Organochlorinated pesticides (OCPs) and PCBs Analysis in environmental samples

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Organic chemistry Sector

POPs Analysis

Extraction

Clean-up

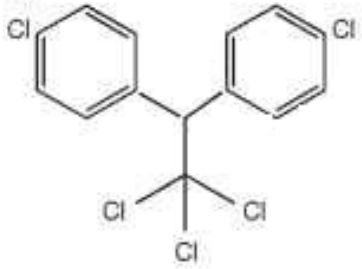
Analysis

Persistent Organic Pollutants (POPs)

Initial POPs	Compounds to be monitored (Air, milk/blood)
Aldrin	Aldrin
Chlordane	Cis/trans-chlordane; cis/trans nonachlor; oxychlordane
DDT	4,4'-DDT; 2,4'-DDT; 4,4´-DDE; 2,4´-DDE; 4,4´-DDD; 2,4´-DDD
Dieldrin	Dieldrin
Endrin	Endrin
Hexaclorobenzene (HCB)	HCB
Heptaclor	Heptaclor ; heptachlorepoxide
Mirex	Mirex
PCB	(7 indicators: 28,52, 101, 118, 138, 153 e 180)
Dl-PCBs	(12 congeners with TEFs: 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 189)
PCDD / PCDF	17 congeners 2,3,7,8-substituted
Toxaphene	Congeners P26, P50, P62

POPs – Analytical standards

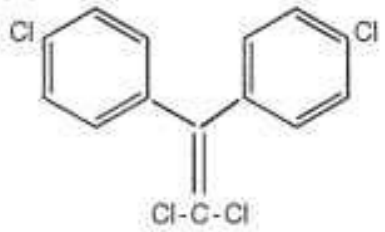
DDT: Isomers and degradation compounds



DDT:
pp´- DDT
op´- DDT

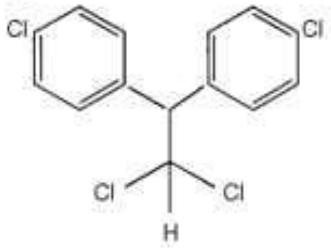
DDE:

pp´- DDE
op´- DDE



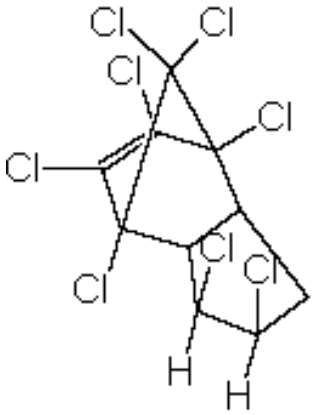
DDD:

pp´- DDD
op´- DDD

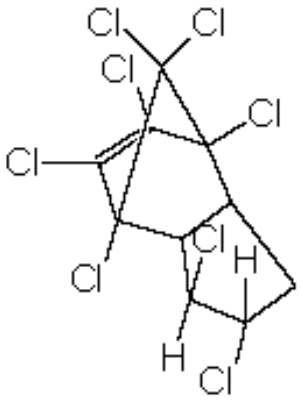


POPs – Analytical standards

<u>Chlordane:</u> mixture of more than 140 compounds, ~120 identified (ATSDR, 94)



cis - chlordane

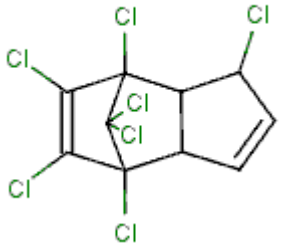


trans - chlordane

Isomers cis and trans:
60-85%
technical chlordane

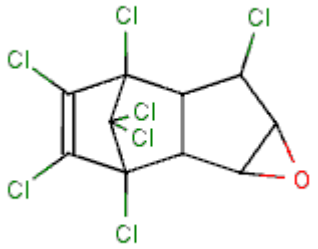
POPs – Analytical standards

Heptachlor



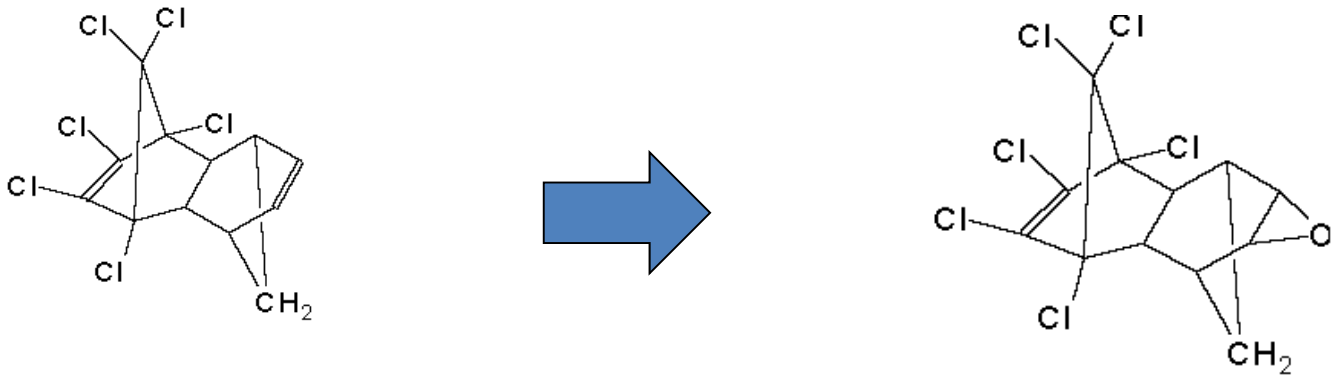
Heptachlorepoxide

Persistence



Heptachlor: ~20% of technical chlordane is heptachlor (ATSDR, 2007)

POPs – Analytical standards

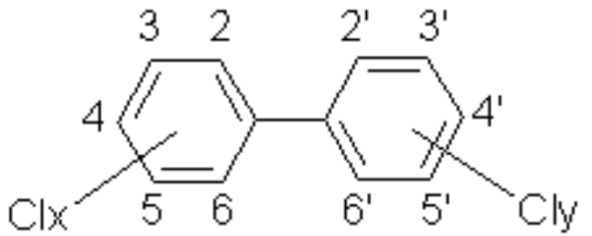


Aldrin

Dieldrin

In the environment, aldrin is rapidly epoxilated and converted to dieldrin, which is more resistant to biotransformation and abiotic degradation

PCBs - Standards



IUPAC	ISOMERS	MW	% CHLORINE	TYPE
MONOCHLOROBYPHENYL	3	188,7	18,8	HOMOLOG
DICHLOROBIPHENYL	12	223,1	21,8	HOMOLOG
TRICHLOROBIPHENYL	24	257,5	41,3	HOMOLOG
TETRACHLOROBIPHENYL	42	292,0	48,6	HOMOLOG
PENTACHLOROBIPHENYL	46	326,4	54,3	HOMOLOG
HEXACHLOROBIPHENYL	42	360,9	58,9	HOMOLOG
HEPTACHLOROBIPHENYL	24	395,3	62,8	HOMOLOG
OCTACHLOROBIPHENYL	12	429,8	66,0	HOMOLOG
NONACHLOROBIPHENYL	3	464,2	68,7	HOMOLOG
DECACHLOROBIPHENYL	1	498,7	71,2	HOMOLOG
TOTAL	209			CONGENERS

PCBs - Standards

dl-PCBs

PCB 77
PCB 81
PCB 126
PCB 169
PCB 105
PCB 114
PCB 118
PCB 123
PCB 156
PCB 157
PCB 167
PCB 189

PCBs indicators

PCB 28
PCB 52
PCB 101
PCB 118
PCB 153
PCB 138
PCB 180

Total PCBs :

→ **Total of 209 individual congeners : not possible to separate all of them**

→ **Technical mixture: aroclor 1242, 1254, 1260....**

PCBs – Commercial mixtures

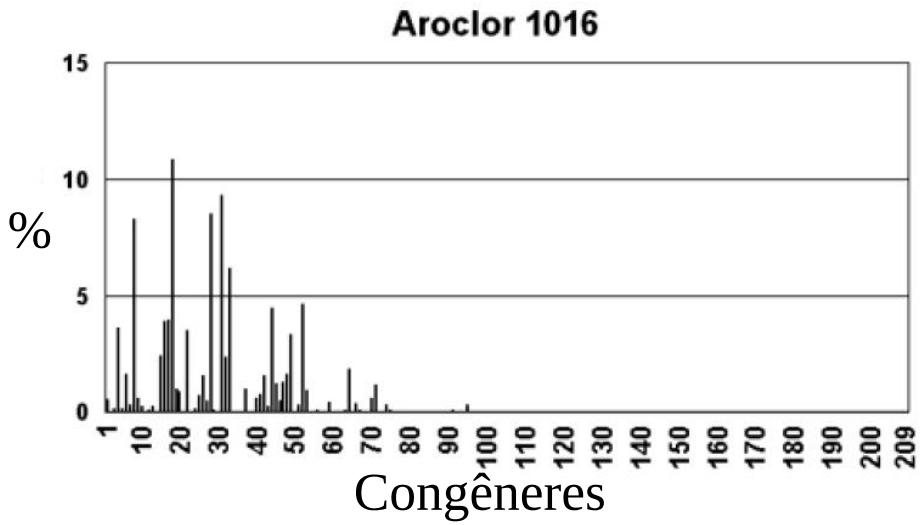
Aroclor	USA
Phenochlor	France
Clophen	Germany
Kanechlor	Japan
Ascarel	Brazil

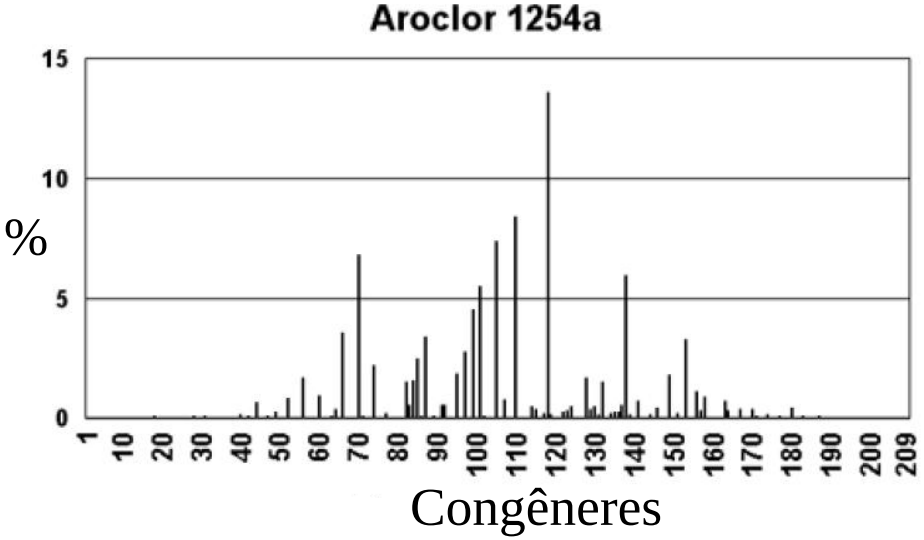
PCB uses	Aroclor tipe
Electrical Capacitors	1221, 1254
Electrical Transformers	1242, 1254, 1260
Vacuum Pump	1248, 1254
Hidraulic Fluids	1232, 1242, 1248, 1254, 1260
Plasticizers Resins	1248, 1254, 1260, 1262, 1268
Plasticizers for rubber	1221, 1232, 1242, 1248, 1254, 1268

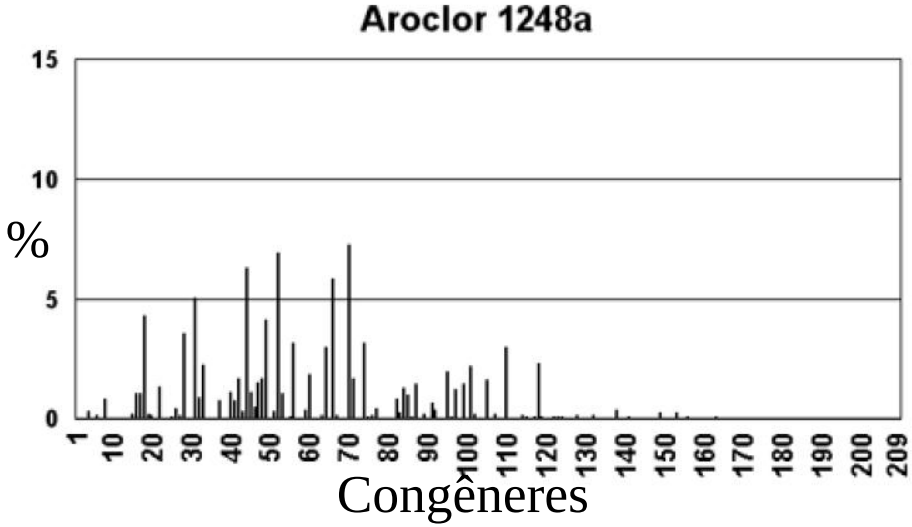
PCBs – Commercial mixtures

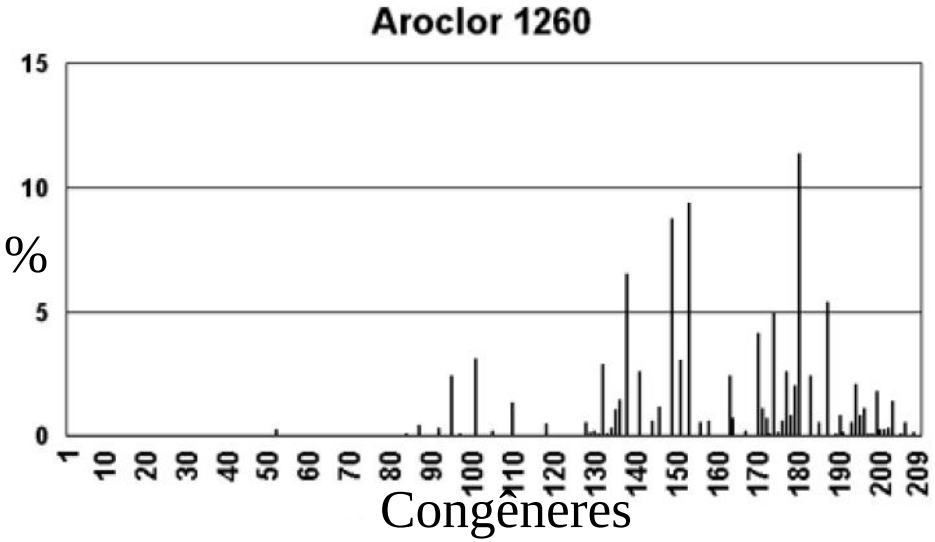
Commercial PCB product	Percent Chlorine (%)
Aroclor 1232	32
Aroclor 1016	41
Aroclor 1242	42
Aroclor 1248	48
Aroclor 1254	54
Aroclor 1260	60

