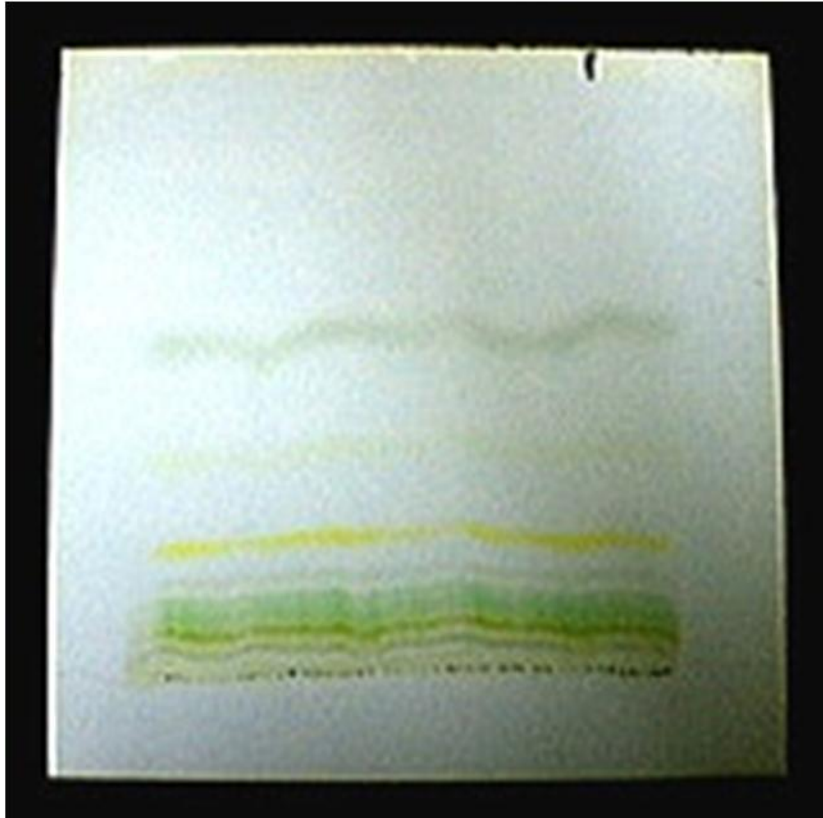


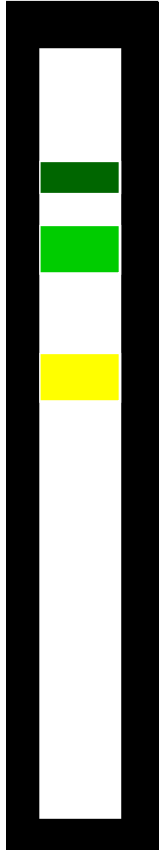
Chromatography and Structure Elucidation

Invention of Chrommatography (1910)



Mikhail Tswett
Russian Botanist
(1872-1919)

Principle of Chromatography



- Glass column filled with stationary phase (silica, aluminum oxide, sugars and sugar polymers, cellulose)
- Mixture of analytes is applied to the column head and eluted with solvents of different polarity
- Analytes are separated into zones.
- Tswett separated pigments and got colored zones (chromatography)

Development



Richard Kuhn

1931:

Richard Kuhn and Edgar Lederer demonstrated the applicability of this method to purify compounds (e.g. vitamine B2).

Development



Richard L. M. Synge

Richard L.M. Synge und Archer J.P. Martin Kieselgel standardized silica gel as stationary phase for column chromatography.

Silica seved as adsorbens for water (stationary phase). The mobile phase is a mixture of organic solvents that do not mix with water.

