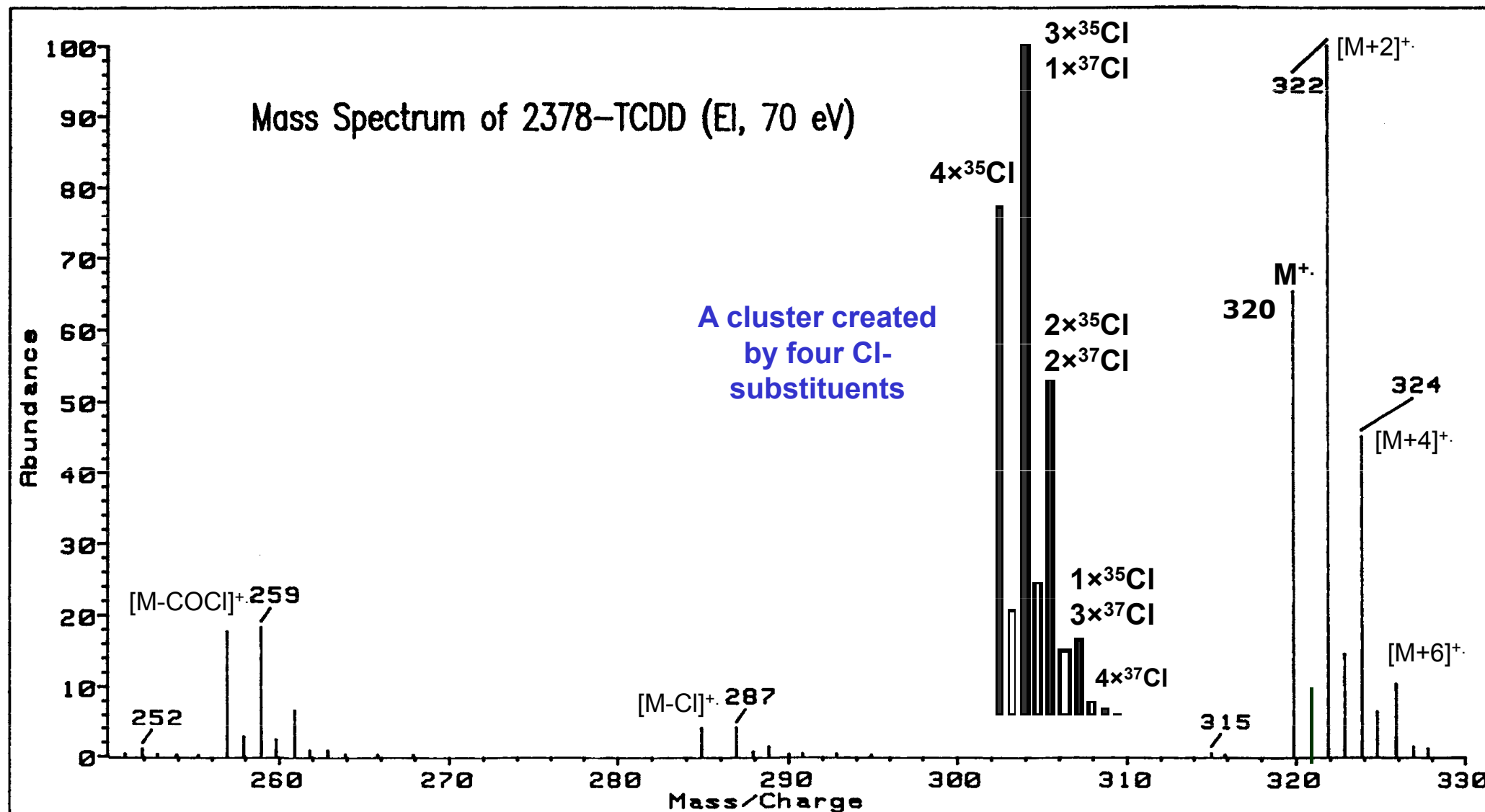
What is the SCAN Mode in Mass Spectrometry ?

- The **scanning** mode provides mass spectra. They are recorded (scanned) at regular intervals (typically 0.5 – 1 /s; much faster if TOFMS is used) during the GC separation and stored in the instrument data system for subsequent qualitative or quantitative evaluation.

- From mass spectra, it is often possible to deduce structural features (mass spectral interpretation) but this requires experience and can be very time-consuming, particularly as a complex mixture might contain hundreds of components.