Total PCBs

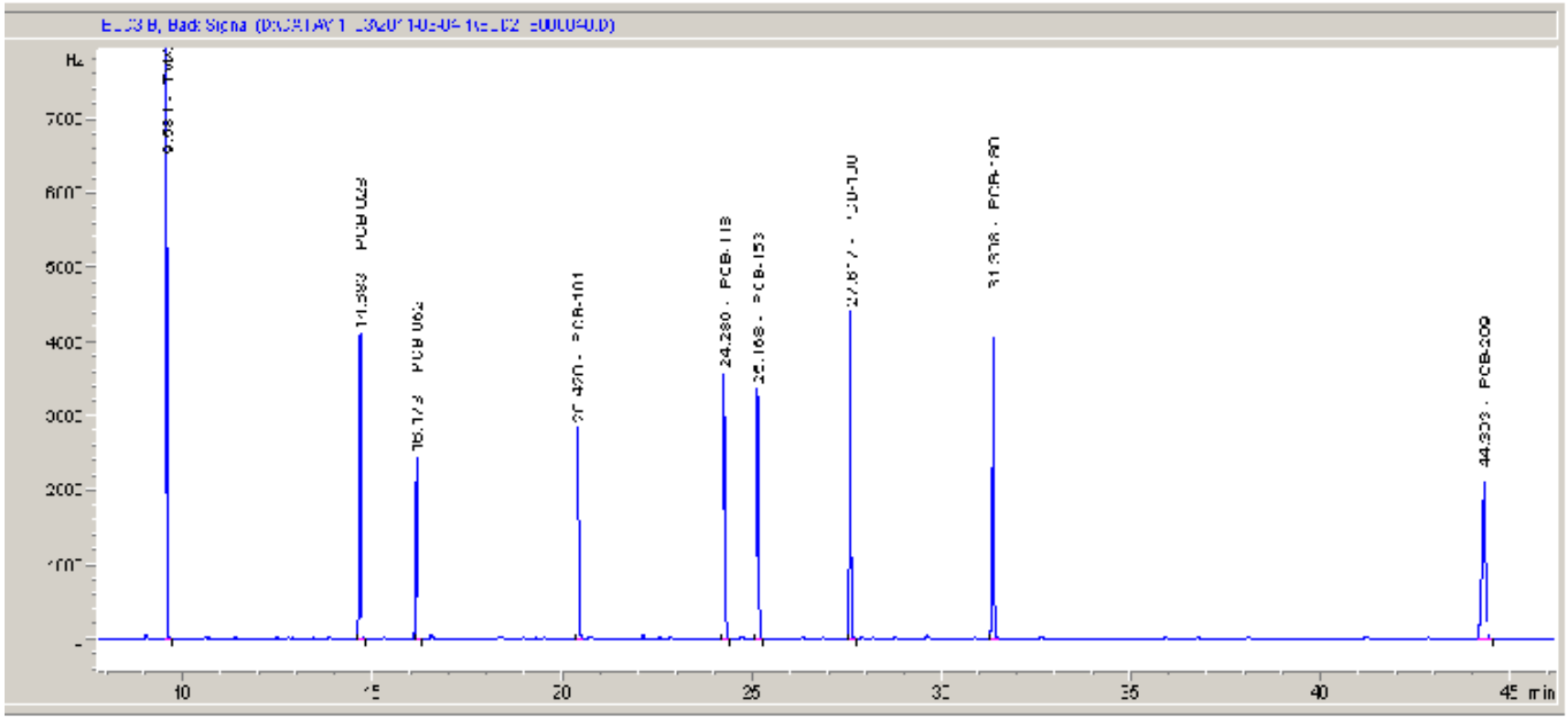

Aroclor 1016
%
Congêneres


Aroclor 1254a
%
Congêneres


Aroclor 1248a
%
Congêneres


Aroclor 1260
%
Congêneres

PCB - INDICATORS

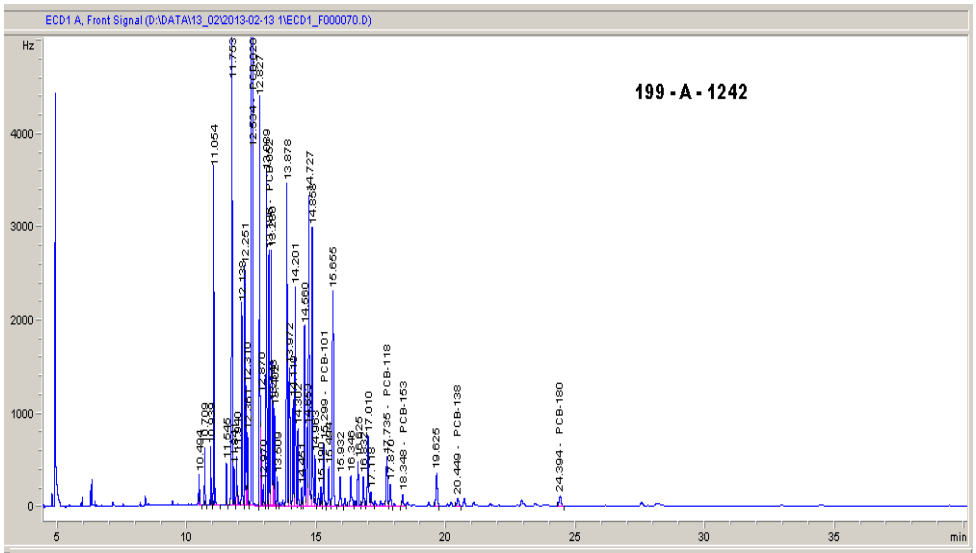


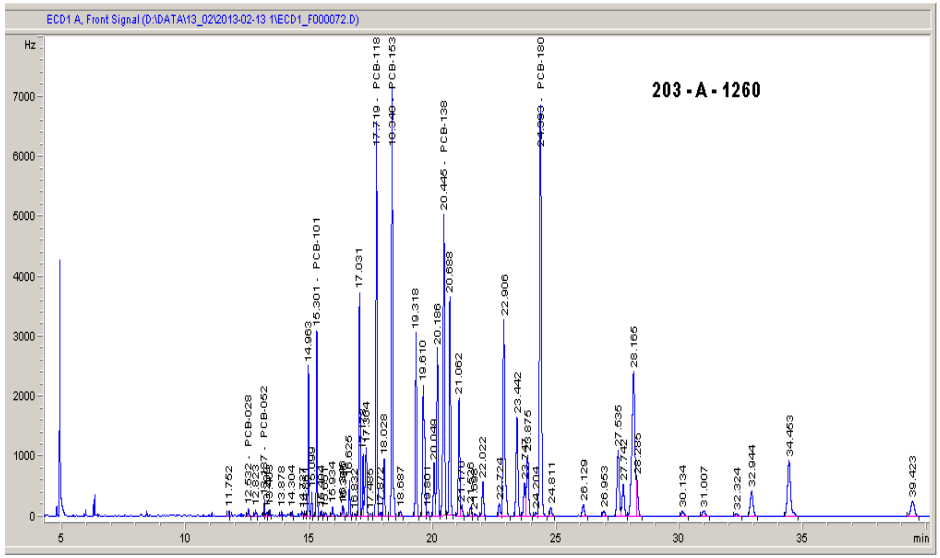
GC-ECD

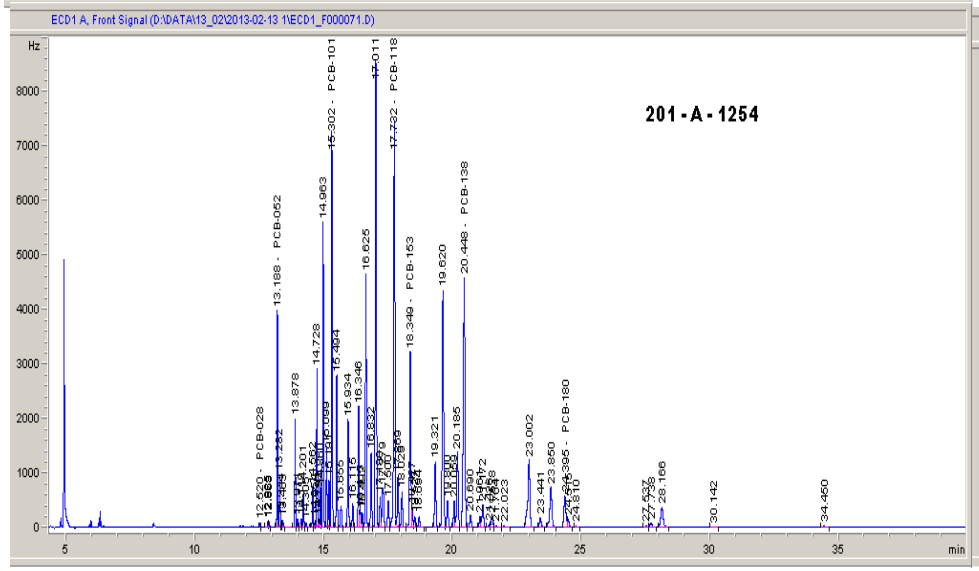
Column: VF-35MS (60mx0,25mmx0,25µm)

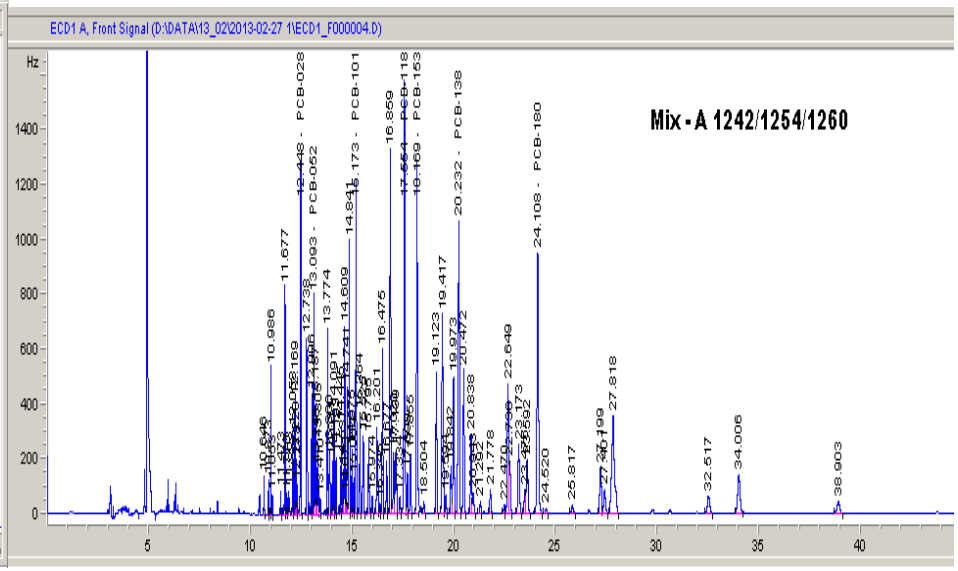
Concentration: 100 µg/L each

PCBs - Aroclors chromatograms (GC-ECD)

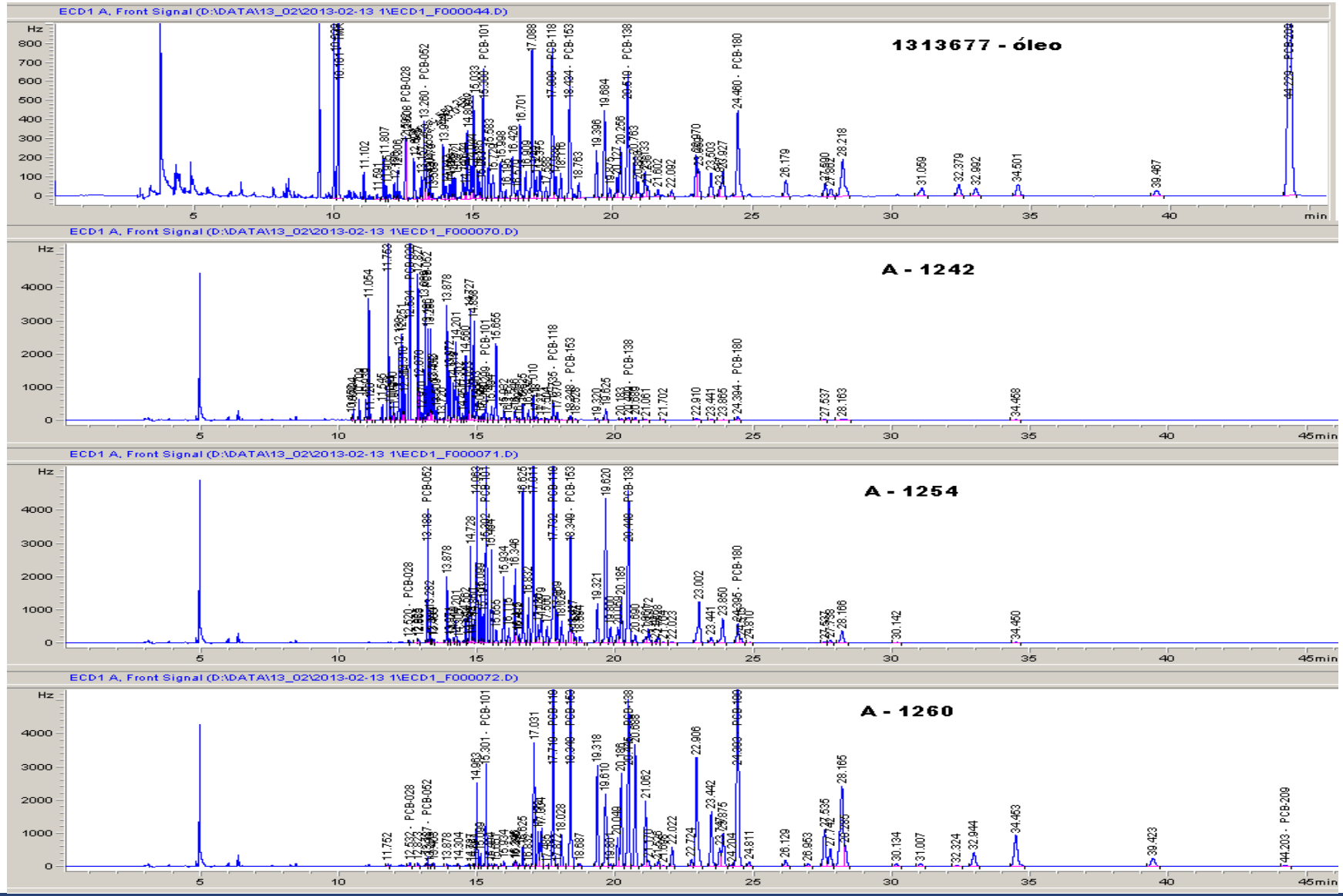




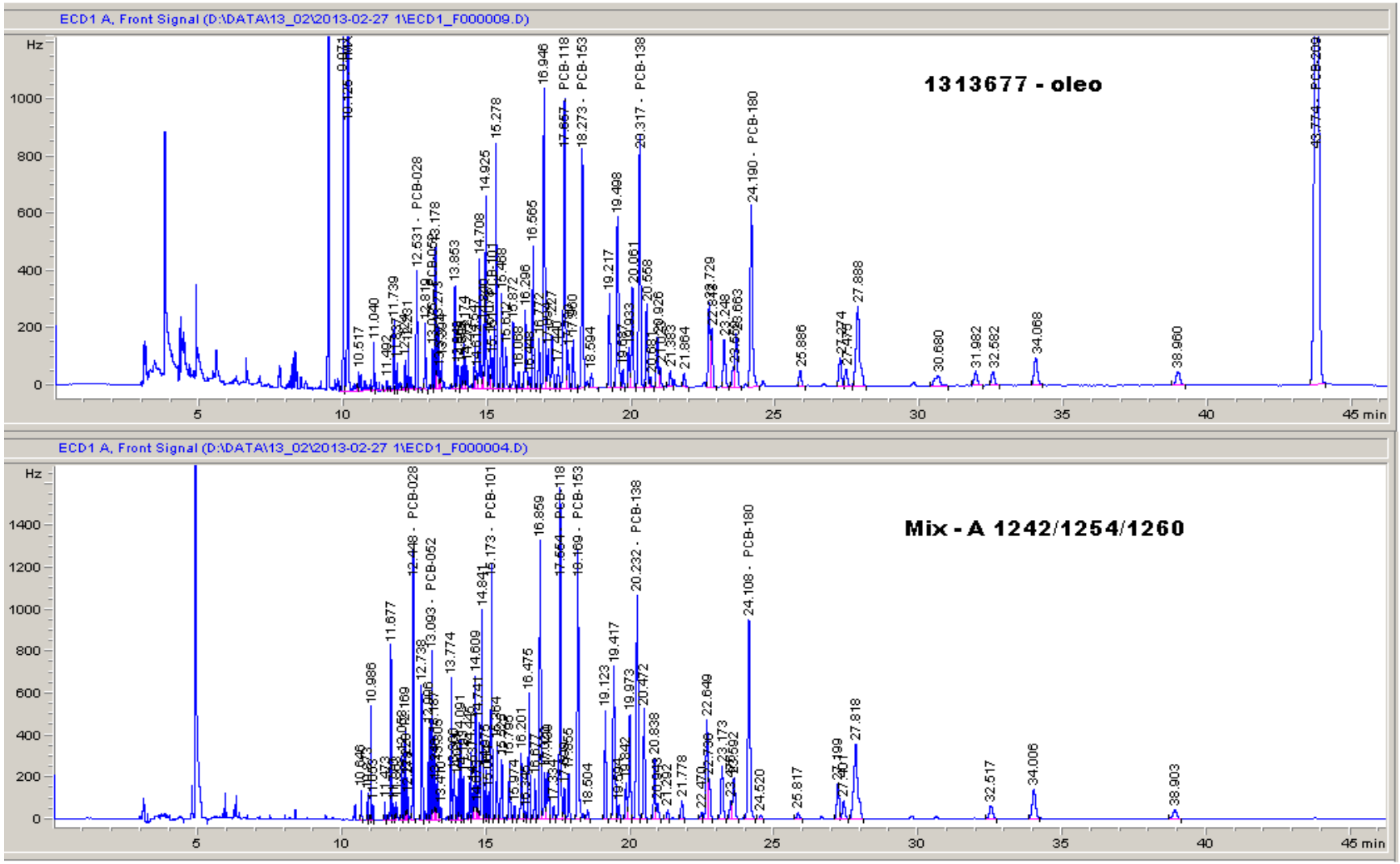




Transformer oil sample/standards



PCBs - Tranformer fluid sample chromatogram



PCB in transformer fluid

United States
Environmental Protection
Agency

Environmental Monitoring & Support
Laboratory
Cincinnati OH 45268

Research and Development

EPA-600/4-81-045 Sept. 1982

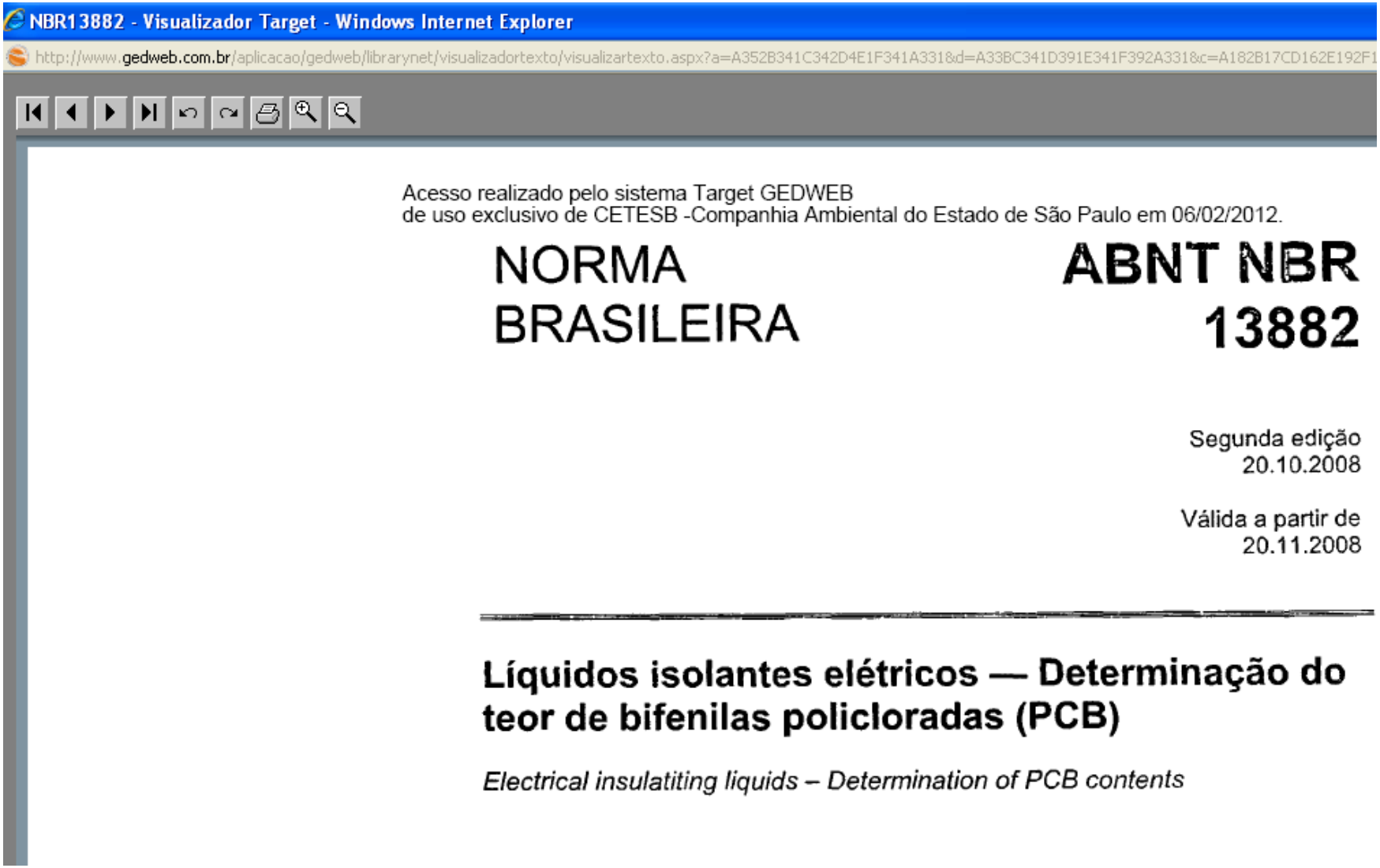
EPA

Test Method

The Determination of Polychlorinated Biphenyls in Transformer Fluid and Waste Oils

Thomas A. Bellar and James J. Lichtenberg

PCB in transformer fluid



NBR13882 - Visualizador Target - Windows Internet Explorer

http://www.gedweb.com.br/aplicacao/gedweb/librarynet/visualizadortexto/visualizartexto.aspx?a=A352B341C342D4E1F341A331&d=A33BC341D391E341F392A331&c=A182B17CD162E192F1

Acesso realizado pelo sistema Target GEDWEB
de uso exclusivo de CETESB -Companhia Ambiental do Estado de São Paulo em 06/02/2012.