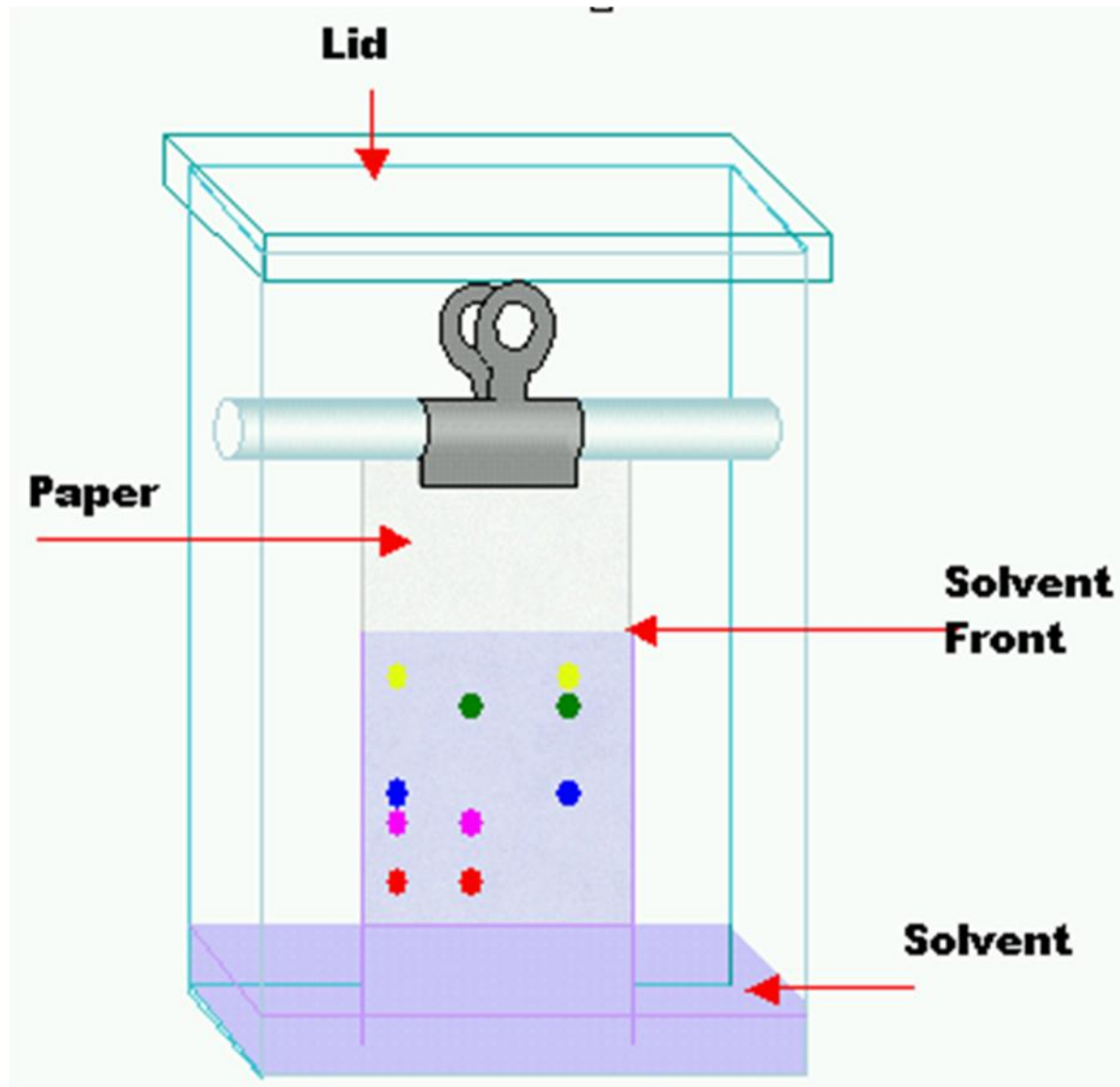


Archer J.P. Martin

The bad reproducibility of this method led to the establishment of paper chromatography in the 40ies.

Synge and Martin postulated the applicability of gases as mobile phases

Paper Chromatography



Thin Layer Chromatography (TLC)

TLC originally was described by the Russian chemists Maria Schraiber und Nikolai Izmailov in 1938.

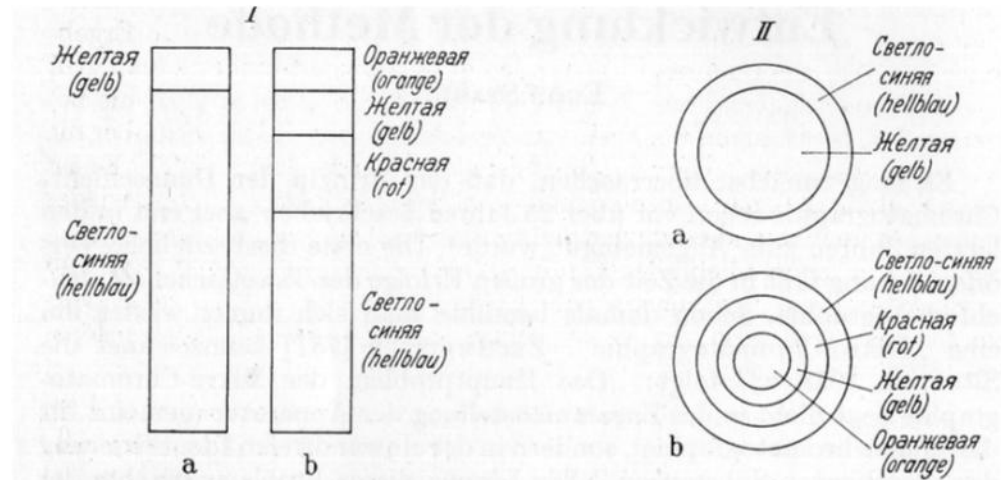


Abb. 1. Vergleich der Fluoreszenzfarben eines Aluminiumoxid-Säulenchromatogramms (I) von Toll-chen-Tinktur mit einem „Tropfenchromatogramm“ (II) vor dem Entwickeln (a) und nach dem Entwickeln mit Spiritus (b). — Die Abbildung ist der Arbeit von IZMAILOV und SCHRAIBER [313] entnommen und lediglich die deutsche Übersetzung der Fluoreszenzfarben zugefügt

Die russisch geschriebene Arbeit schließt mit folgender Zusammenfassung:

“A method for chromatographic adsorption analysis is elaborated based on the observation of the division of substances into zones on a thin layer of adsorbent, using one drop of the substance.

The results obtained by the method proposed are qualitatively the same as those obtained by the usual chromatographic adsorption method of analysis. The method enables to obtain satisfactory results using one drop of the substance under test, very small quantities of the adsorbent and minimal time.

The method may be used for the evaluation of galenical preparations and their identification as well as for preliminary test of the adsorbent and the kind of the developer. Sixteen galenical preparations are studied using the method proposed.”