- The spectra can also be compared with those stored in mass spectral libraries. Although library searching is a very useful and timesaving technique, it is important to remember that such searches do not identify compounds – analysts do!
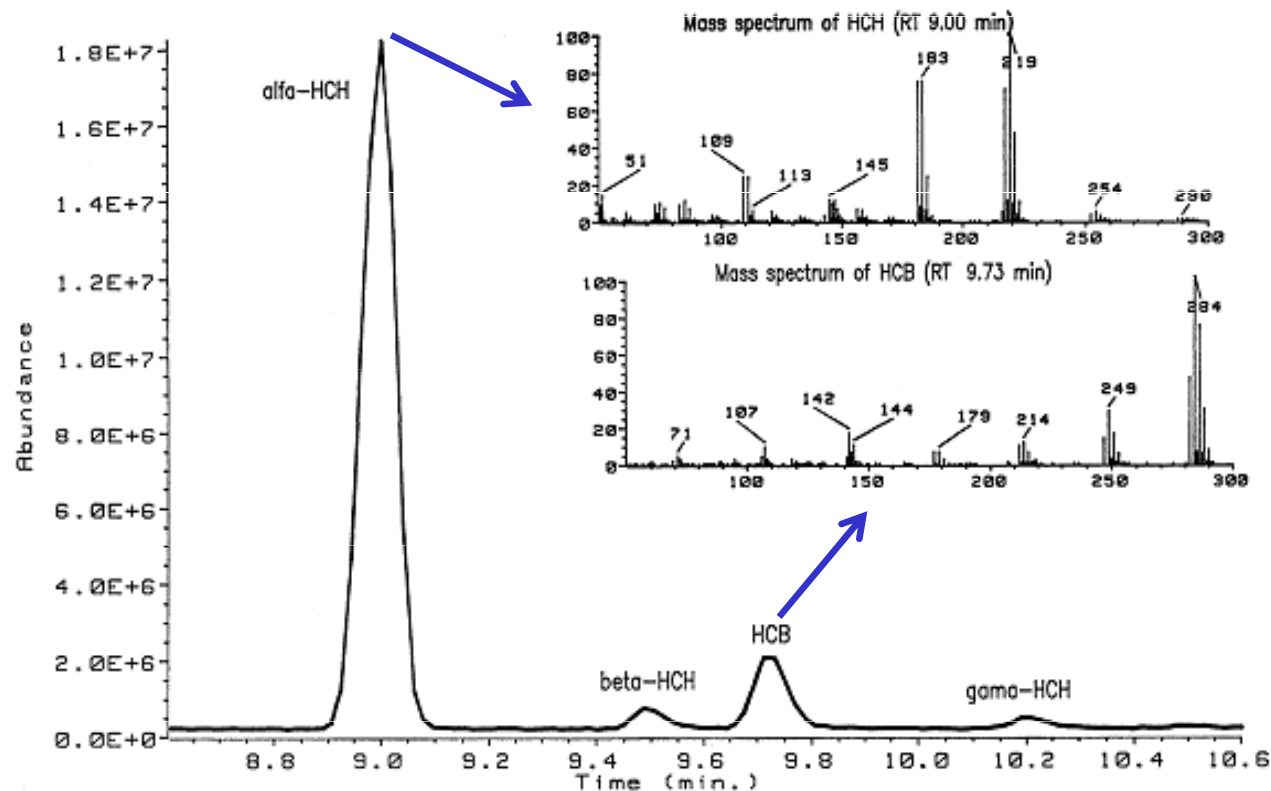
What is the SIM (or MID) Mode in Mass Spectrometry ?

- SIM (Selected Ion Monitoring) or MID (Multiple Ion Detection) is much more sensitive technique suitable for trace quantitative analysis. Here, instead of scanning a whole spectrum, only a few ions (generally, the most abundant but characteristic selected from the mass spectrum) are detected during the GC run.

- This can result in as much as a 500-fold increase in sensitivity, at the expense of selectivity. Depending on the analyte, low picogram to even low femtogram amounts can be measured using this powerful technique.