NORMA BRASILEIRA

ABNT NBR 13882

Segunda edição
20.10.2008

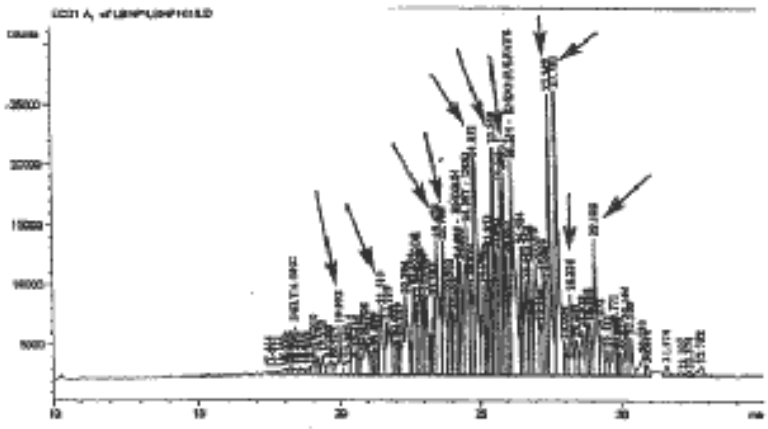
Válida a partir de
20.11.2008

Líquidos isolantes elétricos — Determinação do teor de bifenilas policloradas (PCB)

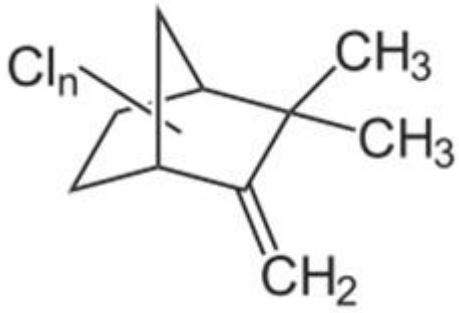
Electrical insulatiting liquids – Determination of PCB contents

POPs – Analytical standards

Toxaphene: Components/Congeners



Technical mixture: > 670 substances



Polychlorinated Bornanes

CHB26/Parlar 26 - Octachlorbornane
CHB50/Parlar 50 - Nonachlorbornane
CHB62/Parlar 62 - Nonachlorbornane

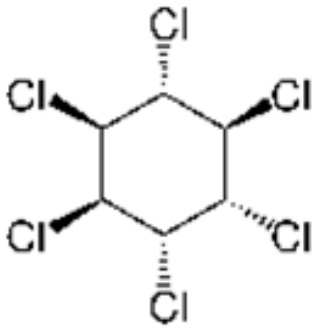
New POPs

POPs (COP 4/5 list)	Compounds to be monitored
Chlordecone	Chlordecone
α-HCH	α-HCH
β-HCH	β-HCH
γ-HCH (lindane)	γ-HCH (lindane)
Pentachlorobenzene	PeCbz
Endosulfan	α, β – endosulfan and endosulfan sulphate
C-penta BDE C- octa BDE	BDE 47, 99, 153,154, 175/183(coeluting), optional BDE 17, 28, 100
hexabromobiphenyl	PPB 153
PFOS	PFOS, PFOSA, NMeFOSA, NEtFOSA, NMeFOSE, NEtFOSE

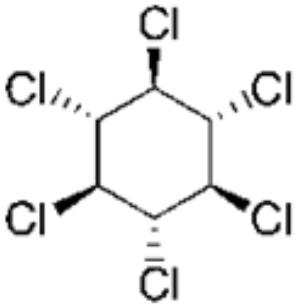
UNEP, 2013

POPs – Analytical standards

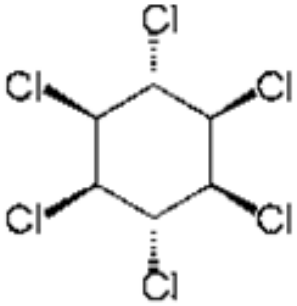
Lindane (γ HCH: gama hexaclorociclohexano): Isomers



α-HCH



β-HCH



γ-HCH
Lindane

65-70%	7-10%	14-15%

HCH- technical

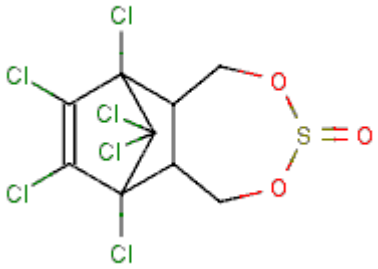
δ – HCH: ~7%
ε – HCH: ~1-2%
Other components: 1-2%

Organochlorinated pesticides

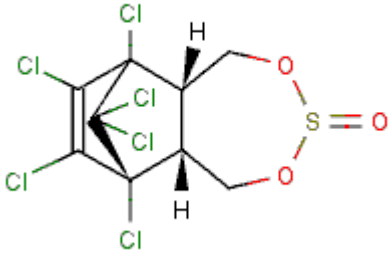
Endosulfan

70% (Endosulfan technical) 30%

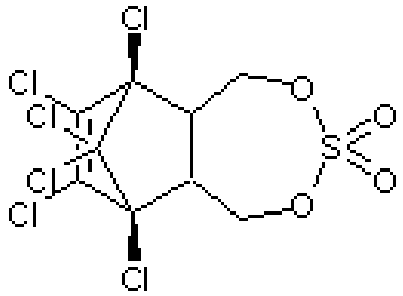
Endosulfan I (alfa)



Endosulfan II (beta)



Endosulfan Sulfate



Surrogates and Internal Standards (GC-ECD)

Internal standard

Quantification

Optional (EPA 8081)

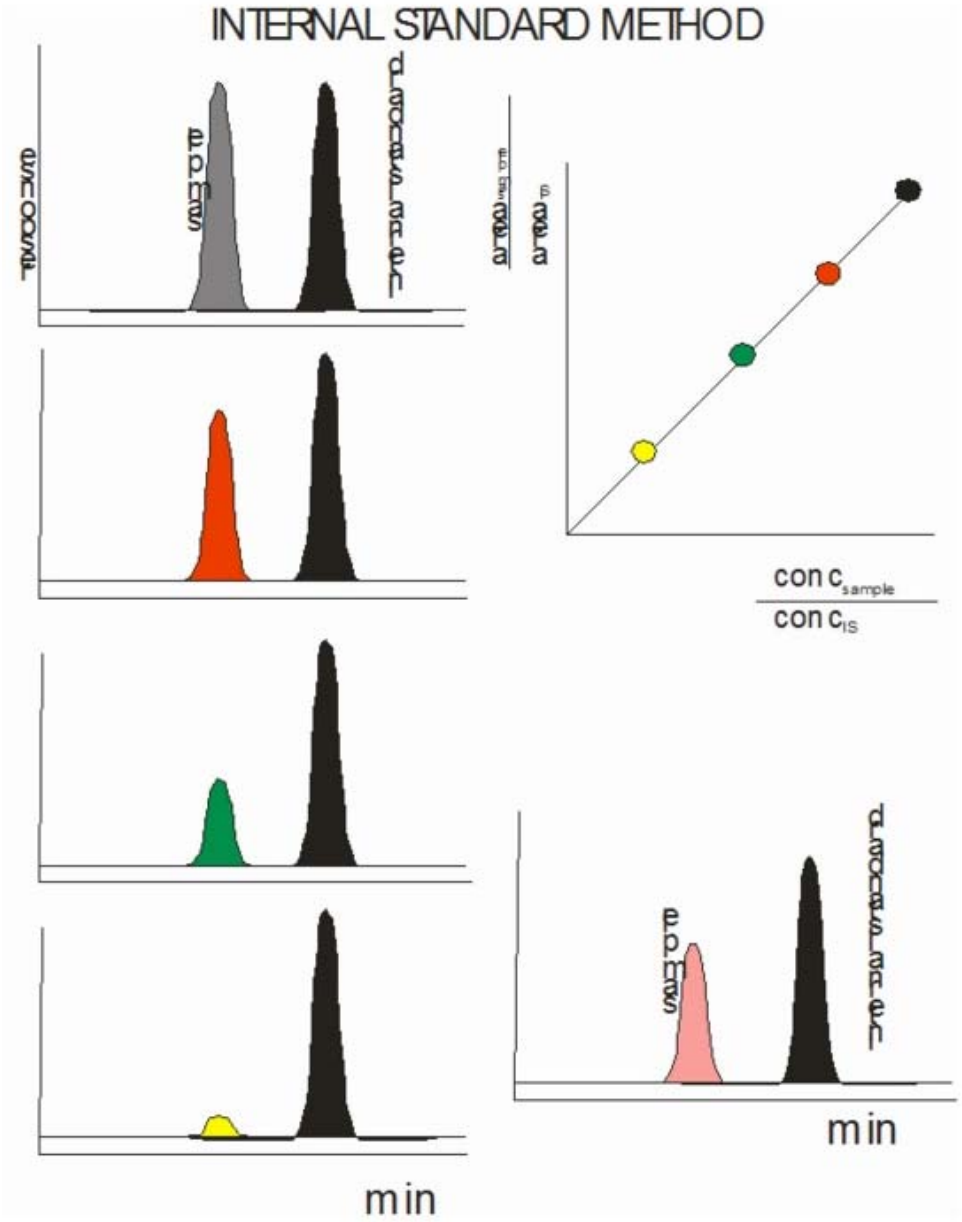
We do not use

Surrogate

Check extraction recovery

- TMX
- PCB-209

Method U.S. EPA 8081 (OCPs)


INTERNAL STANDARD METHOD
conc sample
conc IS
min
min

Surrogates and Internal Standards (GC-MS)

Internal standard

Quantification

- 1,4-diclorobenzeno-d4
- Naftaleno-d8
- Acenafteno-d10
- Fenantreno-d10
- Criseno-d12
- Perileno-d12

Surrogate

Check extraction recovery

- 2-fluorfenol
- Fenol-d6
- Nitrobenzeno-d5
- 2-fluor-1,1-bifenila
- 2,4,6-tribromofenol
- p-terfenil-d14

Method U.S. EPA 8270 (SVOC)

Extraction Methods

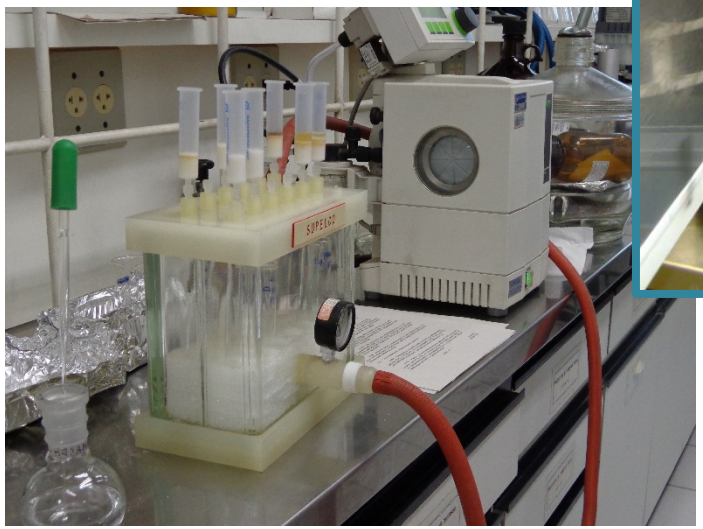
Matrix	Extraction technique	Method
Aqueous	Liquid-liquid	EPA 3510
Aqueous	Solid-phase extraction (SPE)	EPA 3535
Solids/Air (PUF)/Fish	Soxhlet	EPA 3540
Solids/Air (PUF)/Fish	Automated Soxhlet	EPA 3541
Solids/Fish	Microwave	EPA 3546
Solids	Ultrasonic	EPA 3550
Solids	Supercritical Fluid	EPA 3562

Extraction Methods

Liquid-Liquid extraction

Soxhlet

Microwave

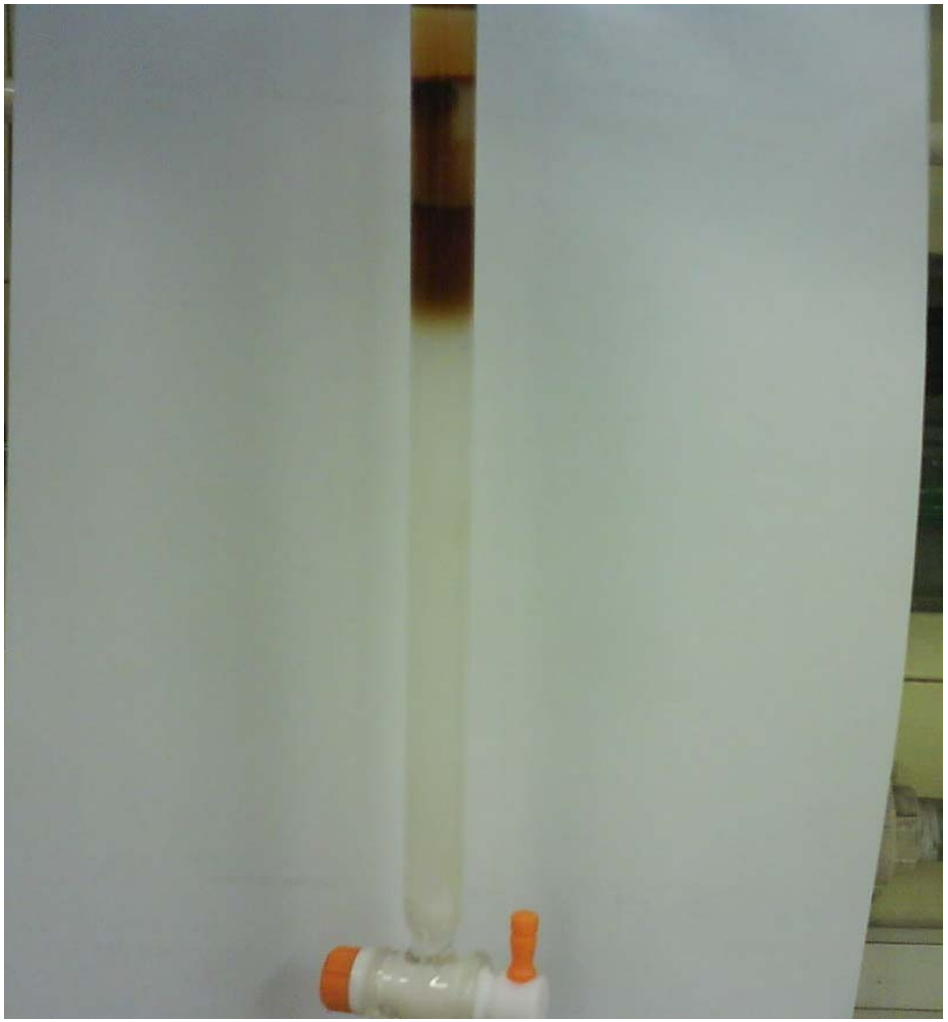


SPE extraction

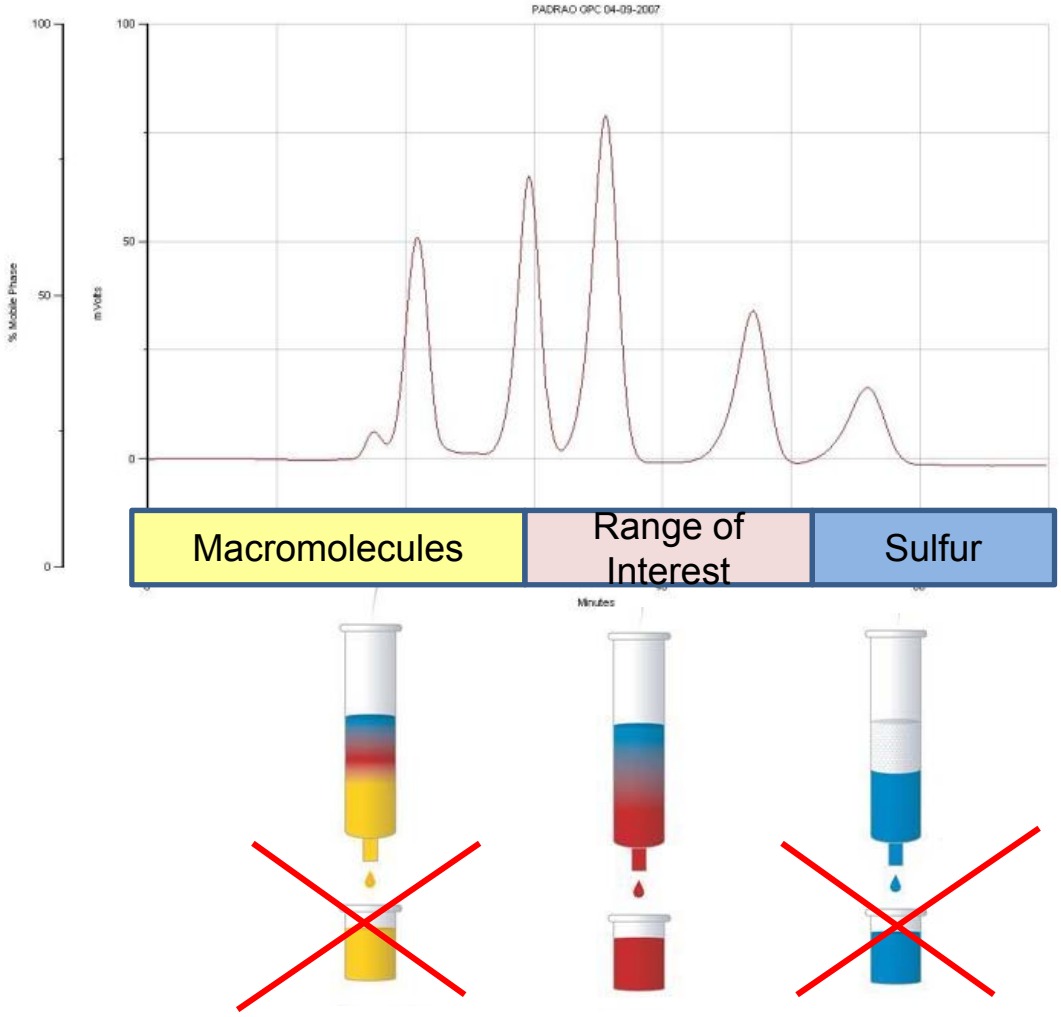
Cleanup Methods

Cleanup technique	**Type**	**Method**
Alumina	Adsorption	EPA 3610
Silica gel	Adsorption	EPA 3630
Florisil	Adsorption	EPA 3620
Gel-permeation (GPC)	Size-separation	EPA 3640
Sulfur	Oxidation/reduction	EPA 3660
Sulfuric acid/permanganate	Oxidation/reduction	EPA3665