Thin Layer Chromatography (TLC)



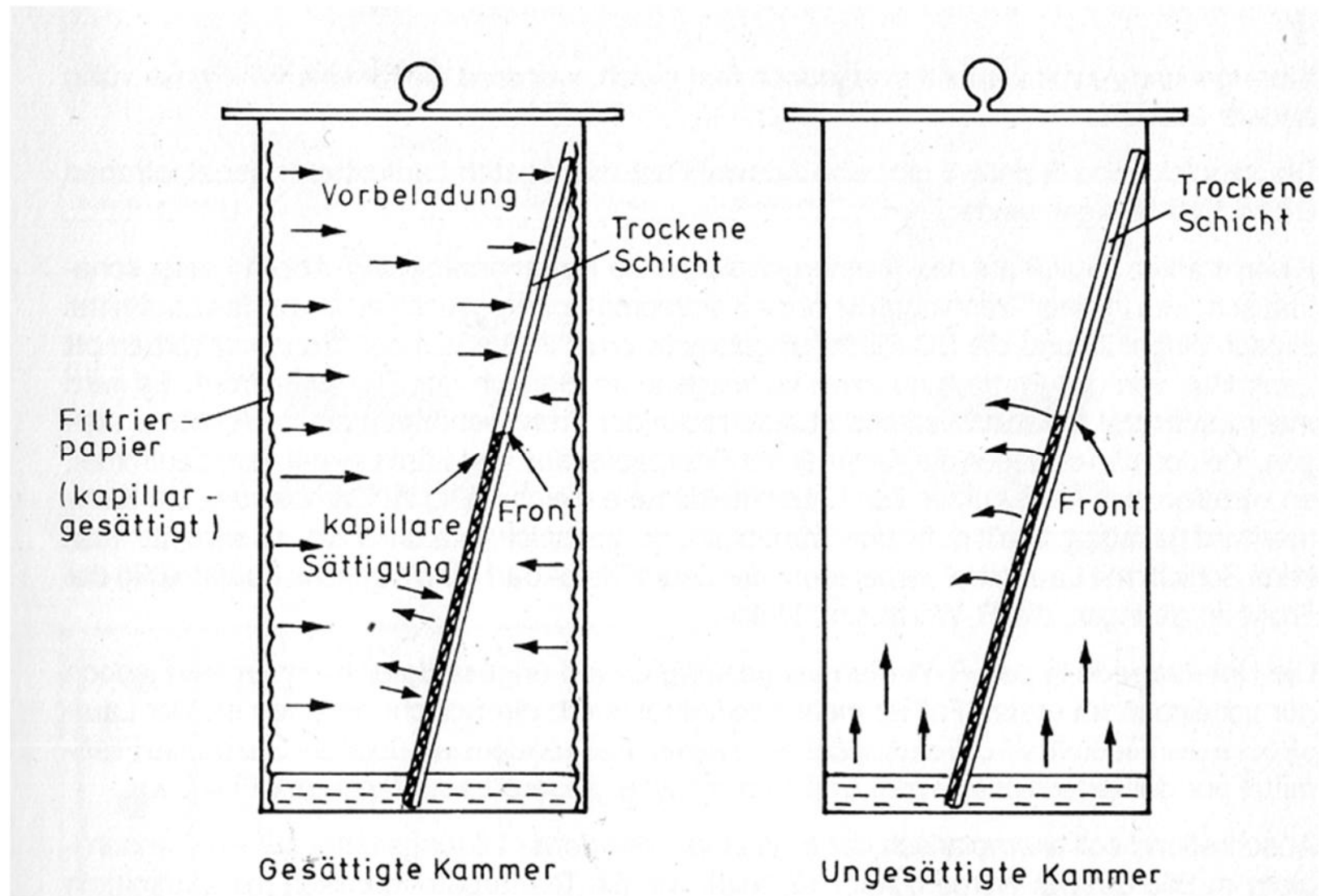
Das Potential dieser Methode wurde jedoch erst von Egon Stahl realisiert, der seine eingehenden Studien 1965 in einem Buch veröffentlichte.



Thin Layer Chromatography (TLC)