- Stable isotope-labeled internal standards can be employed.
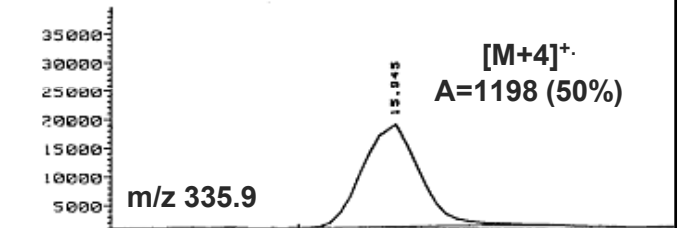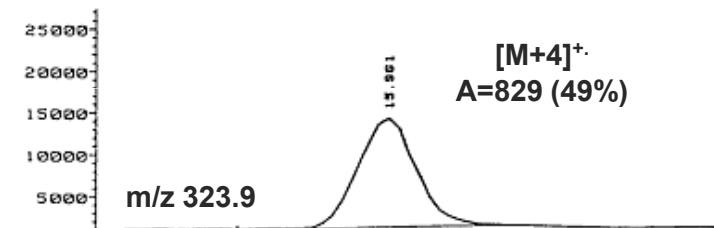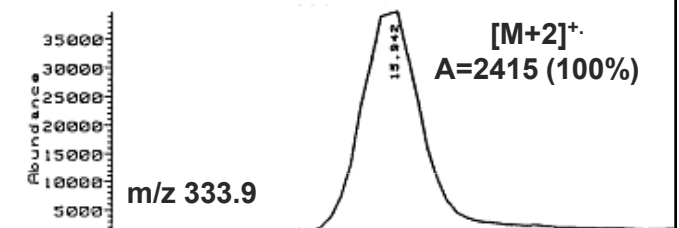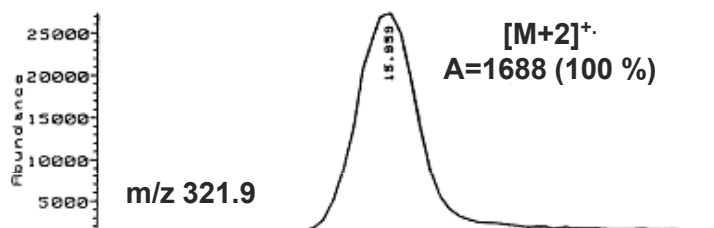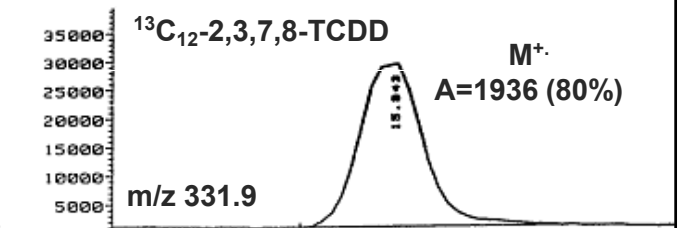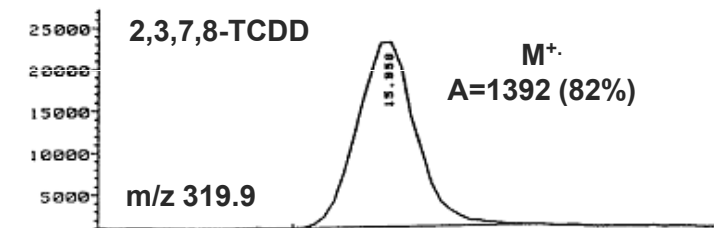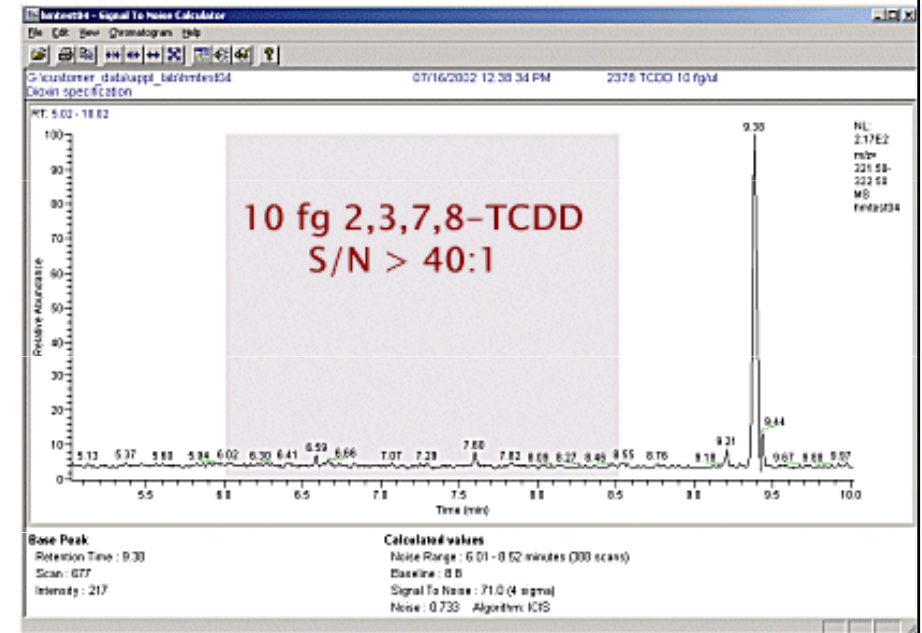
HRMS/LRMS-SIM chromatogram from the analysis of 2378-TCDD in a soil extract by the isotope dilution method