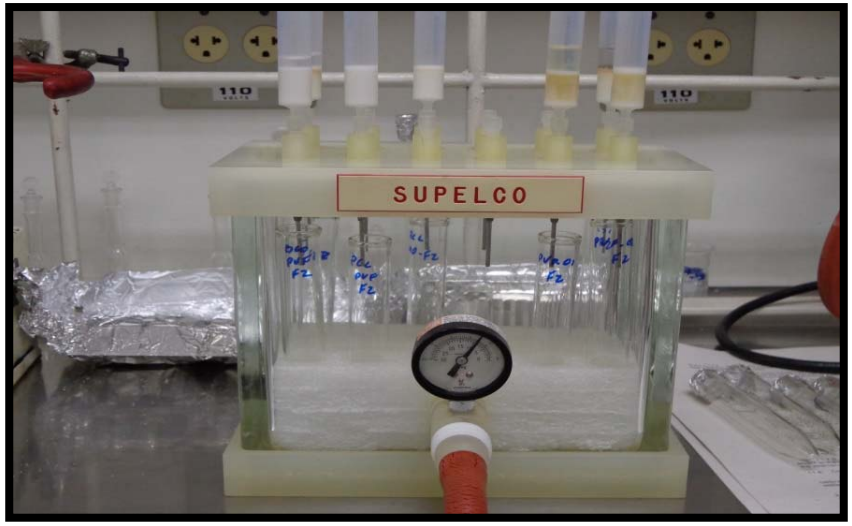
EPA Method 3630 Silica gel column

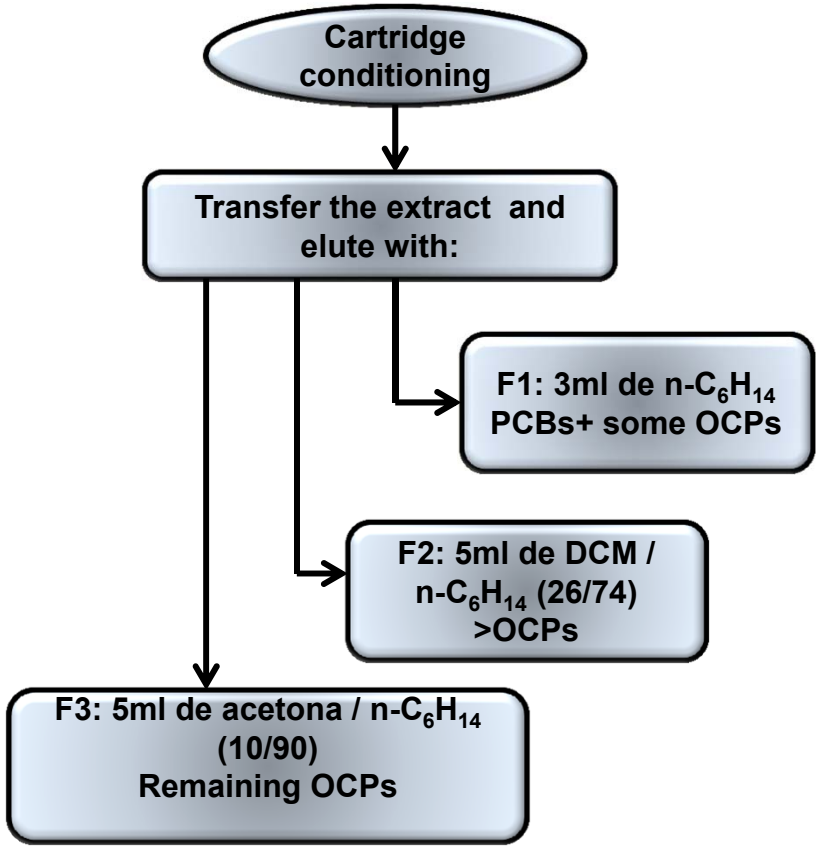


Gel permeation chromatography (GPC)

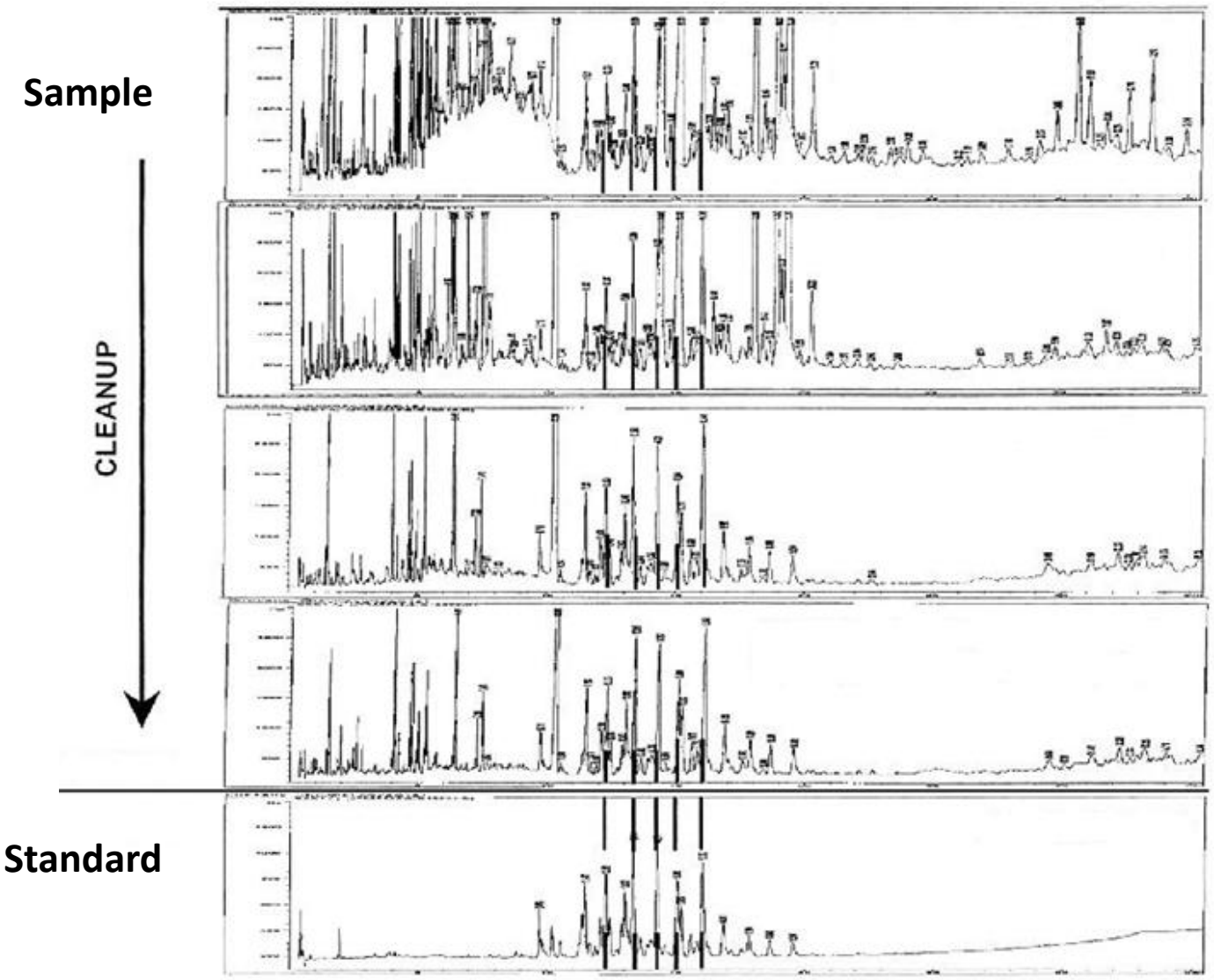


EPA-3620 OCP/PCB cleanup/fractioning
1g Florisil® Cartridge





Importance of Cleanup Steps



USEPA Method- 3600 B Rev02, 1998 .

Extract Concentration

Rotavaevaporator

Syncore

TurboVap

Final volume: 1 - 2mL (OCP)

POPs Analysis equipments

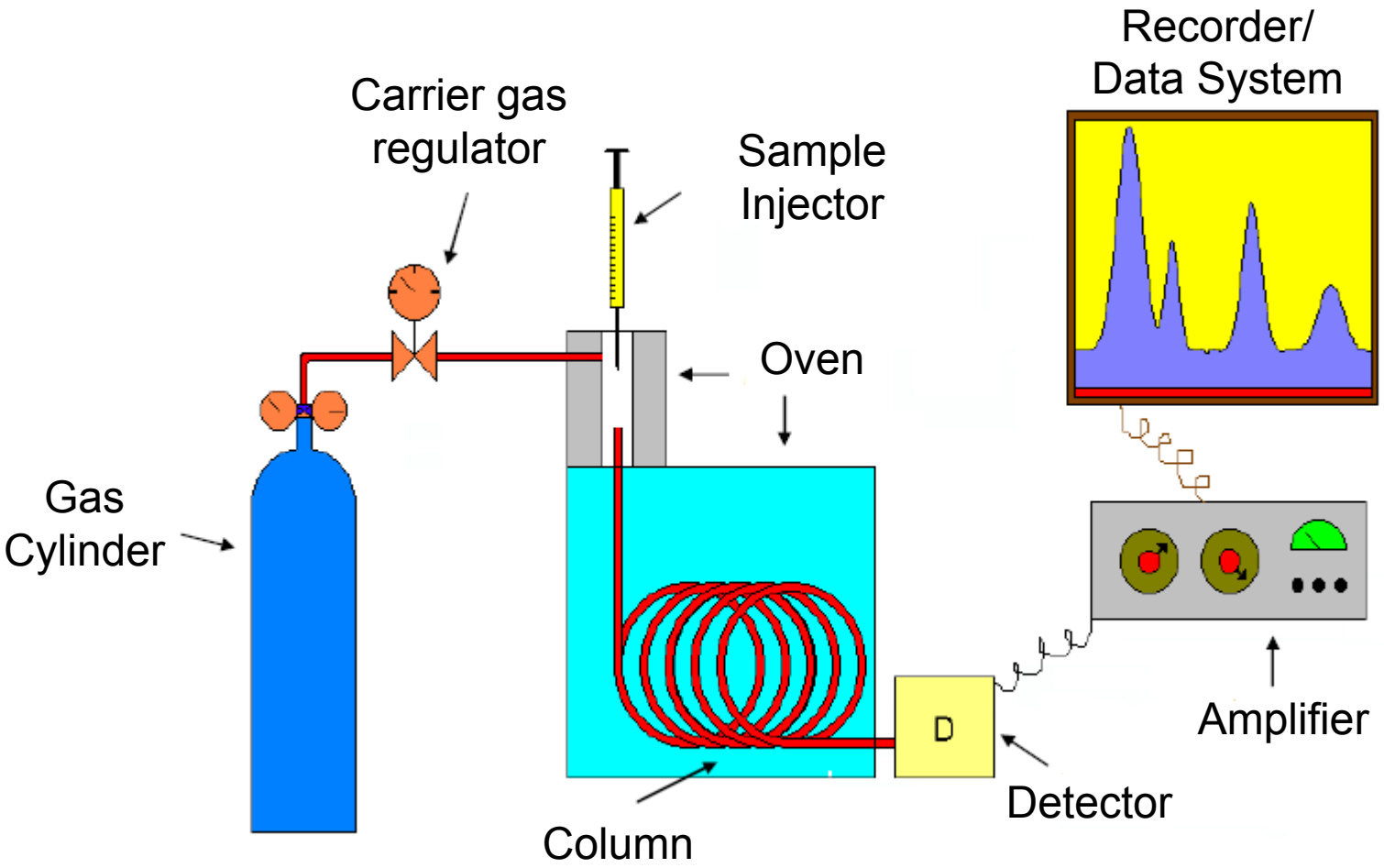
EQUIPMENT	COST (USD)	POPs
GC- ECD	40,000.00	OCPs, PCBs-Ind, PBBs PBDEs
GC-LRMS	140,000.00	OCPs, PCBs-Ind, PBBs, PBDEs
GC-HRMS	700,000.00	DIOXINS, FURANS, PBDEs OCPs, PCBs-Ind, dl-PCBs
LCMSMS (ESI neg) **LCMSMS (APCI)**	200,000.00	PFOS and other anionic PFCs, PFOSA

UNEP – Guidance on the GMP for POPs, 2013

Detection range

	DDT/DDE	HCB	Dieldrin	Toxaphene
GC/ECD	1 pg	0.5 pg		
GC/MS-EI	1-10 pg	1-10 pg	25 pg	500 pg
GC/MS-NCI	0.1 pg	0.1 pg	1 pg	10 pg
GC/HRMS	0.05 pg	0.05 pg	0.1-0.5 pg	10 pg

Gas Chromatography Components



Carrier Gas

Diffusion coefficients D_G (cm^2/s) of n-octane and viscosity η(μP) of most used carrier gases

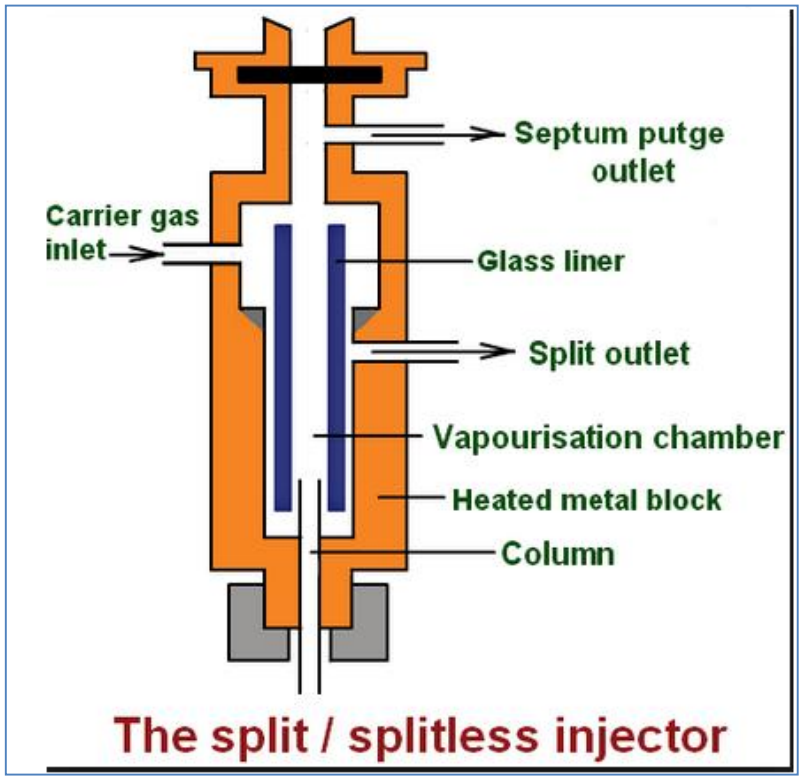
Carrier gas	D_G (30 °C)	η (50 °C)	η (150 °C)
H_2	0.277	94	112
He	0.248	208	249
N_2	0.073	188	227
Ar	0.059	242	296
CO_2	0.059	162	206

Hidrogen:
Disadvantage: explosive in mixture of Air

Advantage: fast separation (low analysis time), separation of strong retained components

Sample injection

→ Capillary Columns



www.chemistry.tutovista.com

SPLIT – Flow splitter

→High number of components

→ <10% of sample

Use: qualitative analysis at high chromatographic resolution/less for quantitative analysis

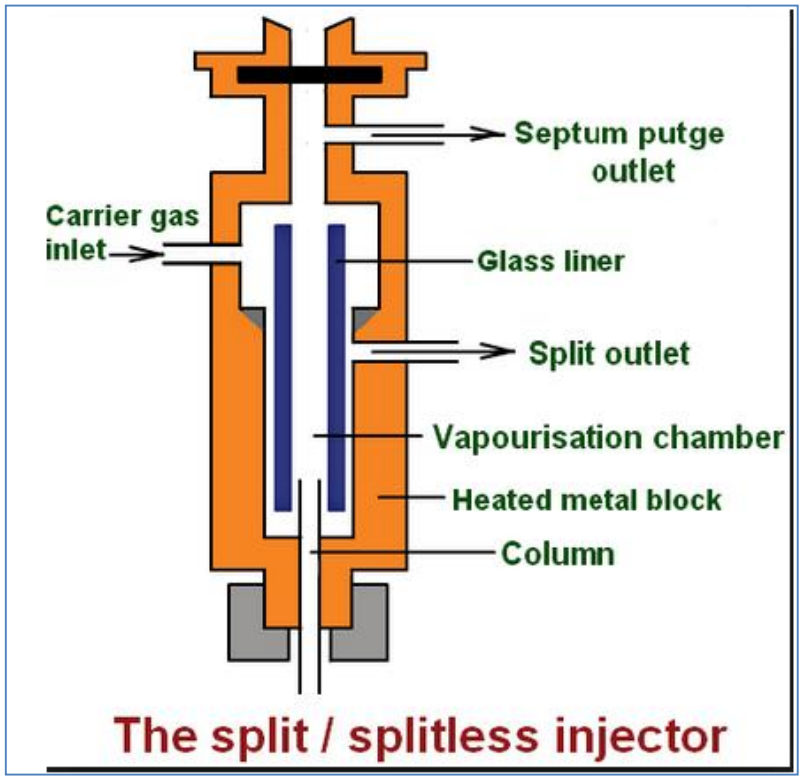
Disadvantages:

→Discrimination of compounds with higher b.p.