Thin Layer Chromatography (TLC): Chamber Saturation



Thin Layer Chromatography (TLC): Eleutotropic Sequence

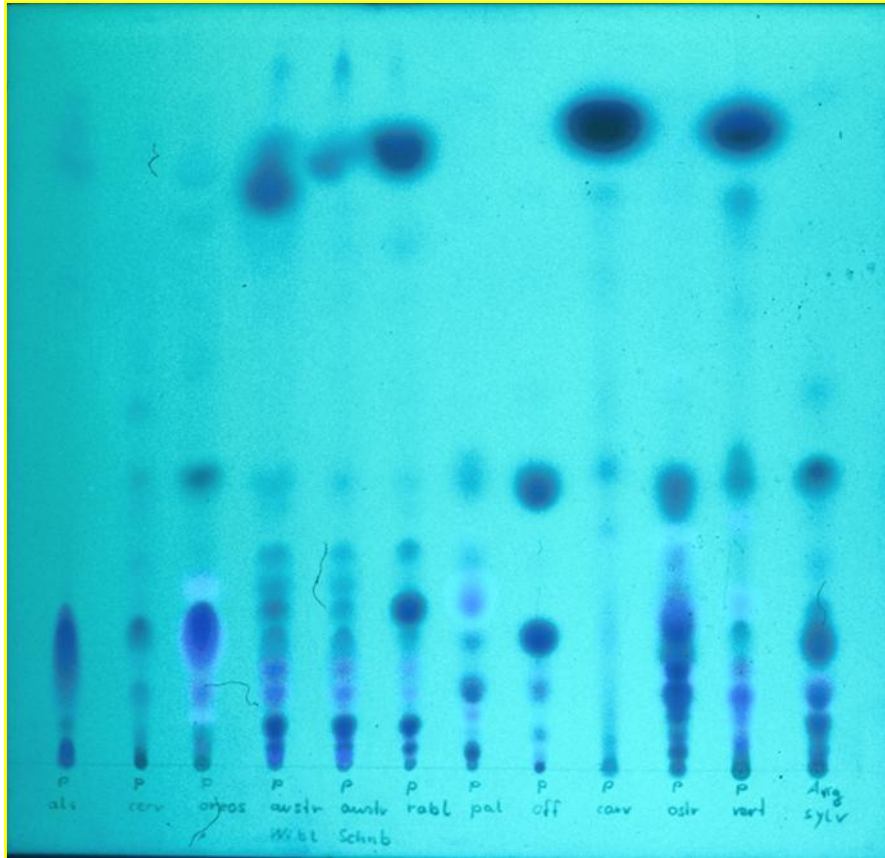
Tabelle 1: Eleutrope Reihe nach HALPAAP [17].

Eleutrope Reihe	Formel	Polaritäts-index nach Snyder*)	Dielektrizitäts-konstante DK (20 bzw. 25 °C)	Molare Masse [g/mol]	Siede-punkt [°C]	Dampf-druck [mbar] (20 °C)	MAK-Wert 1983 [ml/m ³] oder [ppm]
n-Heptan	C ₇ H ₁₆	—	1,9	100,21	98,4	48	500
n-Hexan	C ₆ H ₁₄	0,0	1,9	86,18	68,9	160	100
Cyclohexan	C ₆ H ₁₂	0,0	2,0	84,16	80,7	104	300
Isooctan	C ₈ H ₁₈	0,4	1,9	114,23	99,2	51	500
1,1,2-Trichlor-trifluorethan	Cl ₂ FC ₂ CF ₂	—	2,4	187,38	47,7	368	1000
Tetrachlor-kohlenstoff	CCl ₄	1,7	2,2	153,82	76,5	120	10
Toluol	C ₆ H ₅ CH ₃	2,3	2,4	92,14	110,6	29	200