10 fg 2,3,7,8-TCDD
S/N > 40:1

2,3,7,8-TCDD

$M^{+\cdot}$
A=1392 (82%)

m/z 319.9

$[M+2]^{+\cdot}$
A=1688 (100 %)

m/z 321.9

$[M+4]^{+\cdot}$
A=829 (49%)

m/z 323.9

$^{13}C_{12}$-2,3,7,8-TCDD

$M^{+\cdot}$
A=1936 (80%)

m/z 331.9

$[M+2]^{+\cdot}$
A=2415 (100%)

m/z 333.9

$[M+4]^{+\cdot}$
A=1198 (50%)

m/z 335.9

The Use of the Isotope Dilution HRGC/HRMS in the SIM Mode

A. Kočan, Slovak Medical University

CHRO: 1613-db5-1-t0013   09-Apr-02   Elapse: 29:25.8   934
Samp: Vial 4 T0014,flyash,DIOX fr,pr.0026   Start : 17:19:15   2587
Comm: DB5ms#29,5.04g,250ulES(1-2),20ulDS(1)
Mode: EI +VE +LMR ESCAN LR NRM   Study : 7th Intercalibratio
Oper: AK, c.a.803   Client: van Bavel   Inlet : GC   Vial 4

Fly ash
PentaCDFs

DB5ms, 60 m

m/z:339.8598

27:40 28:06 28:23 28:37 28:47 29:03 29:16 29:31 29:42 30:04 30:12 30:42 31:27 32:29

M+2   E+06 1.620

m/z:341.8569

28:06 28:23 28:37 28:47 29:03 29:16 29:31 29:42 30:04 30:12 30:41 31:26 32:29

M+4   E+06 1.083

m/z:351.9000

29:14 **12378-PeCDF (13C12)**   30:18 **23478-PeCDF (13C12)**

M+2   E+04 4.663

m/z:353.8970

29:15 **Sampling standard**   29:31   30:18 **Extraction standard**

M+4   E+04 3.050

28:00 28:30 29:00 29:30 30:00 30:30 31:00 31:30 32:00 32:30

Dioxins and Related Compounds Analysis (1)

PCDDs

PCDFs

PCBs

HCB

PBDEs

p,p'-DDE
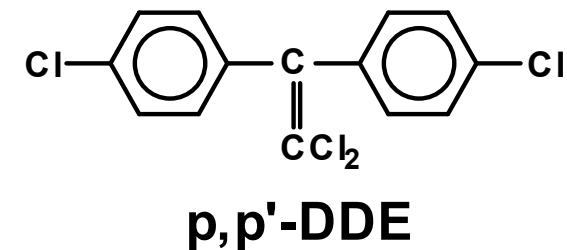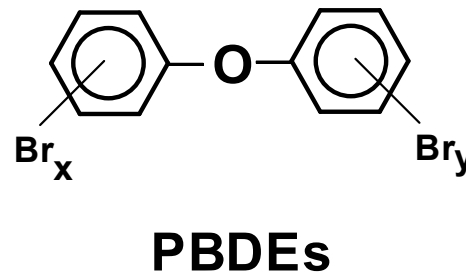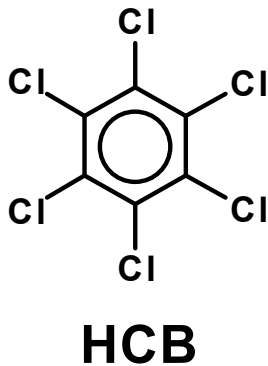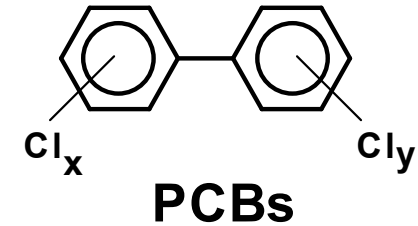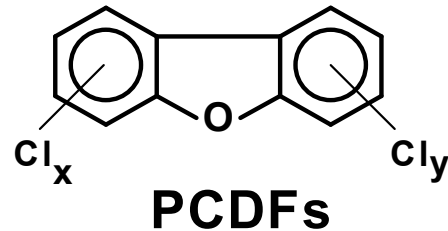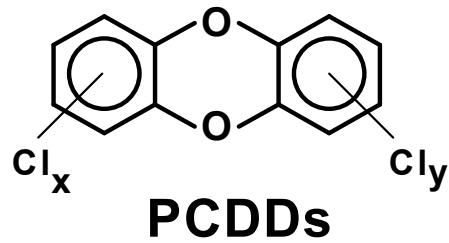
Types of samples:

- Emissions from combustion processes (stack aerosol, flyash)
- Wastes (obsolete chemicals, disposal sites)
- Environmental samples (air, soil, water sediment, biota)
- Foods (meat, fish, milk, eggs, oils)
- Human biological material (milk, blood, adipose tissue)