→Loss of injected sample according to split opening

Sample injection

→ Capillary Columns



www.chemistry.tutovista.com

SPLIT LESS – without Flow splitter

→ diluted samples

→ injection of aprox. 80% of sample

Splitter closed 1-2 minutes, sample enters column any longer

Injection ways:

→Into hot column

→Into cold column (temp. 20-30ºC lower than b.p. of solvent used)

Disadvantages:

→Analysis of diluted samples without preconcentration

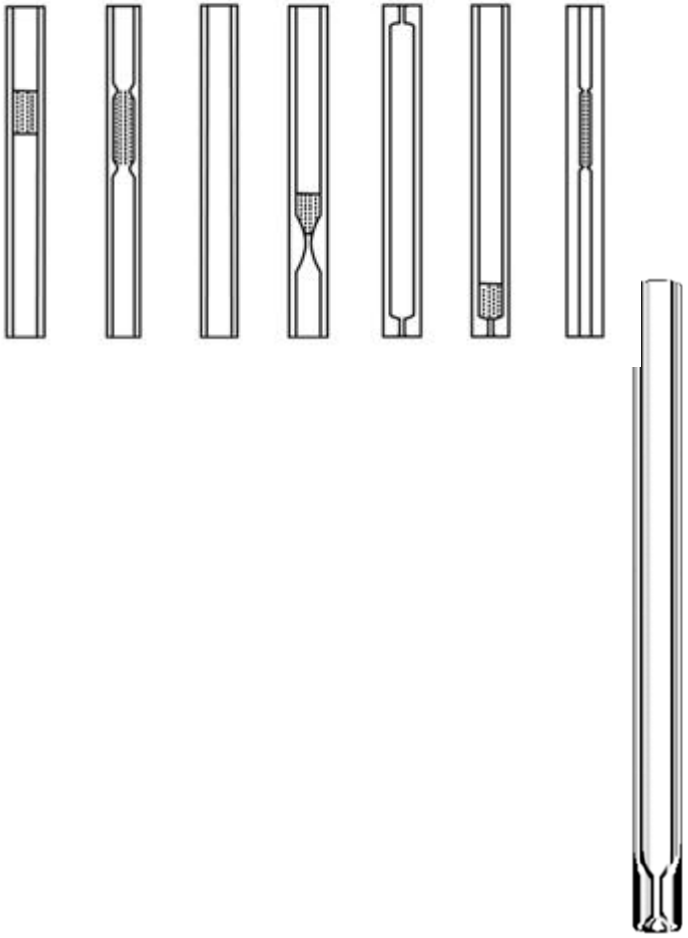
→Analysis of impure samples (change of injection liner and retention gap)

Sample injection liners

Liner design should:

- Minimize mass discrimnation by ensuring complete vaporization of the sample before it reaches the column entrance
-The volume of the inlet liner must be larger than the volume vaporized sample and solvent
- must not react with the sample (specially important for polar solutes where the liner should be deactivated)
- Addition of quartz wool increase the vaporization surface area for the sample and promote efficient mixing of the sample and carrier gas
- The position of quartz wool should be optimized corresponding to the needle depth in the liner
- Recommended not to use wool for low level pesticides such as DDT and Endrin

Different liner design



Degradation of Endrin and DDT (injection port)

DDT → DDE + DDD

Endrin → Endrin Aldehyde + Endrin ketone

Degradation of Endrin and DDT (injection port)

$$DDT \rightarrow DDE + DDD$$

$$\%breakdown = \frac{\sum DDE+DDD}{\sum DDT+DDE+DDD} \times 100$$

$$Endrin \rightarrow End.\ Aldehyde + End.\ Ketone$$

$$\%breakdown = \frac{\sum End.\ aldehyde + ketone}{\sum Endrin+End.aldehyde+ketone} \times 100$$

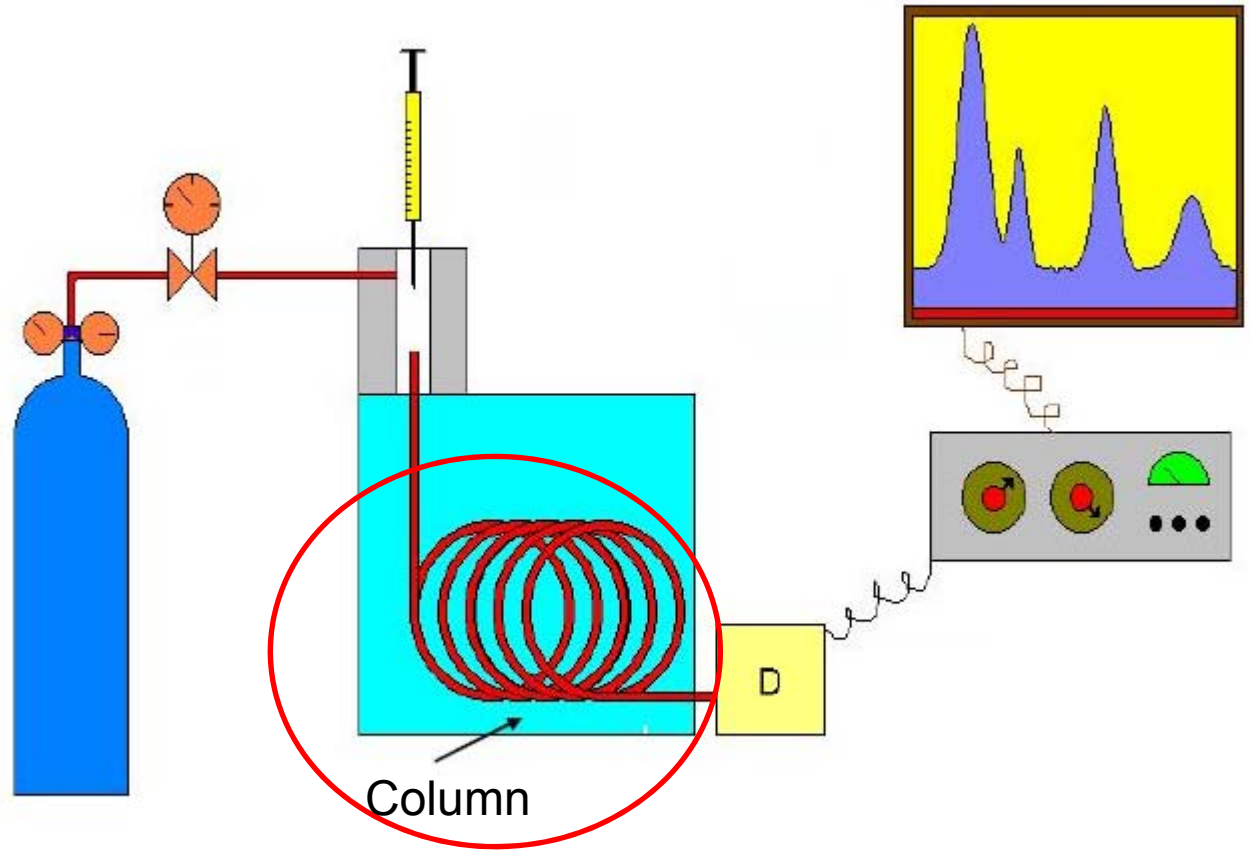
Aceptance criterium: degradation $\leq$ 20%

Degradation data: real example

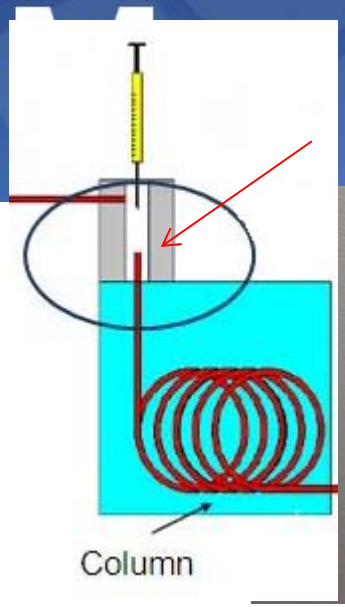
	1) Initial situation	2) liner/ septum change	3) liner/septum and column change
% Endrin degradation	61	30	8
% DDT degradation	82	49	13

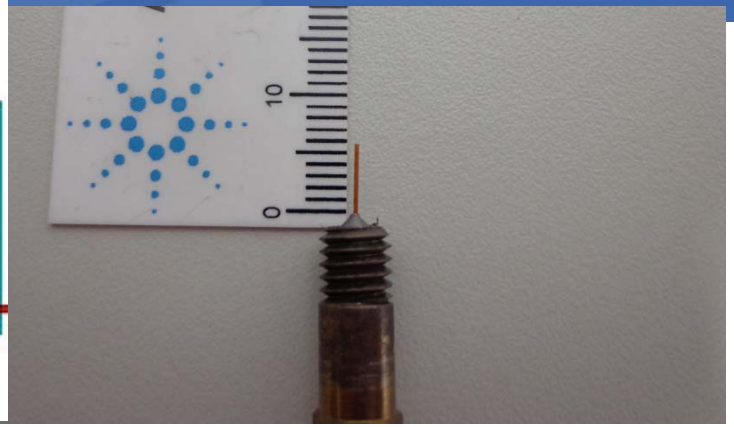
Aceptance criterium: degradation $\leq$ 20%

GC columns

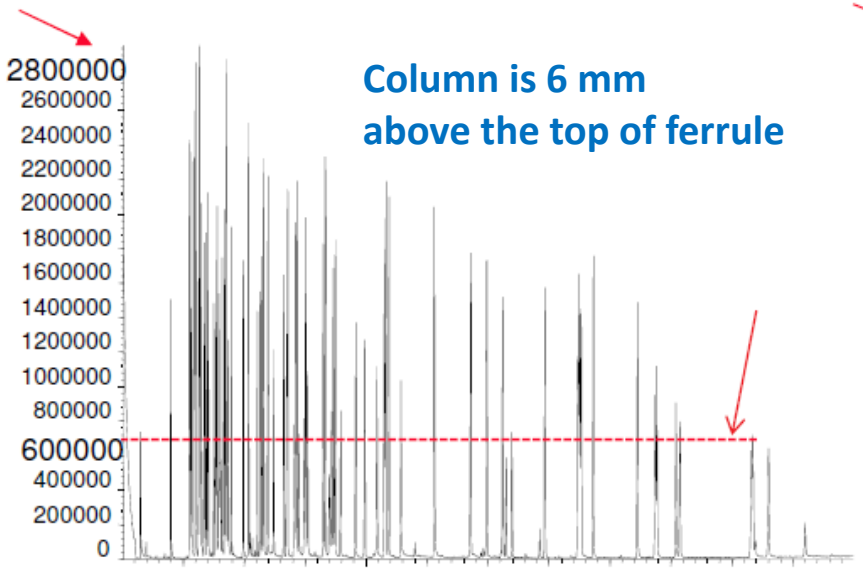


Column installation

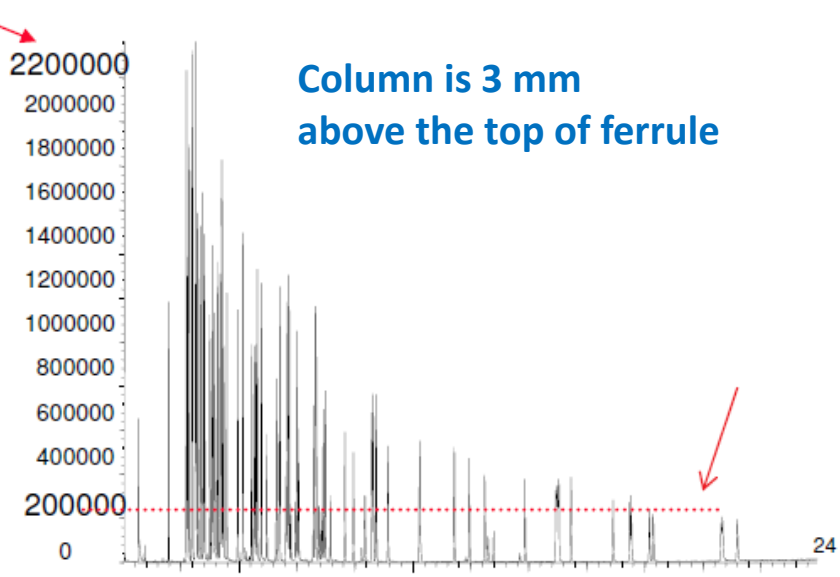




Installation!
can affect the response

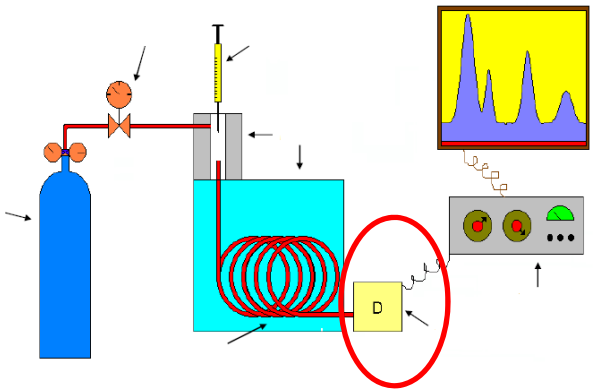


Column is 6 mm above the top of ferrule



Column is 3 mm above the top of ferrule

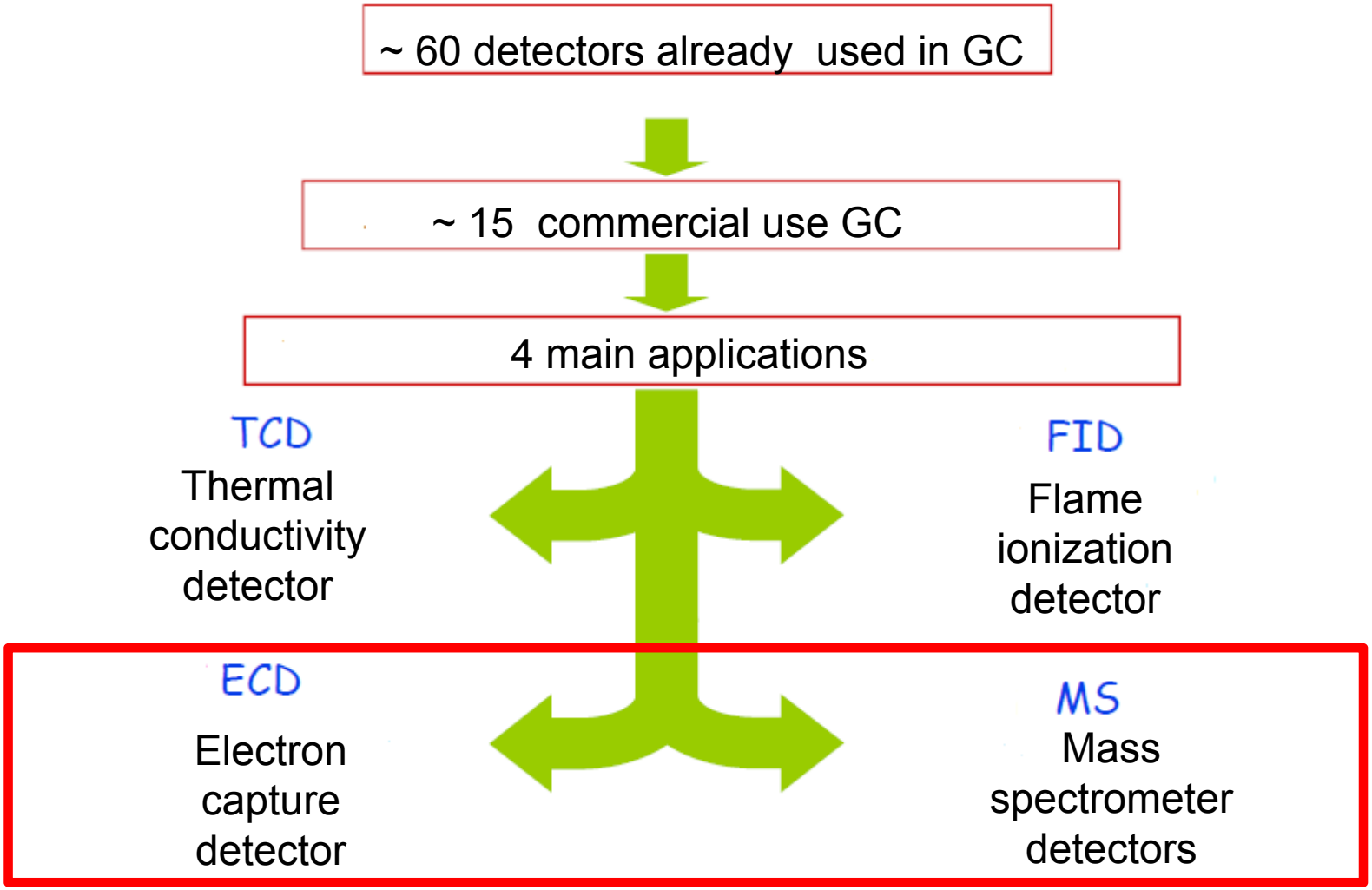
Detectors



Detector requirements

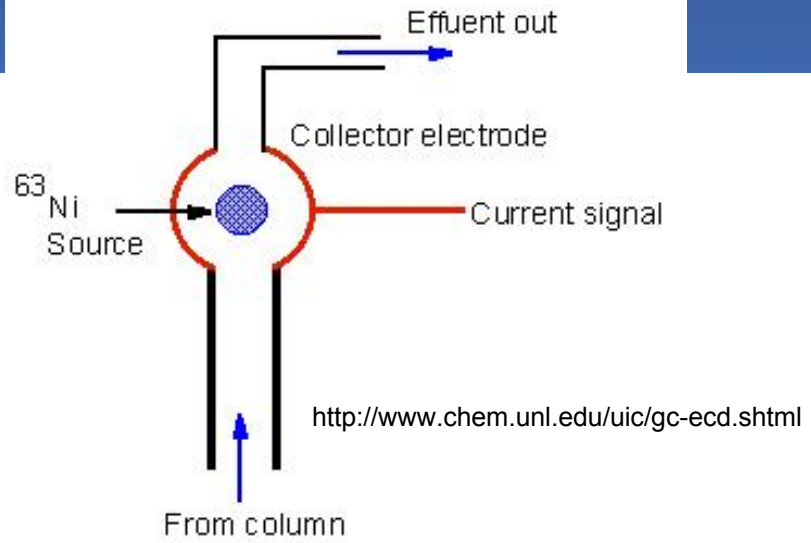
- ✓ High sensitivity
- ✓ Low limit of detection
- ✓ Good stability and repeatability of signal
- ✓ Low noise and drift of signal
- ✓ Linear response over several orders

Detectors



OCPs, PCBs and PCDD/F analysis

ECD – Electron capture detector



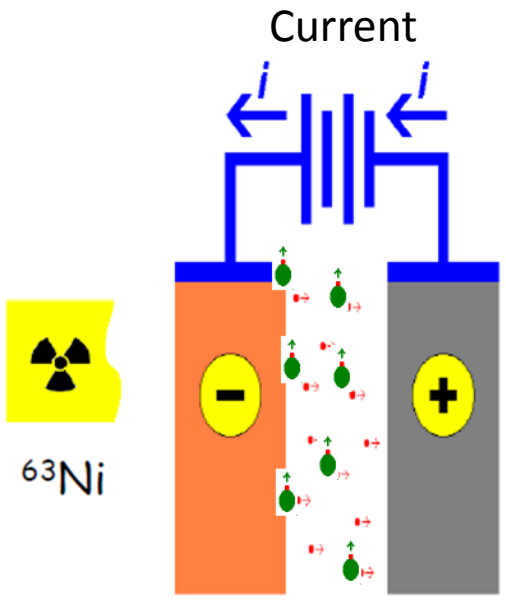
http://www.chem.unl.edu/uic/gc-ecd.shtml

^{63}Ni (source) → β emitter

$\beta + N_2$ (carrier gas) → $N_2^* + e^-$
Constant current (base line)

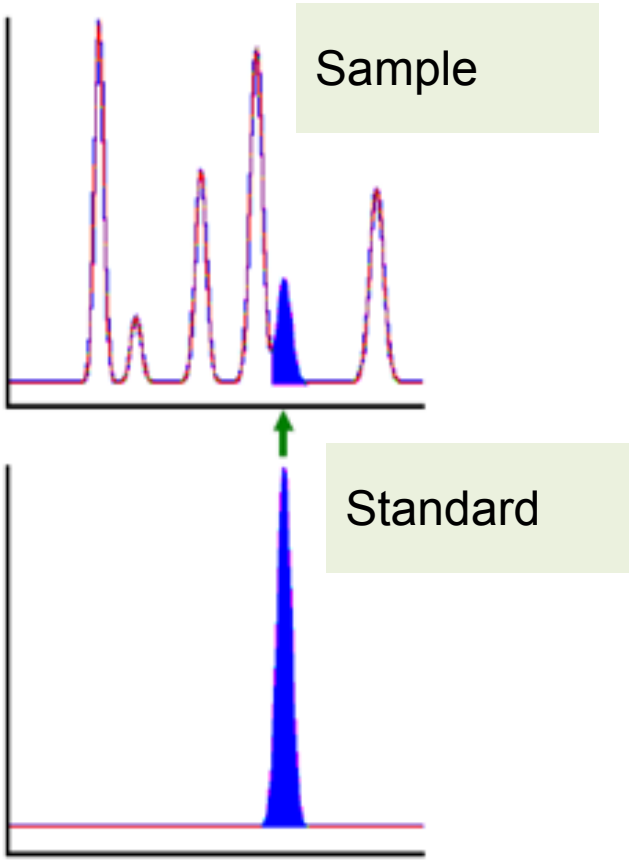
M-Cl + e^- → Decrease the current

The ECD uses a radioactive Beta emitter (electrons) to ionize some of the carrier gas and produce a current between a biased pair of electrodes.
When organic molecules that contain electronegative functional groups, such as halogens, phosphorous, and nitro groups pass by the detector, they capture some of the electrons and reduce the current measured between the electrodes. After processing, this signal creates the chromatogram