Chloroform	CHCl ₃	4,4	4,8	119,38	61,7	210	10
Dichlor-ethan	ClCH ₂ CH ₂ Cl	3,7	10,6	98,97	83,4	87	20
Dichlor-methan	CH ₂ Cl ₂	3,4	9,1	84,93	40,0	453	100
1-Butanol	CH ₃ (CH ₂) ₃ OH	3,9	17,8	74,12	117,2	6,7	100
Acetonitril	CH ₃ CN	6,2	37,5	41,05	81,6	97	40
2-Propanol	CH ₃ CH(OH)CH ₃	4,3	18,3	60,10	82,4	43	400
Ethylacetat	CH ₃ COOC ₂ H ₅	4,3	6,0	88,10	77,1	97	400
Aceton	CH ₃ COCH ₃	5,4	20,7	58,08	56,2	233	1000
Ethanol	C ₂ H ₅ OH	5,2	24,3	46,07	78,5	59	1000
1,4-Dioxan	C ₄ H ₈ O ₂	4,8	2,2	88,11	101,0	41	50
Tetra-hydrofuran	C ₄ H ₈ O	4,2	7,4	72,11	66,0	200	200
Methanol	CH ₃ OH	6,6	32,6	32,04	65,0	128	200
Wasser	H ₂ O	9,0	80,2	18,01	100,0	23	—

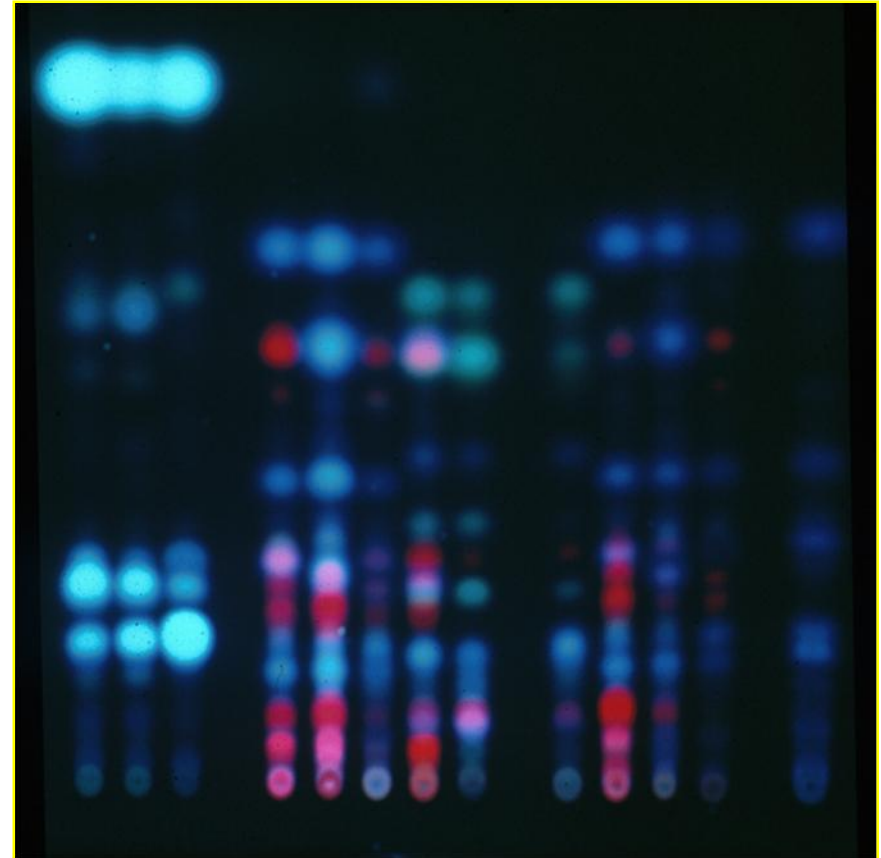
*) L. R. Snyder: J. Chromatogr. 92, 223–230 (1974)