Flow chart of a clean-up procedure for stack emission samples

A. Kočan, Slovak Medical University
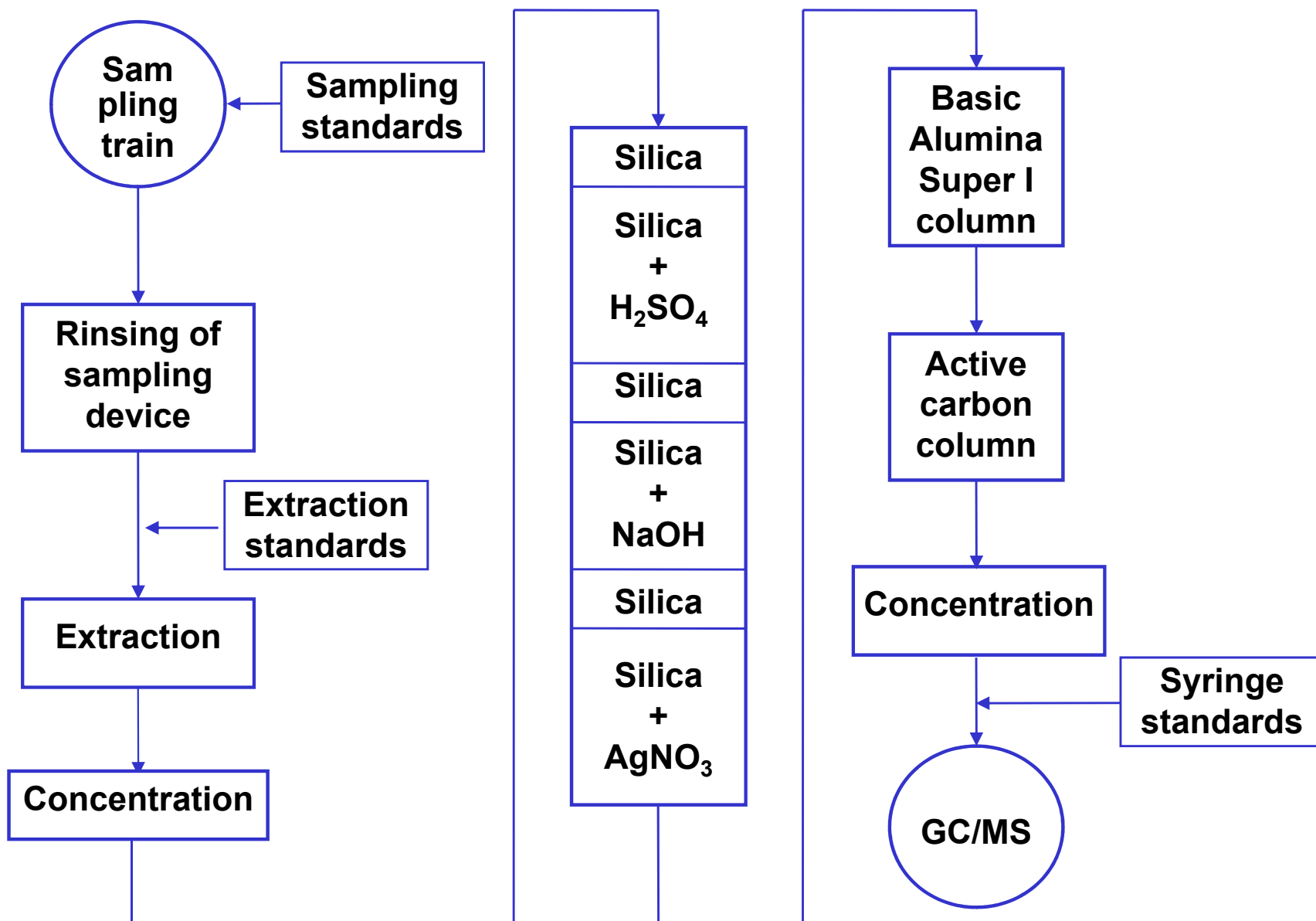
Sampling:

- **The collection of samples should represent the situation, process or the species studied**

- **Attention must be paid to the appropriate timing of collections of samples, their frequency and site layout**

- **Sampling media, particularly those for air and emission sampling should be spiked before sampling with selected isotope labeled surrogates (^{13}C-sampling standards) to make corrections of losses of analytes during the sampling**

Sampling extraction & cleanup:

- **Addition of isotope labeled standards (^{13}C) before extraction**

- **Solvent extraction (Soxhlet, ASE, ultrasonic, SPE, SFE, MASE)**

- **Removal of matrix substances (lipids, oils, etc.) – H_2SO_4, GPC, SPM dialysis**