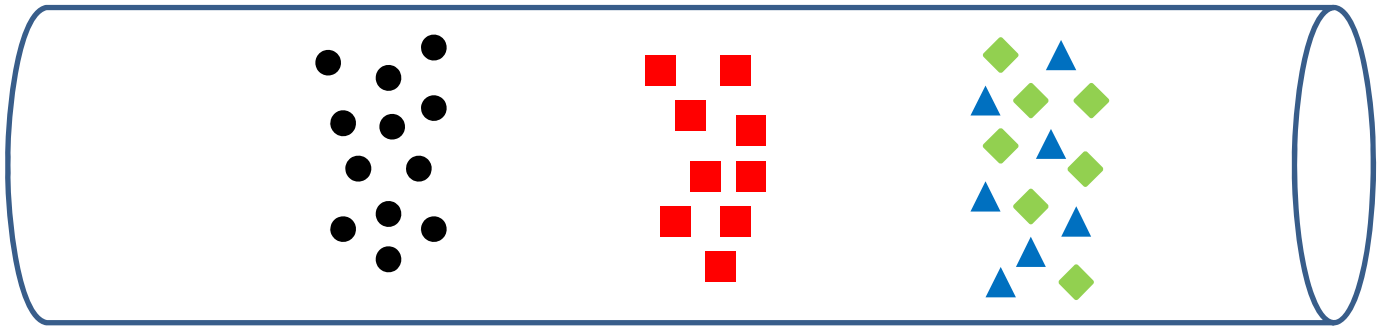


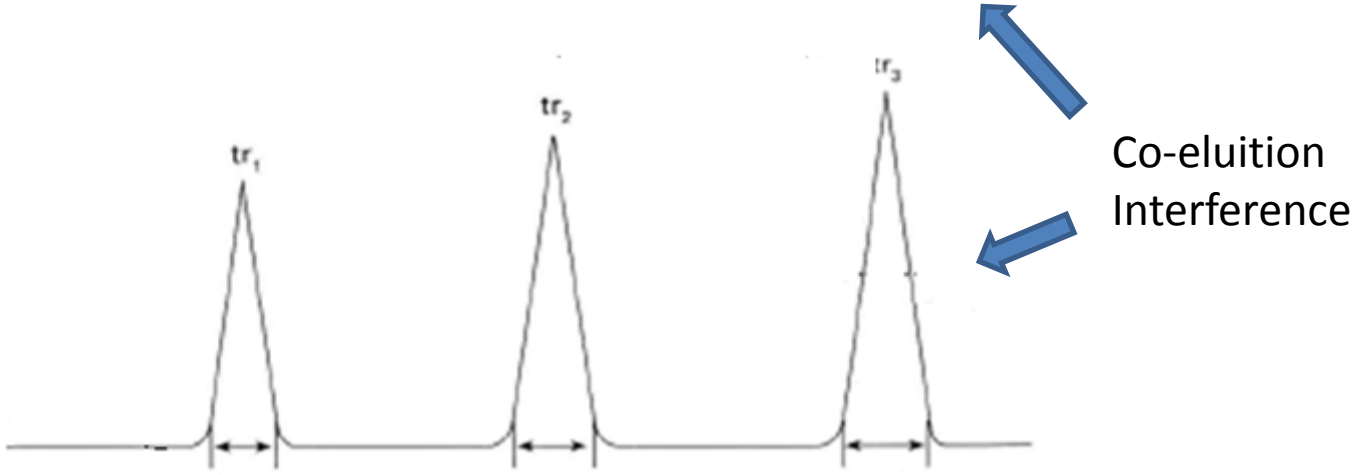
Identification by retention time

Retention time Comparison

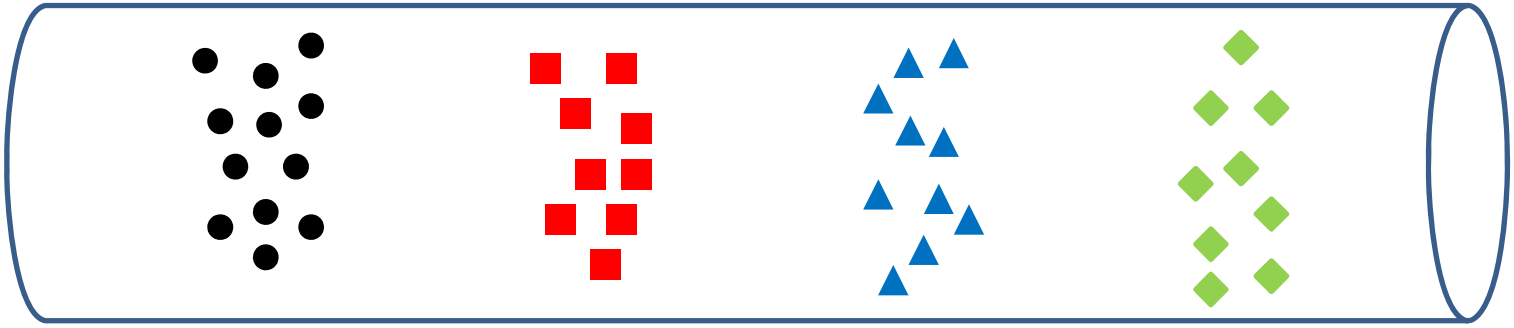


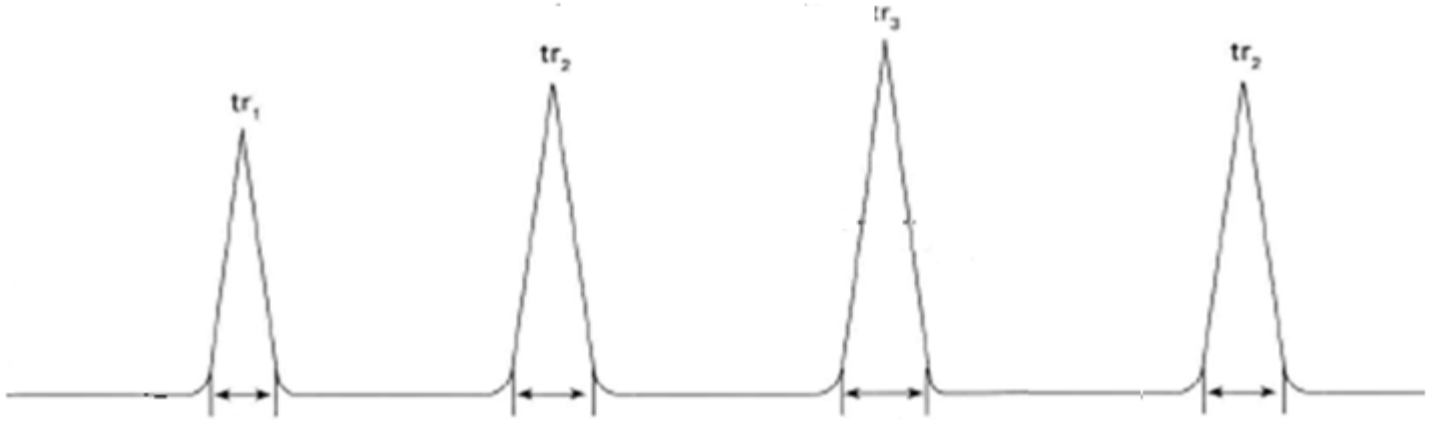
Interferences





2nd Column confirmation

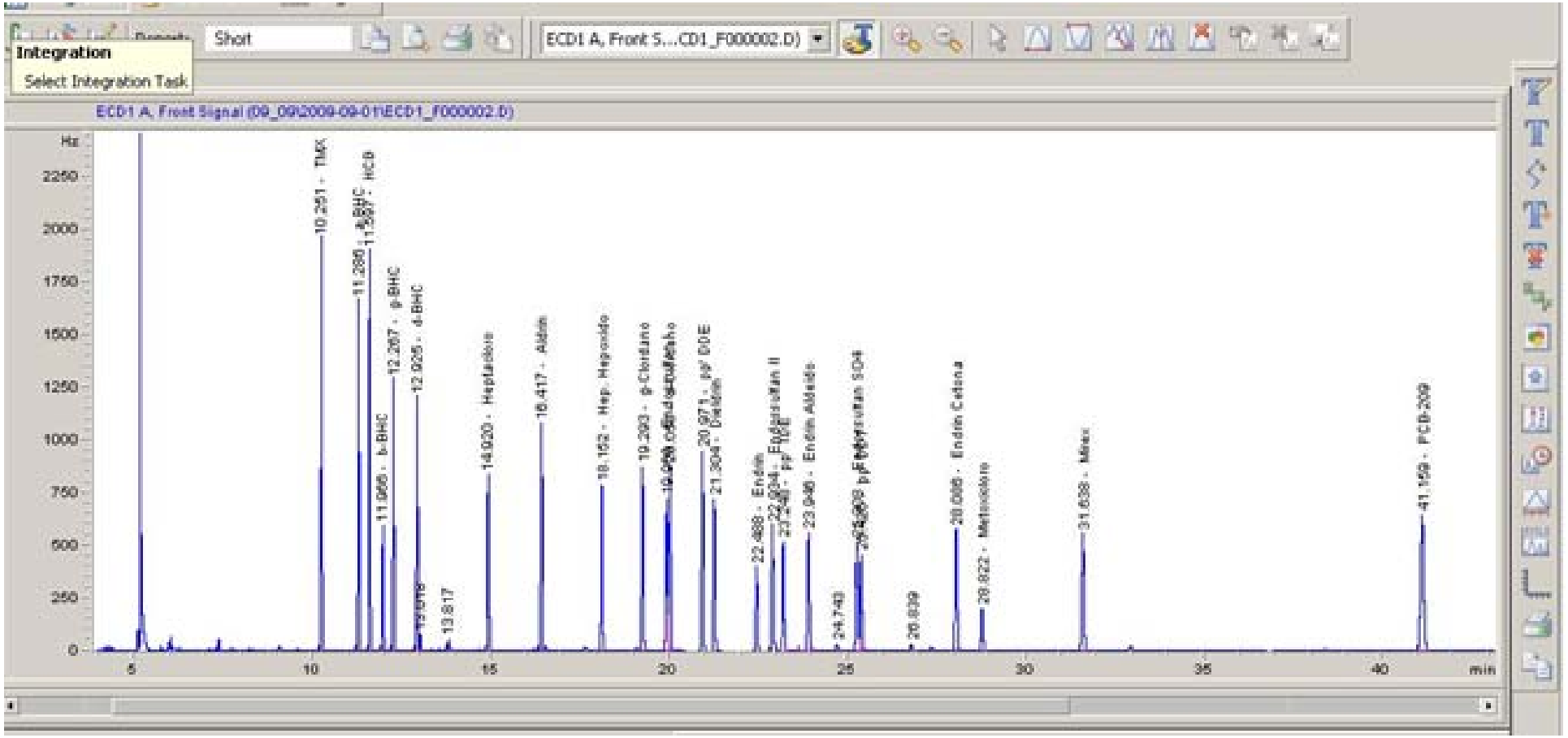




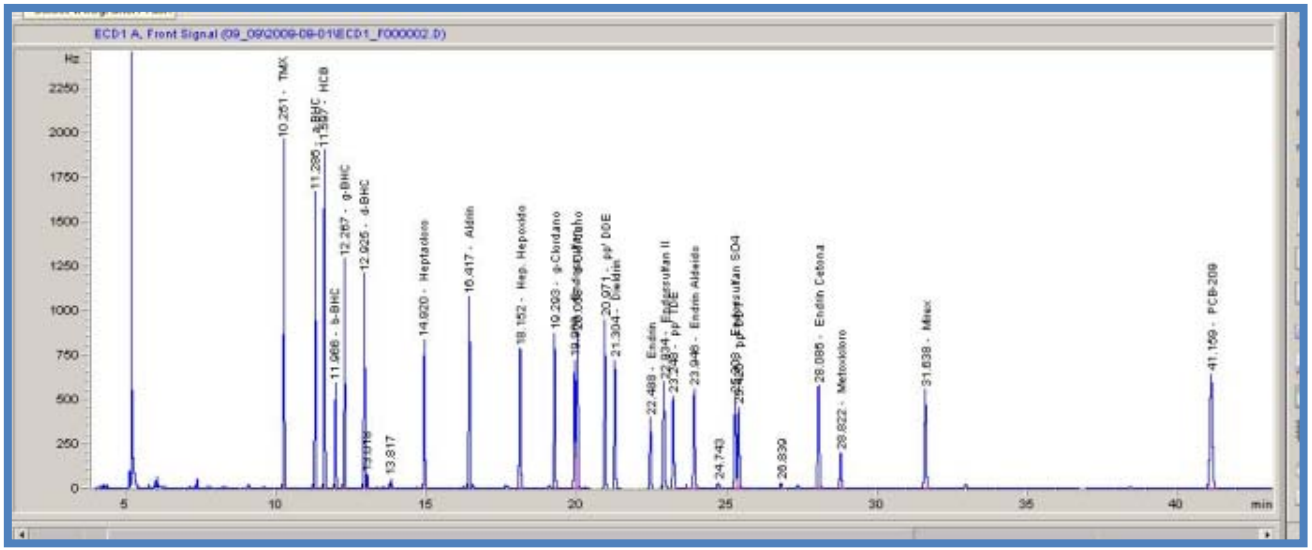
GC-ECD analysis: 2º column confirmation or GCMS confirmation

Standard chromatograms – ECD detection

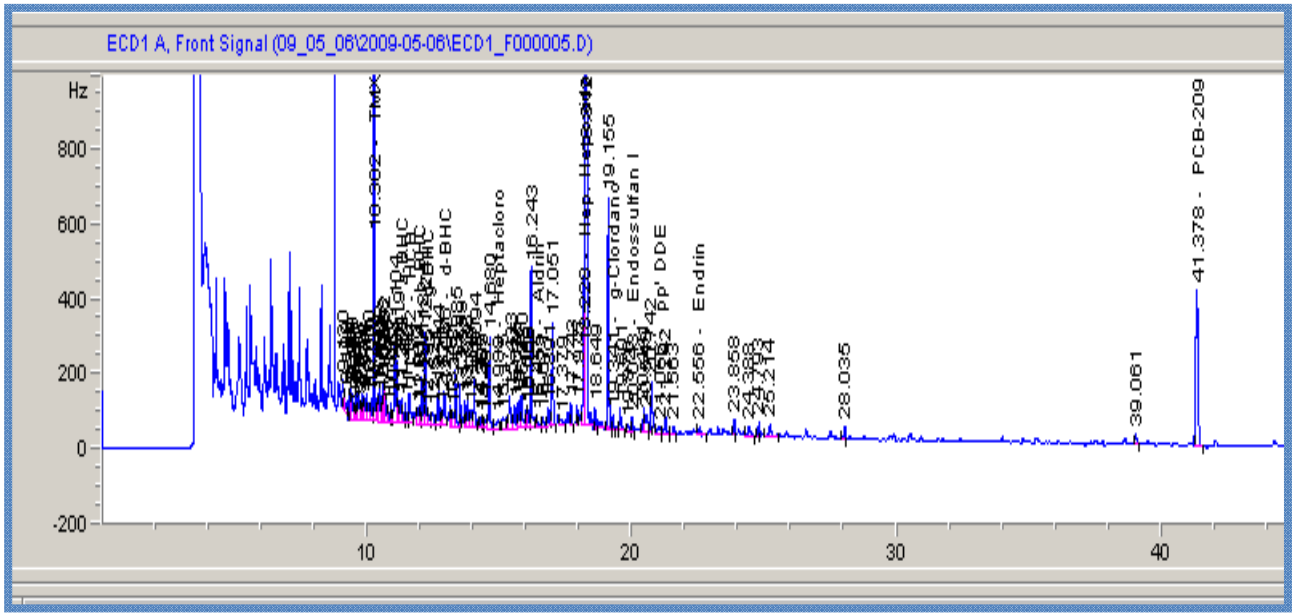
OCP standards + surrogates: TMX and PCB-209