Thin Layer Chromatography (TLC): Detection 1

UV



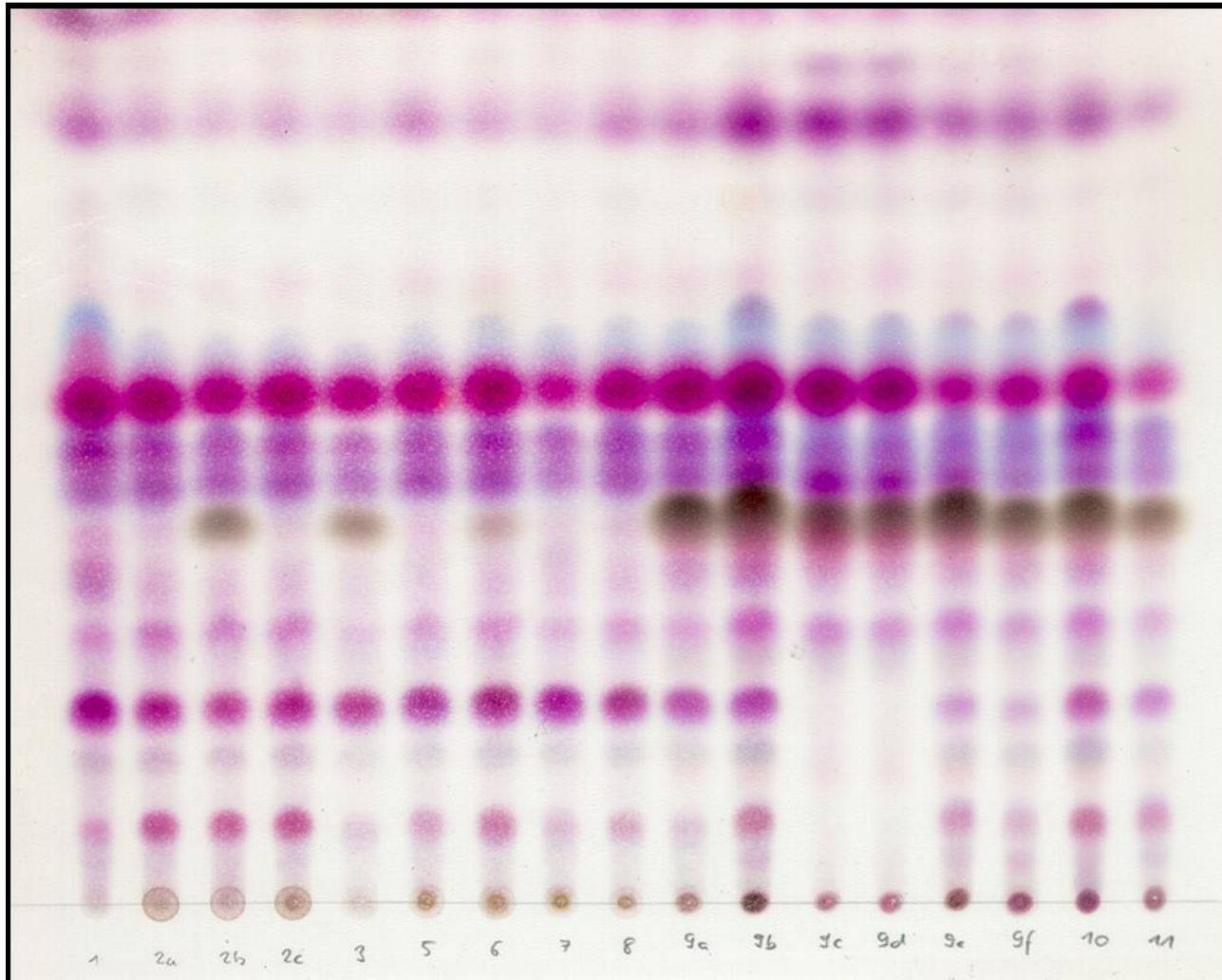
254 nm



366 nm

Thin Layer Chromatography (TLC): Detection 2

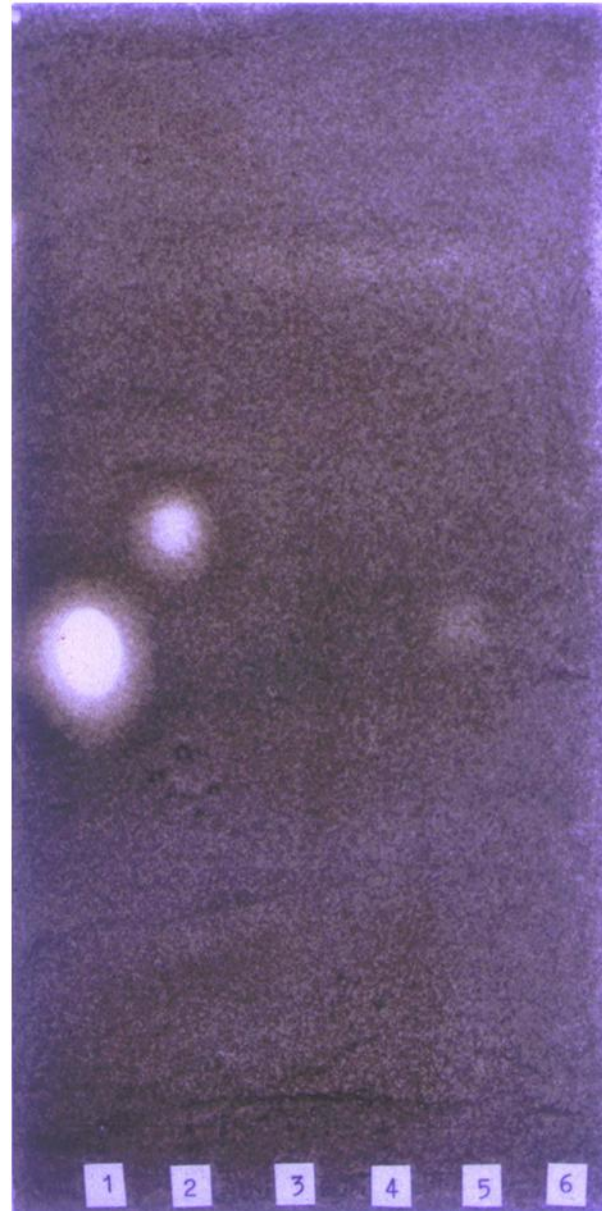
Chemical
derivatisation



Anis aldehyd-sulphuric acid

Thin Layer Chromatography (TLC): Detection 3

Bioautography

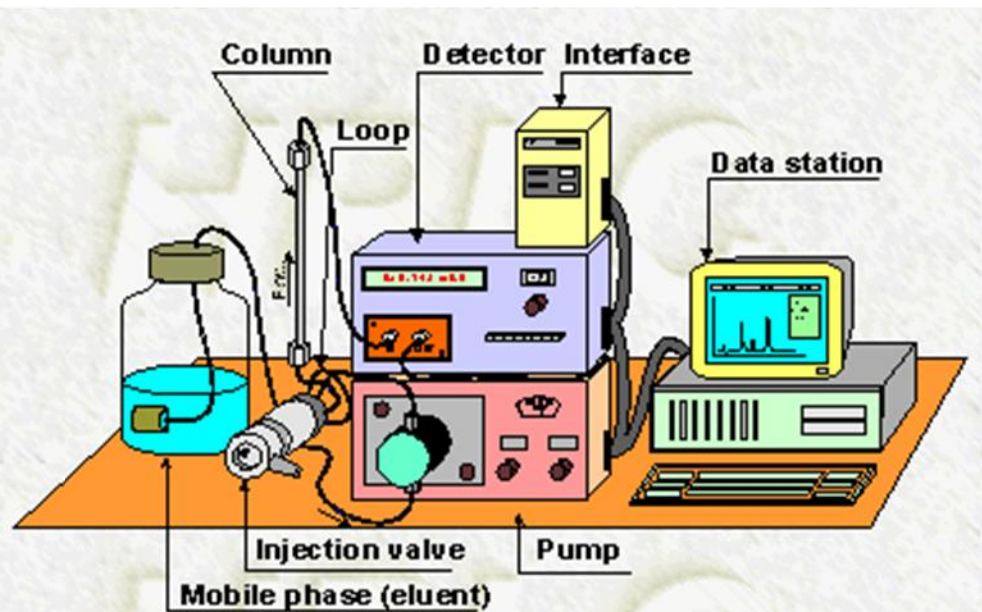


Separation on silica

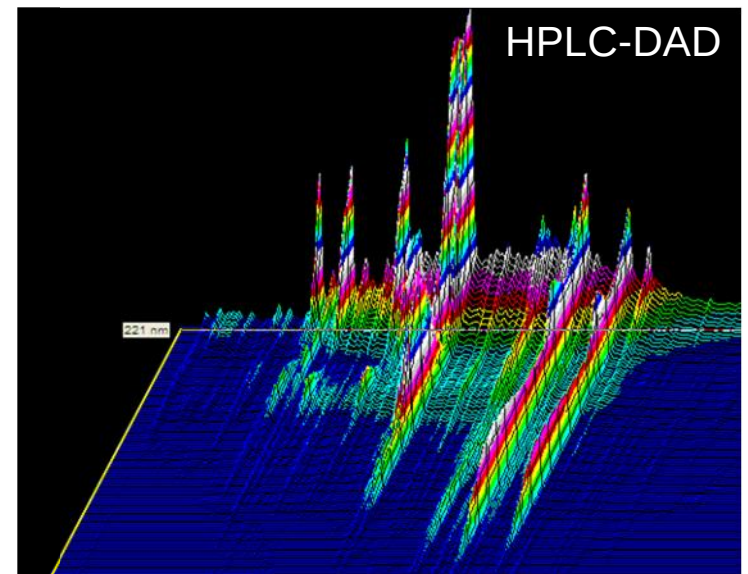


- Absorption
Pore structure of stationary phase
- Partition