SPE of blood serum
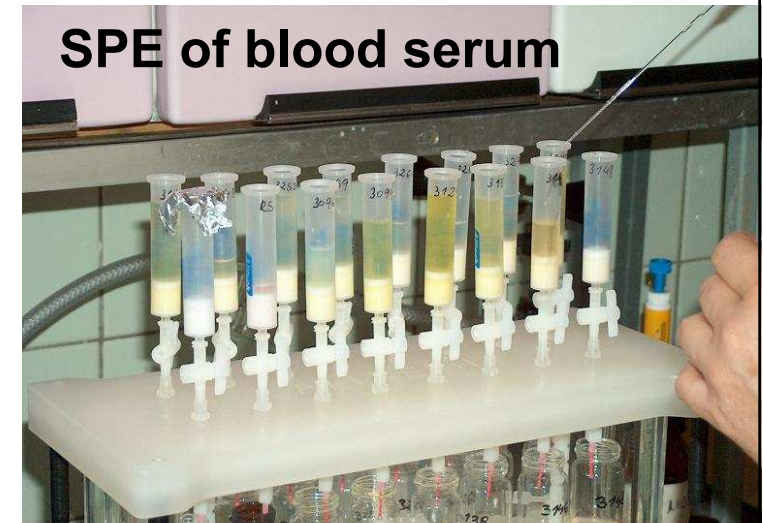
- **Removal of interfering coextracted compounds (PCBs, PCDPEs, etc.) – acid/base modified silica, alumina, active carbon, semi-preparative HPLC)**

- **Concentration of the analytes**

Cleanup on silica/H_2SO_4 columns
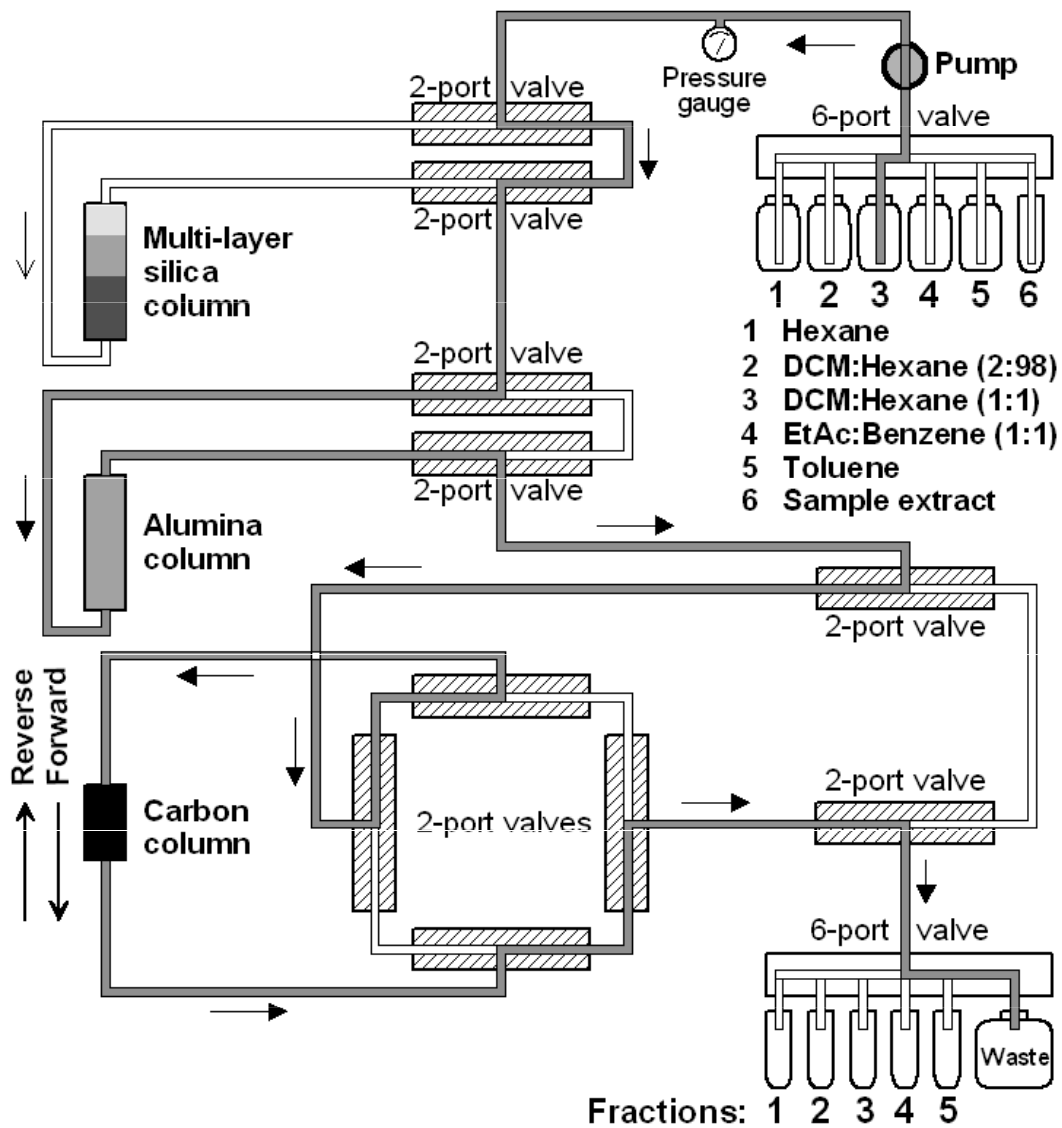
Schematic of a semi-automatic equipment for sample cleanup