OCPs Analysis: Chromatograms



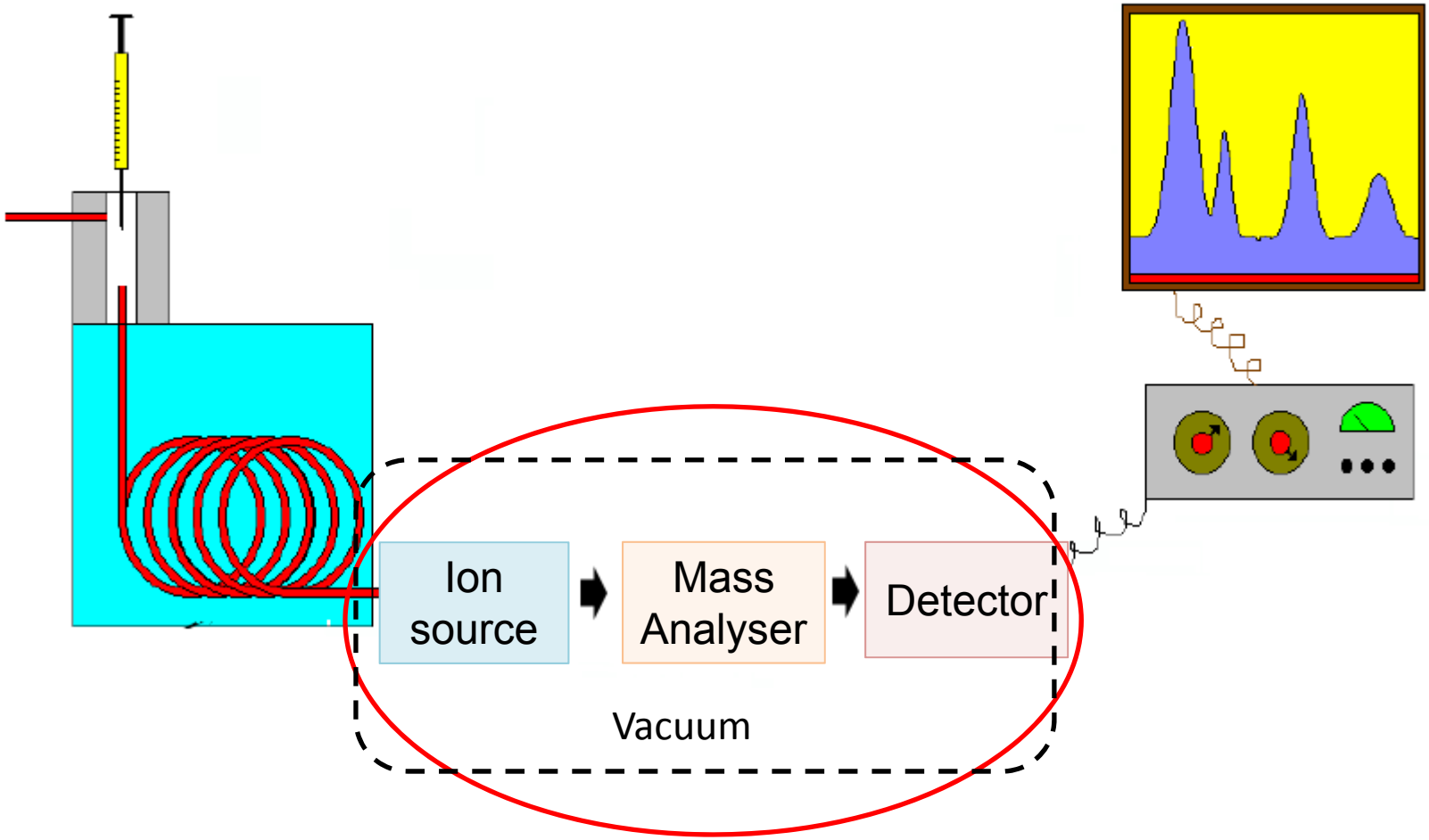
Standard



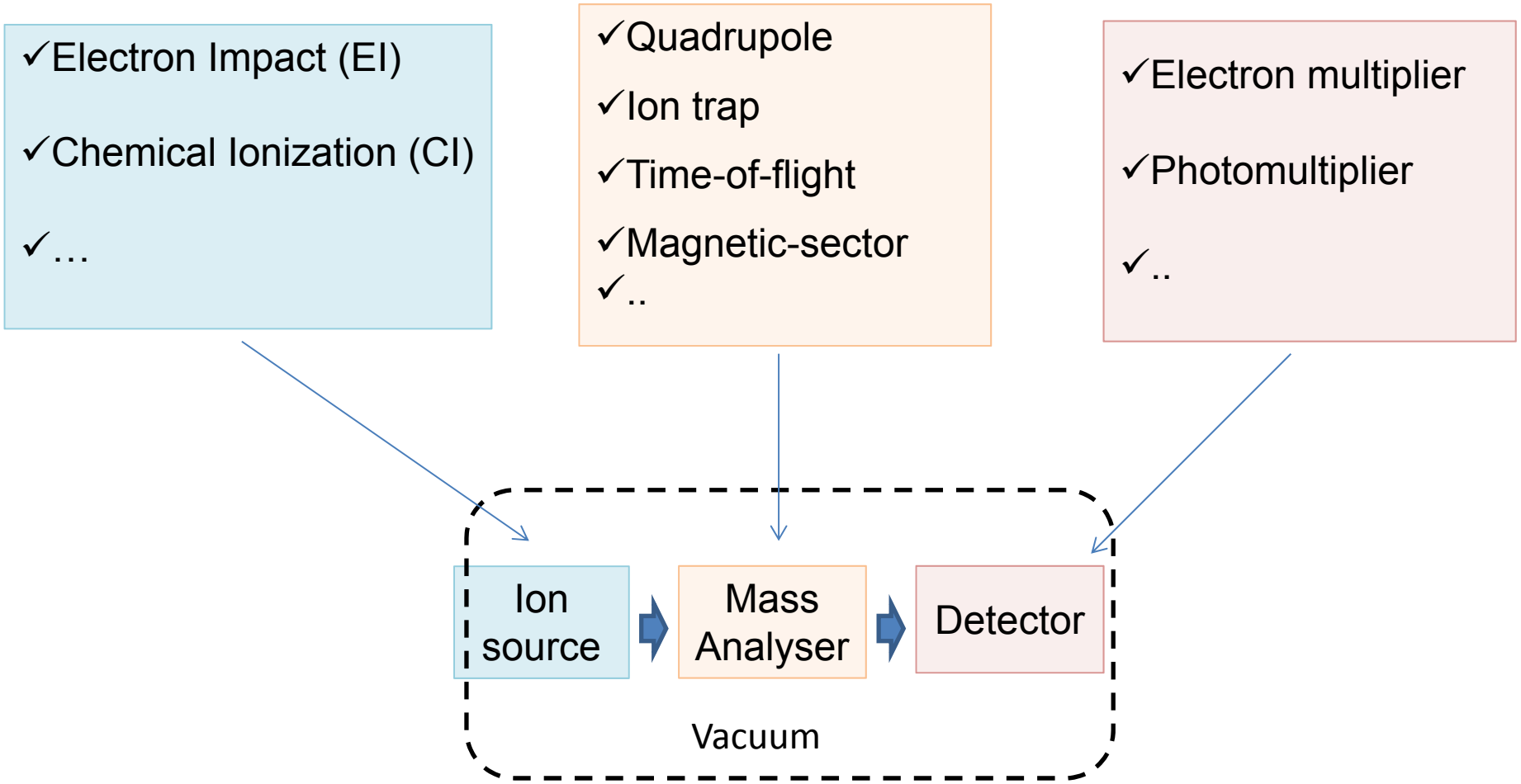
Sediment

Sample extract

Mass Spectrometry - MS



Mass Spectrometry - MS

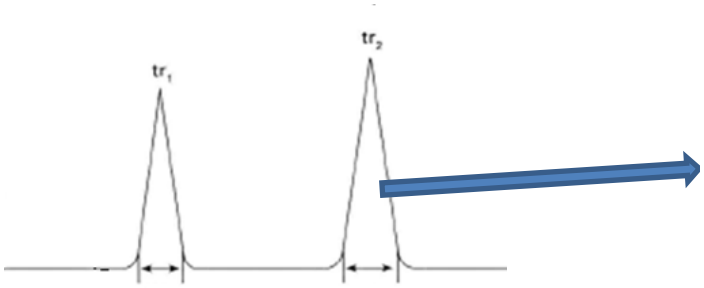


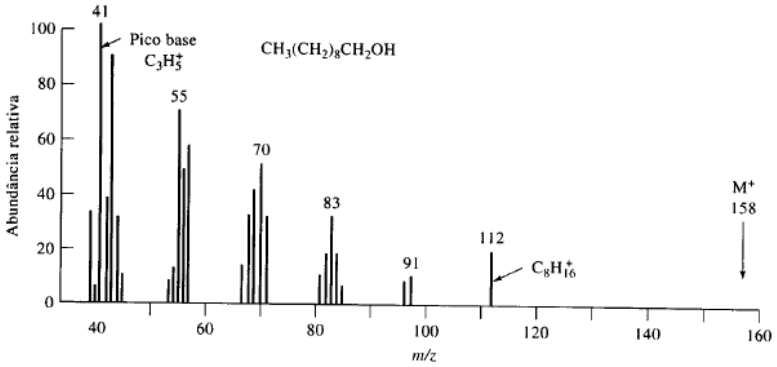
Ion Source

EI: electron impact ionisation: beam of electrons through the gas-phase sample.
Produces molecular ions or fragment ions.
Typically 70eV. Sample heated.
(+): Reproducible, structural information
(-): sample must be volatile and stable, molecular ion often abscent, almost impossible to distinguish between isomers (GC separation needed)
mass range: < 1000Da

MS

- Better identification of the compounds than ECD
- ID confirmaed by retention time and mass spectra
- Use of internal standard for quantification (ex: deuterated standards)

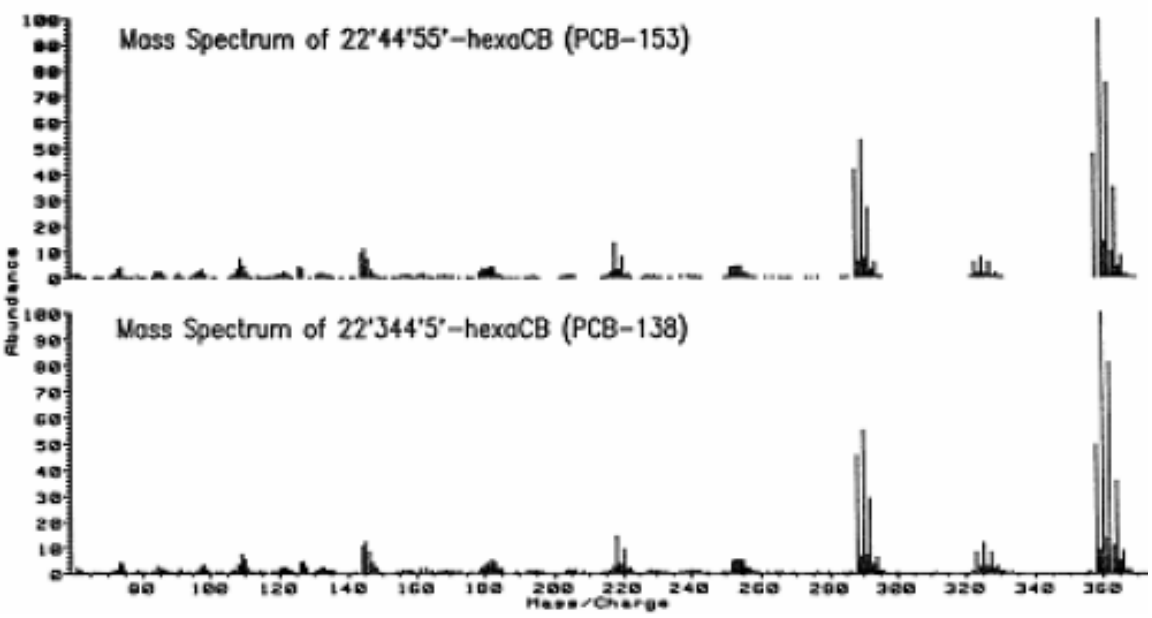




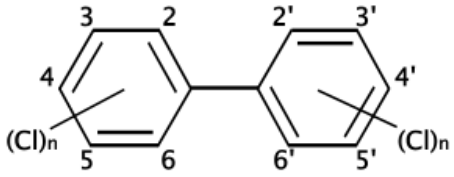
Fragments generated by the ion source

EI – Electron Impact

- 70 eV is usually used for scanning mass spectra
- The fragmentation gives structural information



Almost impossible distinguish between isomers
(GC separation needed)



n= 6

CI – Chemical Ionization

Methane gas ions react with the analyte

Generate positive ions – positive chemical ionization – PCI

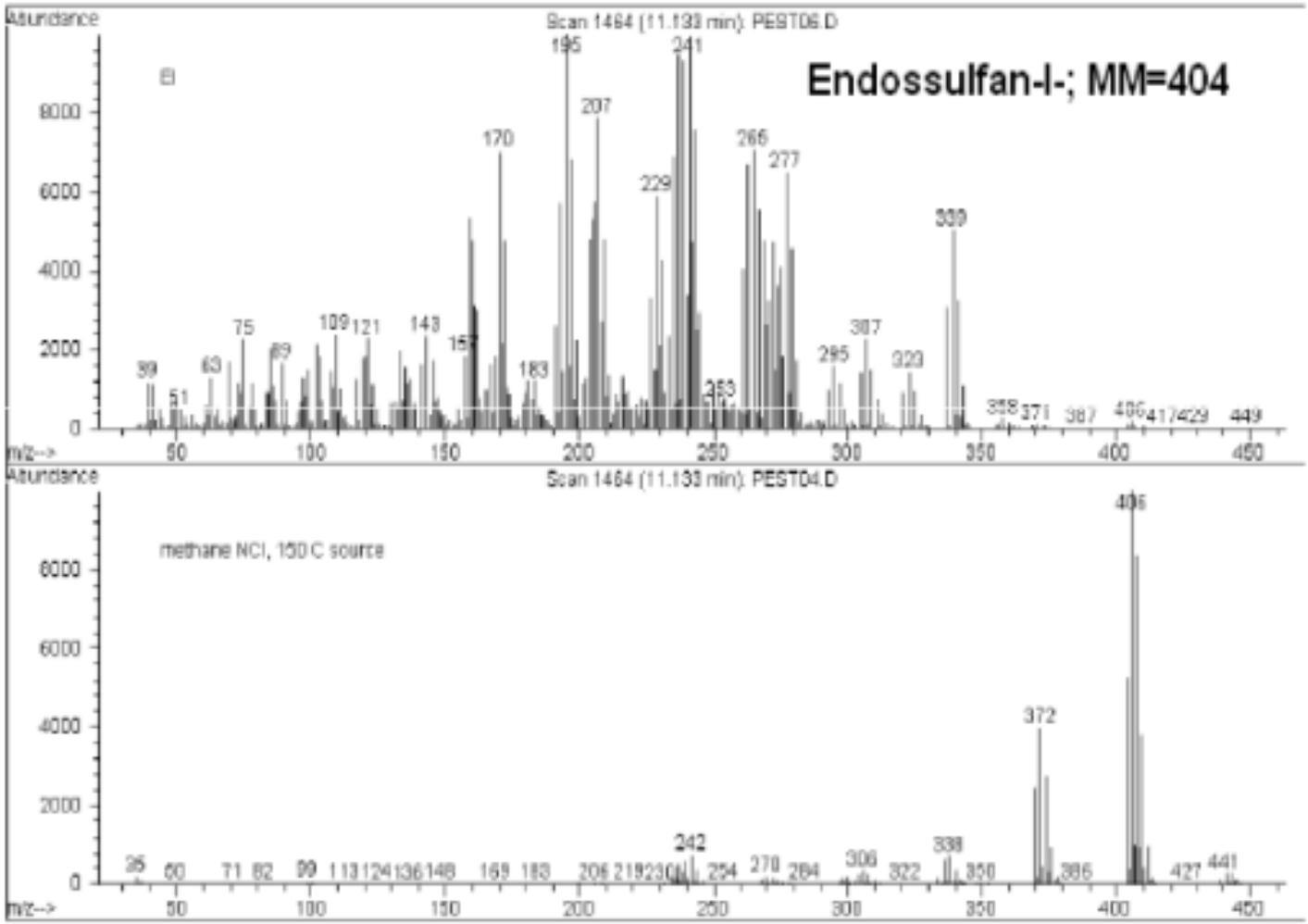
Generate negative ions – negative chemical ionization – NCI

Halogenated compounds present high eletronegativity,
, have a great hability to capture (receive) electrons
and gennerate negative ions

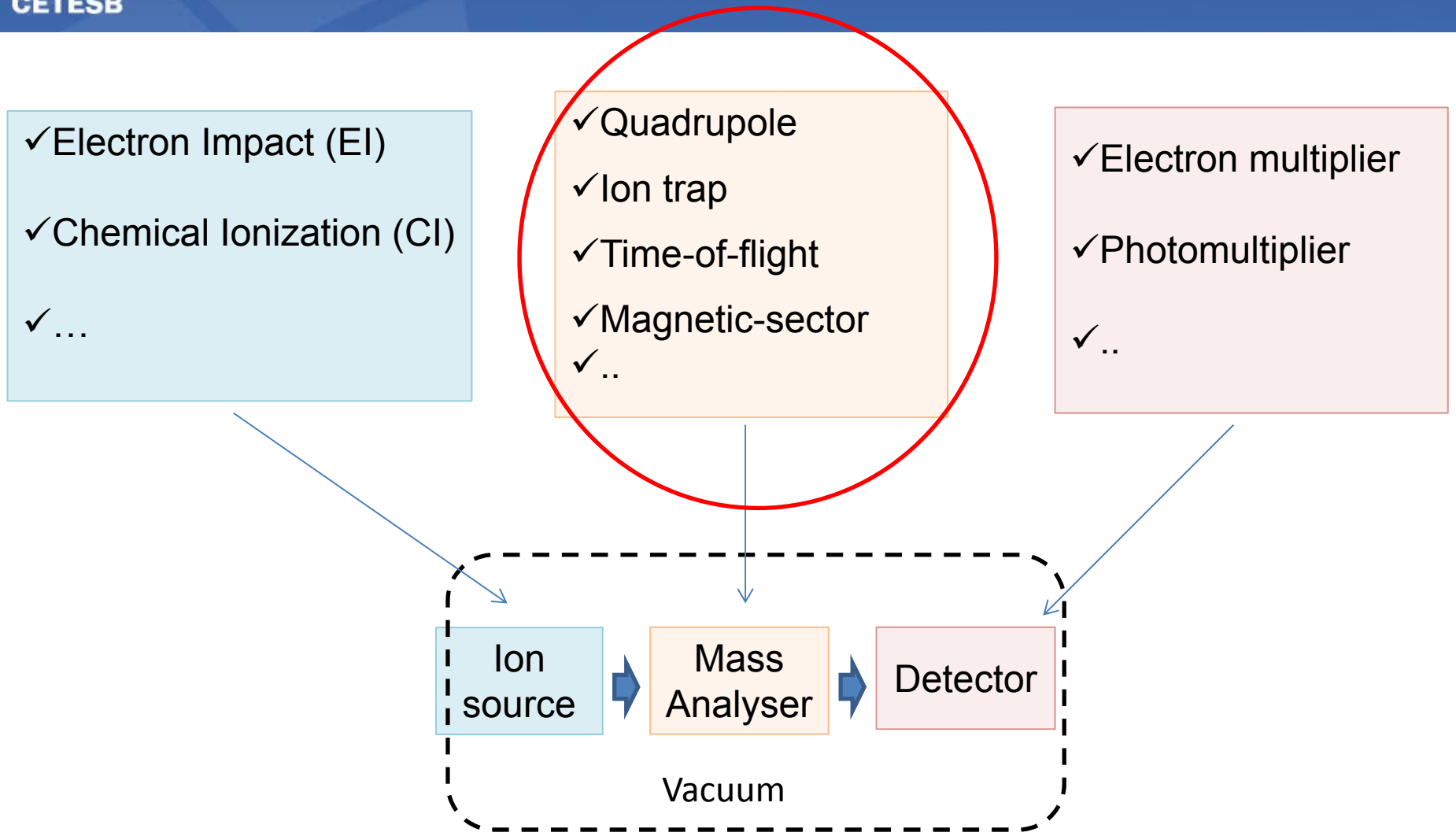
CI -Chemical Ionization

- CI generate less fragmentation than EI
- CI is more “soft” ionization than EI
- Reagent gas pressure increases→ the number of collisions increases
- Operation conditions: temperature and pressure should be controlled

Example EI x NCI



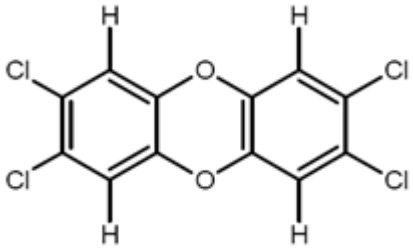
Mass Analyser



Low x High Resolution

monitored mass accuracy

Element	Isotope Nominal Mass	Relative Abundance (%)	Average Mass	Exact mass
H	1	100	1.008	**1.0078**
	2	0.016		**2.041**
C	12	100	12.011	**12**
	13	1.08		**13.0034**
N	14	100	14.007	**14.0031**
	15	0.38		**15.0001**
O	16	100	15.999	**15.9949**
	17	0.24		**16.9991**
F	19	100	18.998	**18.9984**
P	31	100	30.974	**30.9738**
S	32	100	32.06	**31.9721**
	33	0.78		**32.9715**
	34	4.4		**33.9679**
Cl	35	100	35.453	**34.9689**
	37	32.5		**36.9659**
Br	79	100	79.904	**78.9183**
	81	98		**80.9163**
I	127	100	126.905	**126.9045**



12 C = 144
4 Cl = 139,8756
4 H = 4,0312
2 O = 31,9898

M (Total) = 319,8966

Response:
Low resolution: 319,9/320
High resolution: 319,8966

Quality Control

- ✓ Blank (method, field)
- ✓ Calibration check standard /verification standard (Equipment)
- ✓ Control standard (spiked blank)
- ✓ Duplicate sample
- ✓ Spiked Sample
- ✓ Surrogate standard
- ✓ Internal standard (GCMS)
- ✓ Certificated Reference Materials (CRMs)
- ✓ Intercalibration Study
- ✓ ISO-17025 accreditation

POPs Analysis –Infrastructure

- ✓Consistent power suplly (UPS- uninterruptible power supply);
- ✓Air conditioning
- ✓Extraction hoods
- ✓Laboratory space to avoid interferences (physical separations)
- ✓Handling area for inflammable products
- ✓GC gas line (Helium, nitrogen)
- ✓Trained personnel to operate and troubleshoot equipment problems
- ✓Equipment maintenance, parts….