between mobile and stationary
liquid

HPLC (High Performance Liquid Chromatography)



Functional schematic of a modern HPLC instrument.



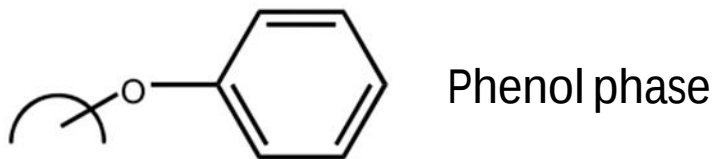
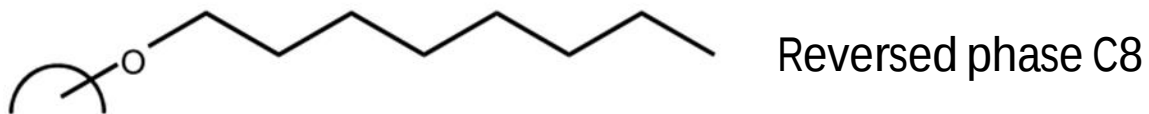
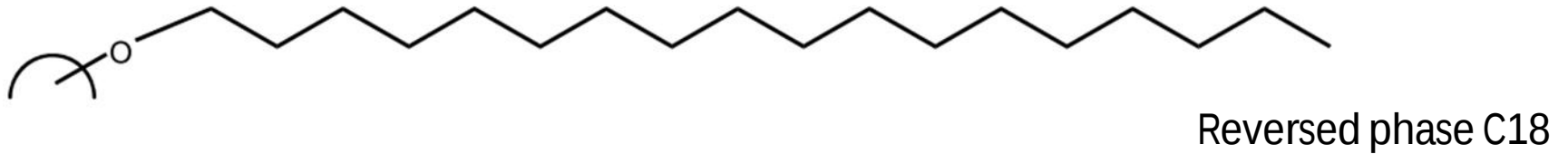
Stationary phases: particle size

Column chromatography 500 μm	Gravity	200-
Thin layer chromatography μm	Capillary force	20
Middle pressure liquid chromatography 15-60 μm	1-10 bar	
High pressure liquid chromatography μm	100-300 bar	1-5