2-port valve · Pressure gauge · Pump · **6-port valve**

Multi-layer silica column

2-port valve

1. Hexane
2. DCM:Hexane (2:98)
3. DCM:Hexane (1:1)
4. EtAc:Benzene (1:1)
5. Toluene
6. Sample extract

2-port valve

Alumina column

Reverse / Forward

Carbon column

2-port valves

2-port valve

6-port valve

Waste

Fractions: 1 2 3 4 5

Semi-automated clean-up equipment

GC separation:

- **Non-polar stationary phase (e.g. DB-5) – used for the samples of animal origin and higher chlorinated congeners**

- **Polar phase (e.g. SP-2330) – used for environmental samples (good separation but shorter lifetime)**

- **Splitless, on-column or large-volume injection**

- **Direct connection of the column to the ion source**

out of splitless injector

to mass spectrometer
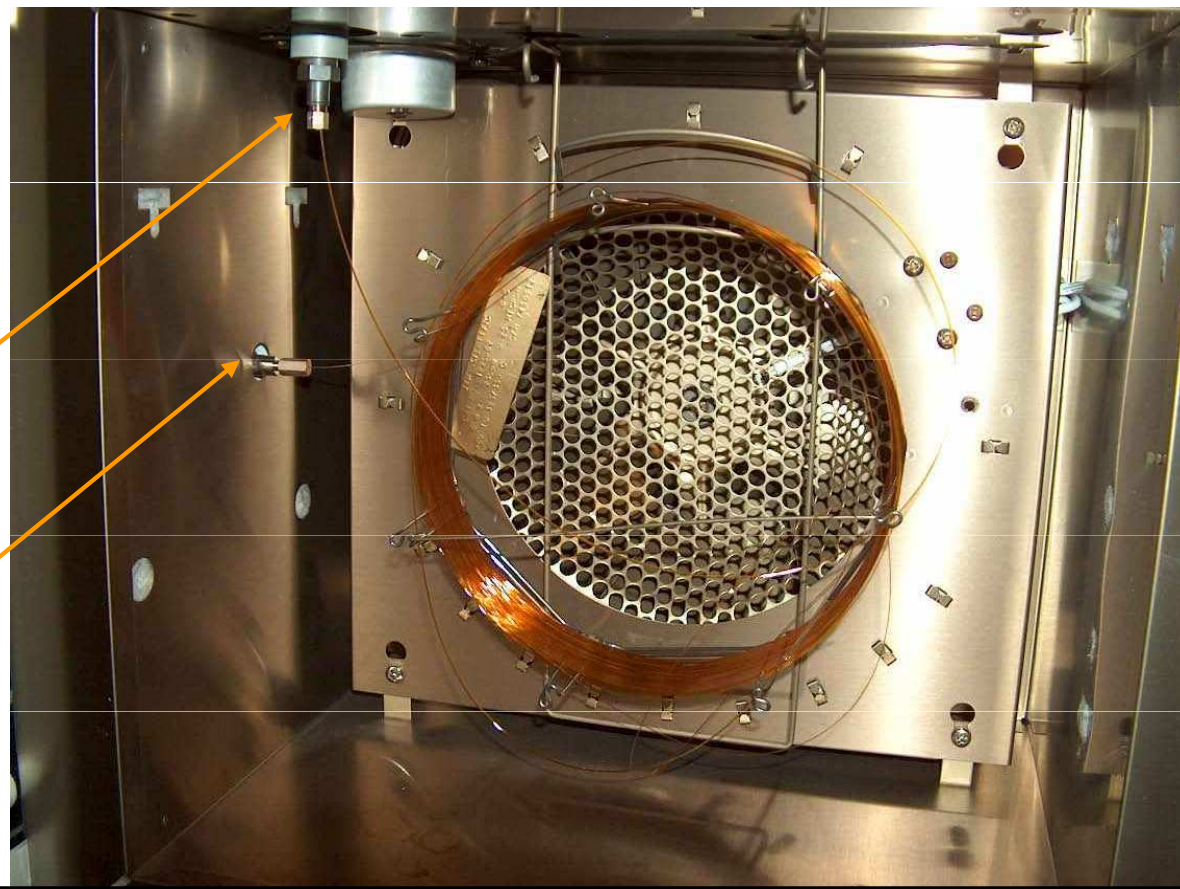
Identification Criteria

(according to
the EN 1948 Standard)

- **The isotope ratio between the ions monitored shall match the theoretical value within ± 20 %**

- **The retention time of a native 2,3,7,8-isomer shall be within a time window of (0s;+3s) based on the retention time of the corresponding $^{13}C_{12}$-labelled isomer in the sample. For hepta- and octachloro-congeners deviations of (-2s;+3s) are acceptable**

- **The GC column shall separate 2,3,7,8-congeners from interfering congeners with a 90 % valley relative to the highest peak. 2,3,7,8-TCDF shall be separated from all other interfering isomers within a 25 % valley**