……..

High cost $$$!!

Thank you!!

Gracias!!

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Physical-chemical Analysis Division
ela_cetesb@sp.gov.br

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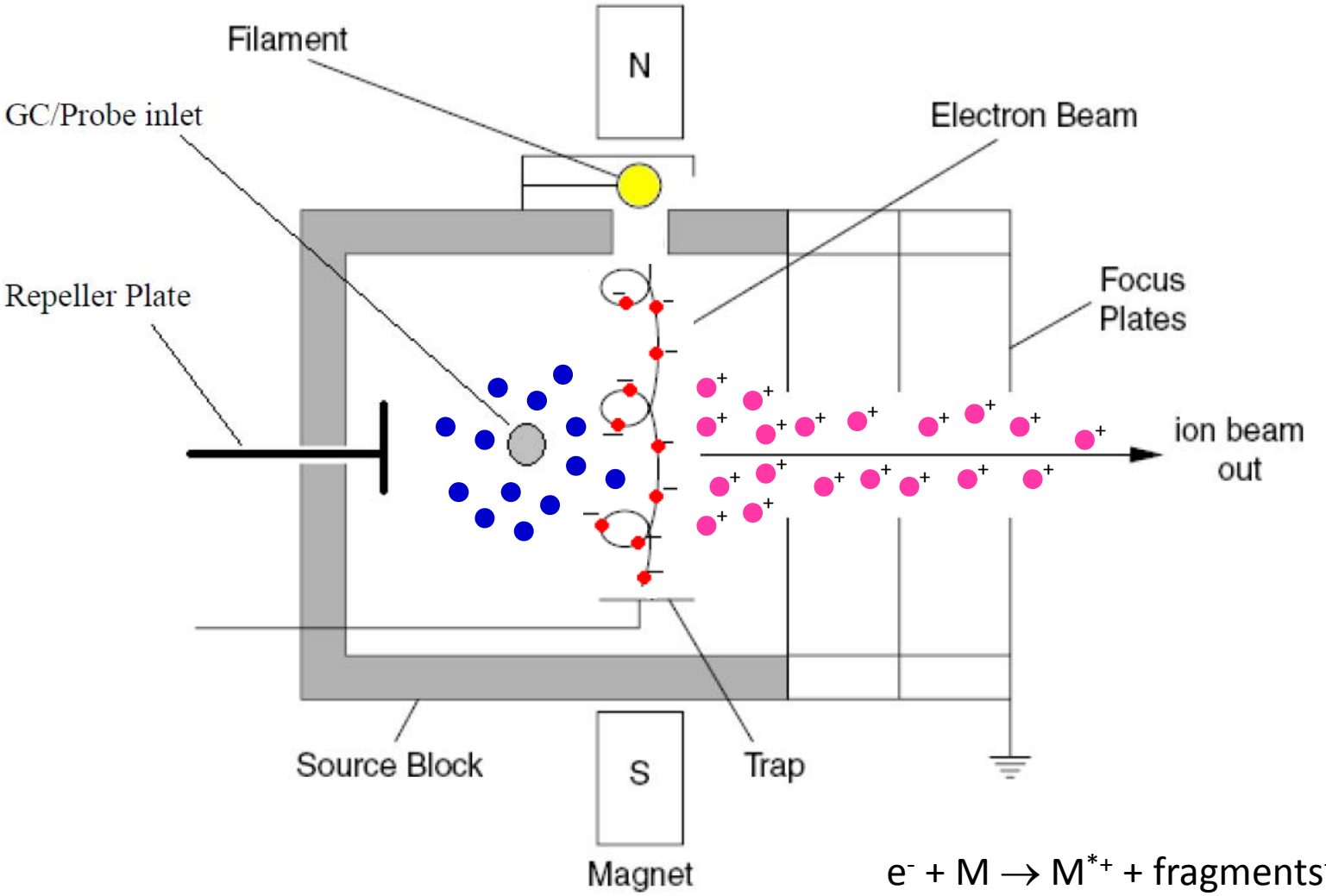
EI -Electron Impact



$e^- + M \rightarrow M^{*+} + fragments^+$

CI -Chemical Ionization

Uses ion molecules reactions to produce ions from the analyte.
The reagent gas (methane, isobutane, or ammonia) is ionized with electrons.
High gas pressure: (R = reagent, S = sample, e = electron, . = radical electron , H = hydrogen)

R + e ---> R+. + 2e

R+. + RH ---> RH+ + R.

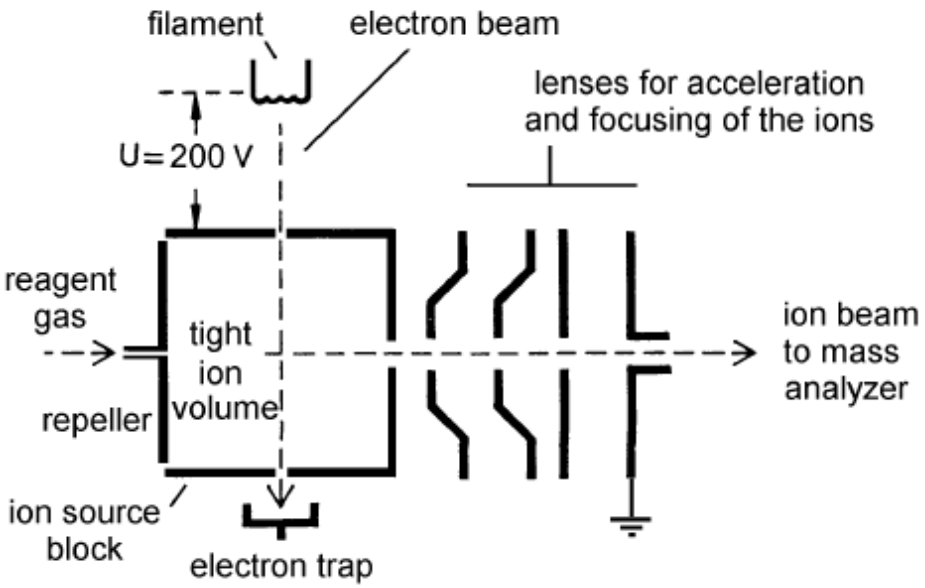
RH+ + S ---> SH+ + R

Heated sample.

+ [M+H]+ often visible, less fragmentation than EI

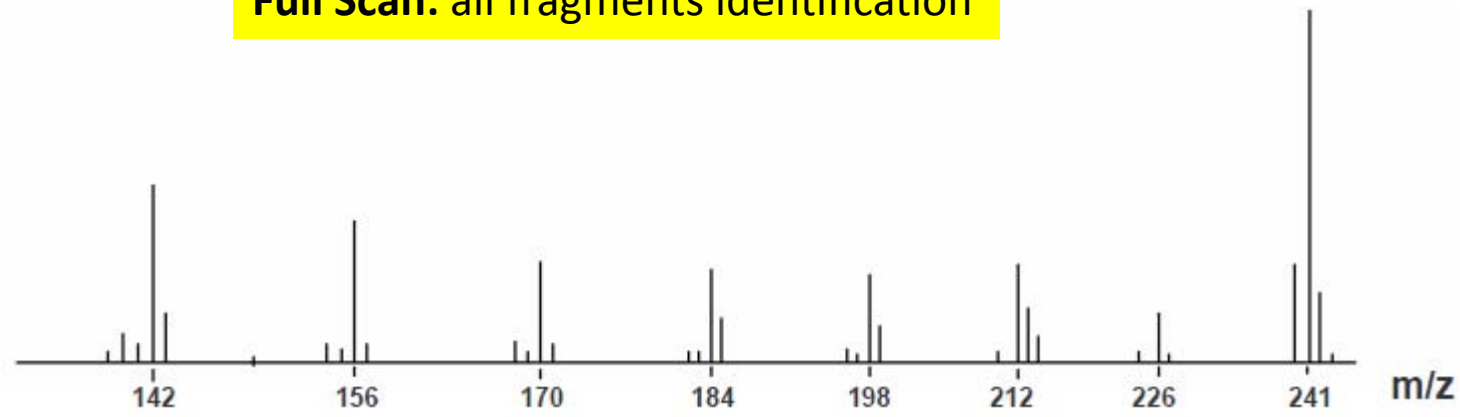
-sample must be volatile and stable, less structural info than EI

mass range: < 1000Da



Full Scan x SIM

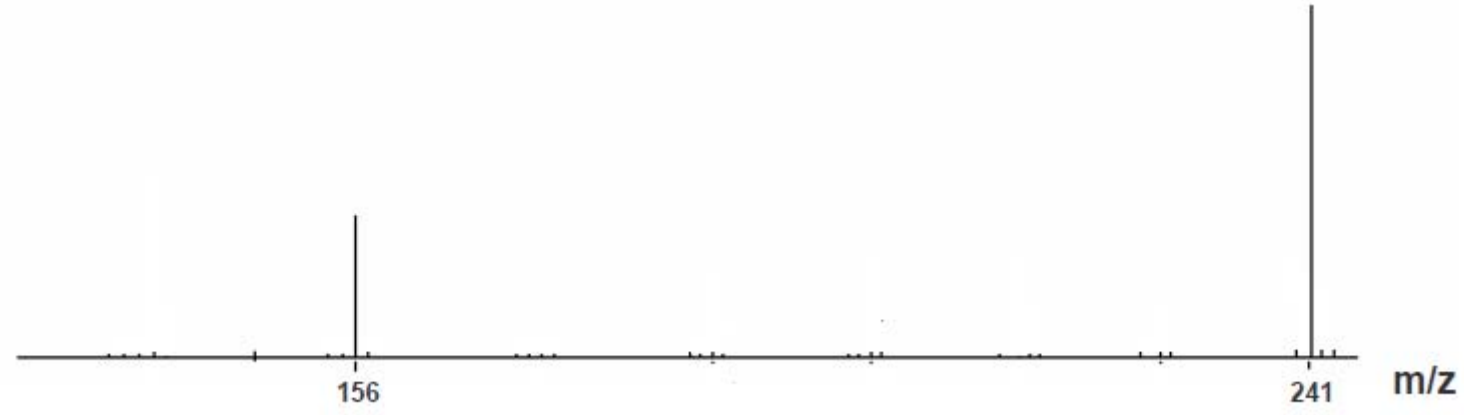
Full Scan: all fragments identification



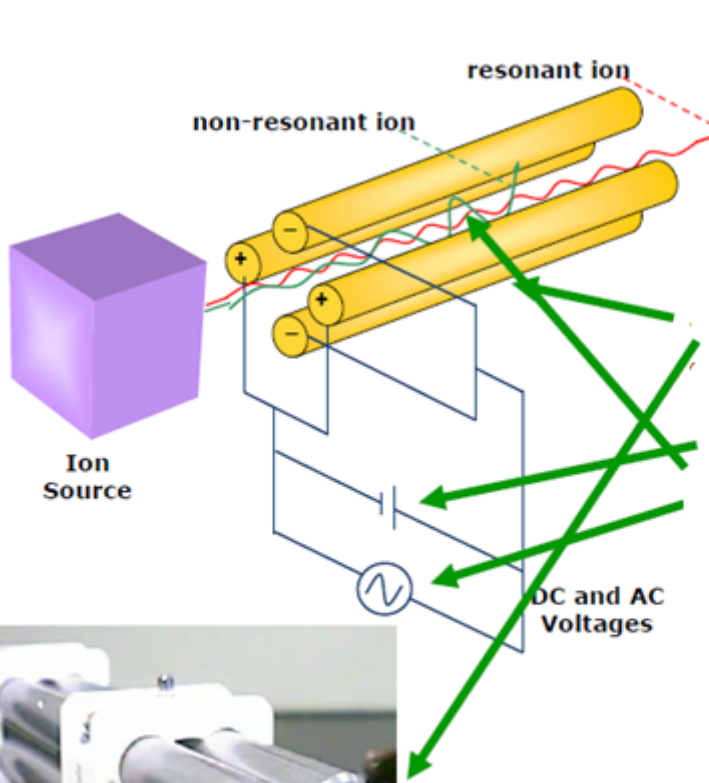
SIM: Single Ion Mode

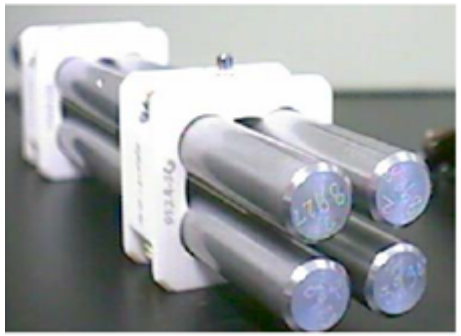
exemple: Selecting 2 fragments, 1 para quantification and the other qualify

Eliminates possible intereferents, increase sensitivity



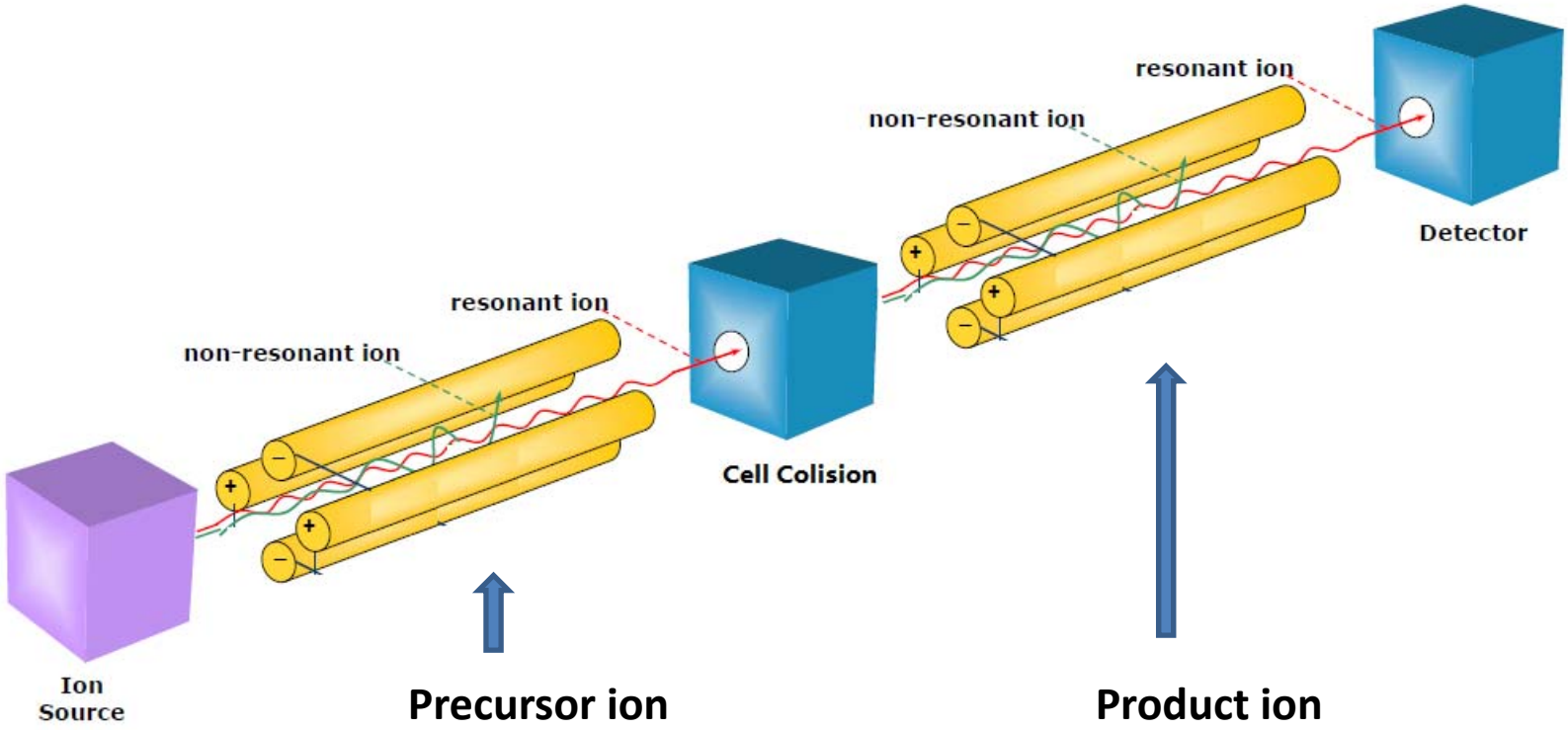
MS - Quadrupole





- Consists of 4 parallel rods (quadrupole)
- 2 opposite voltages DC and AC is apllied between adjacent rods
- The voltage affect the ions trajetory crossing the centre of the roads
- Depending on the voltage DC and AC applied, only some mass/charge ions pass through the filter
- Is obtained the mass spectrum by monitoring the ions passing through the quadrupole filter

MS/MS



Precursor ion
Full Scan
Full Scan
SIM
SiM

Product ion
Full Scan
SIM
SIM
Full Scan

Increases sensitivity, qualitative confirmation

Ion Trap MS

• Ion trap analizer consist of 3 eletrodes with hiperbolic surfaces for “trap” the ions in a small volume – na electrode in a central ring and 2 adjacent. The mass spectrum is obtained by changing the voltage and eject the ions from the trap.

• Compact size , hability to “trap” and accumulate ions to increase the signal-noise

•Easily used in the MS/MS mode