Mass of ions monitored for tetra- to octaCDDs/Fs when HRMS is applied

Group	Ion	% Abundance	Natives	$^{13}C_{12}$-labelled
TetraCDFs	$[M]^{+\cdot}$	77.55	303.9016	315.9418
	$[M+2]^{+\cdot}$	100	305.8987	317.9390
TetraCDDs	$[M]^{+\cdot}$	77.55	319.8965	331.9398
	$[M+2]^{+\cdot}$	100	321.8935	333.9338
PentaCDFs	$[M+2]^{+\cdot}$	100	339.8597	351.9000
	$[M+4]^{+\cdot}$	64.15	341.8567	353.8970
PentaCDDs	$[M+2]^{+\cdot}$	100	355.8546	367.8948
	$[M+4]^{+\cdot}$	64.15	357.8516	369.8918
HexaCDFs	$[M+2]^{+\cdot}$	100	373.8208	385.8610
	$[M+4]^{+\cdot}$	80.54	375.8178	387.8581
HexaCDDs	$[M+2]^{+\cdot}$	100	389.8156	401.8558
	$[M+4]^{+\cdot}$	80.54	391.8126	403.8528
HeptaCDFs	$[M+2]^{+\cdot}$	100	407.7818	419.8220
	$[M+4]^{+\cdot}$	96.52	409.7789	421.8192
HeptaCDDs	$[M+2]^{+\cdot}$	100	423.7766	435.8169
	$[M+4]^{+\cdot}$	96.52	425.7737	437.8140
OctaCDF	$[M+2]^{+\cdot}$	88.89	441.7428	453.7830
	$[M+4]^{+\cdot}$	100	443.7398	455.7801
OctaCDD	$[M+2]^{+\cdot}$	88.89	457.7377	469.7779
	$[M+4]^{+\cdot}$	100	459.7348	471.7750

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Quantification criteria
(according to the EN 1948 Standard)

- **The S/N ratio of the raw data shall be at least 3:1 for the signal used for quantification**

- **The calibration is carried out with at least 5 calibration solutions**

- **The measuring range shall be linear**

- **Daily calibration checks shall be run**

- **HRMS at 6000 to 10000 resolution can be used if the absence of interferences is documented**

- **Other techniques can be used if show meeting the criteria**

Conversion of Analytical Results into the Toxic Equivalent (TEQ)

- **This conversion is based on the assumption that all the 2,3,7,8-substituted PCDDs and PCDFs (17 cong.), as well as the dioxin-like PCBs (12 cong.), bind to the same receptor, the Ah receptor, and show comparable qualitative (toxic) effects, but with different potencies**

- **These differences in toxicity are expressed in the toxic equivalency factors (TEFs)**

- **TEF of the most toxic 2378-TCDD = 1**

Congener	I-TEF	WHO-TEF	Congener	I-TEF	WHO-TEF
2378-TCDD	1	1	2378-TCDF	0.1	0.1
12378-PeCDD	0.5	1	23478-PeCDF	0.5	0.5
123478-HxCDD	0.1	0.1	12378-PeCDF	0.05	0.05
123678-HxCDD	0.1	0.1	123478-HxCDF	0.1	0.1
123789-HxCDD	0.1	0.1	123789-HxCDF	0.1	0.1
1234678-HpCDD	0.01	0.01	123678-HxCDF	0.1	0.1
OCDD	0.001	0.0001	234678-HxCDF	0.1	0.1
			1234678-HpCDF	0.01	0.01
			1234789-HpCDF	0.01	0.01
			OCDF	0.001	0.0001

$$TEQ = (PCDD_i \times TEF_i) + (PCDF_i \times TEF_i) + (PCB_i \times TEF_i)$$

A. Kočan, Slovak Medical University

Calculations Regarding Dioxin Determination by the Isotope Dilution Method

Relative Response Factors

$$rrf_i = \frac{A_{i,12C}}{A_{i,13C}} \times \frac{Q_{i,13C}}{Q_{i,12C}}$$

Quantification

$$Q_{i,12C} = \frac{Q_{i,13C}}{rrf_i} \times \frac{A_{i,12C}}{A_{i,13C}}$$

$$C_i = Q_{i,12C} / V_{nr}$$

$$C_T = \Sigma\,(C_i \times I\text{-}TEF_i)$$

Extraction Standards Recovery

$$R_{i,e} = \frac{100}{Q_{i,e}} \times \frac{Q_{i,sy}}{rrf_i} \times \frac{A_{i,e}}{A_{i,sy}}$$

Sampling Standards Recovery

$$R_{i,sa} = \frac{100}{Q_{i,sa}} \times \frac{Q_{i,e}}{rrf_i} \times \frac{A_{i,sa}}{A_{i,e}}$$

A. Kočan, Slovak Medical University