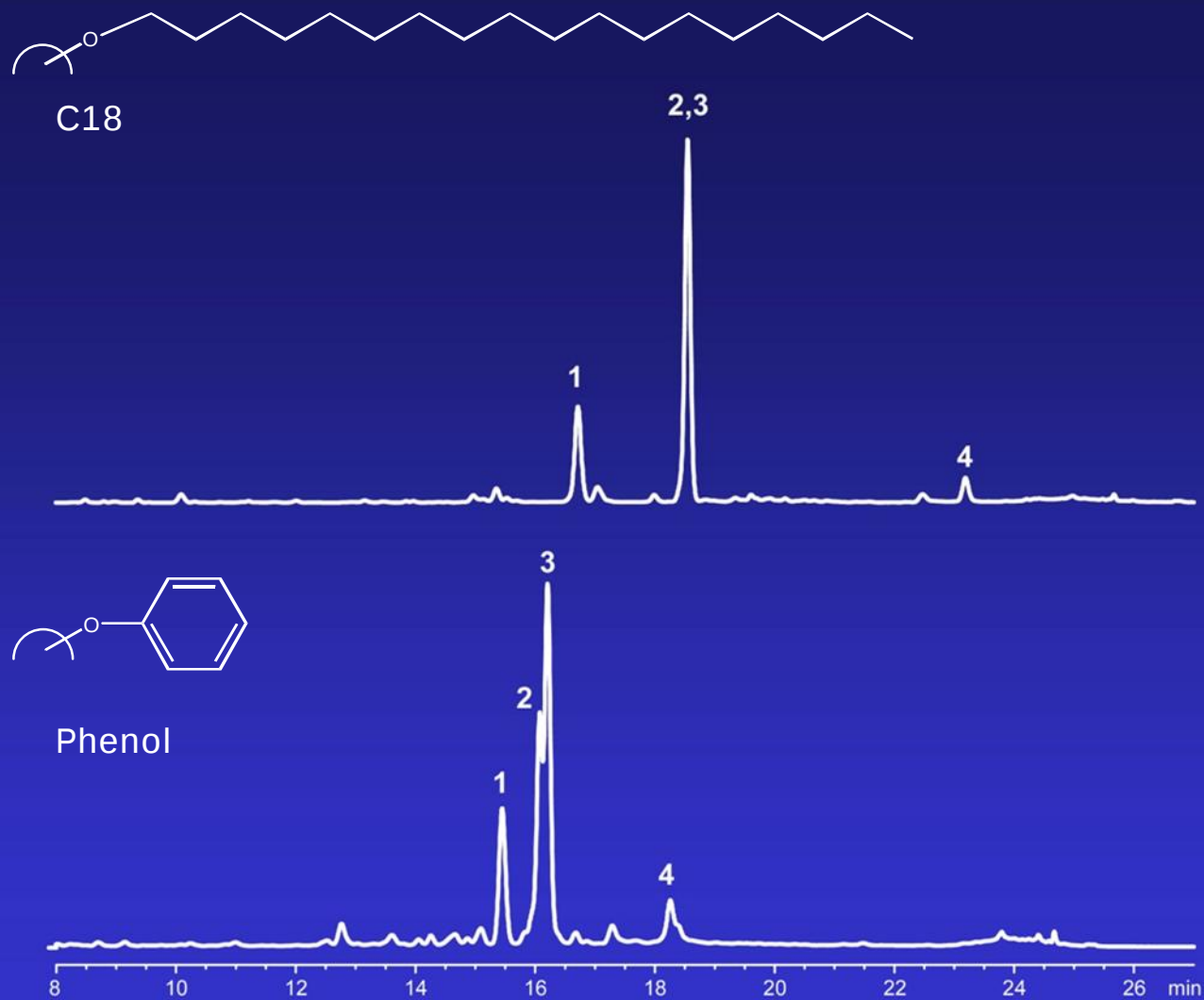
HPLC columns



HPLC columns: stationary phases

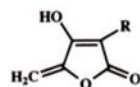


HPLC columns: selectivity

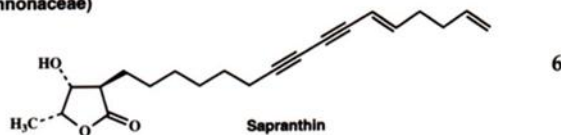


HPLC columns: effect of pH

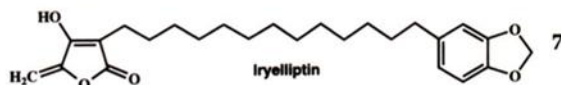
Peucedanum
(Apiaceae)



Sapranthus
(Annonaceae)



Iryanthera
(Myristicaceae)



Enterobacter
(Bacteria)

Agglomerin



Plexaura
(gorgonian)

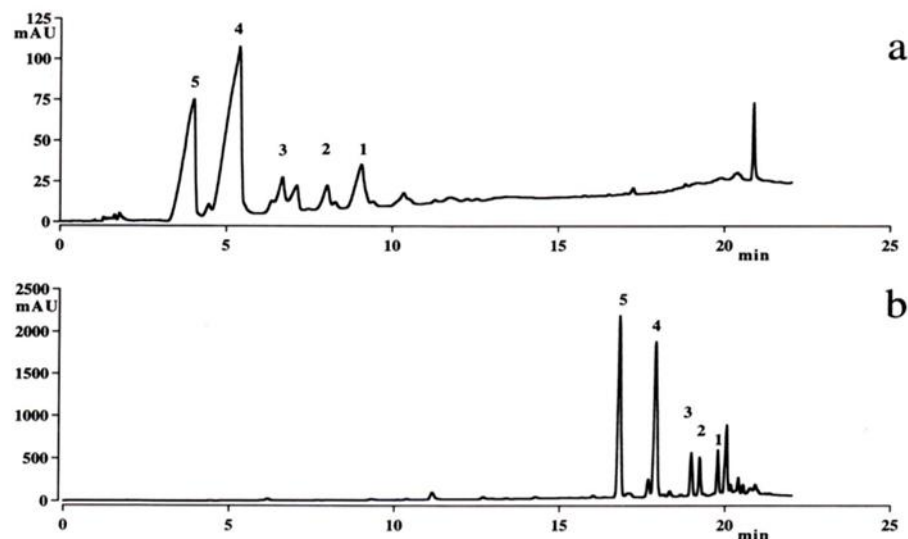
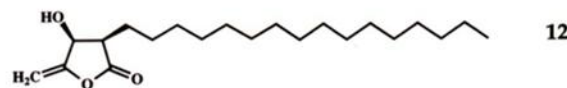
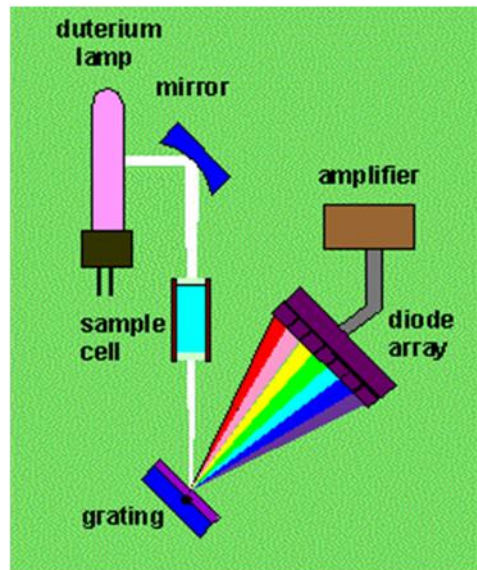
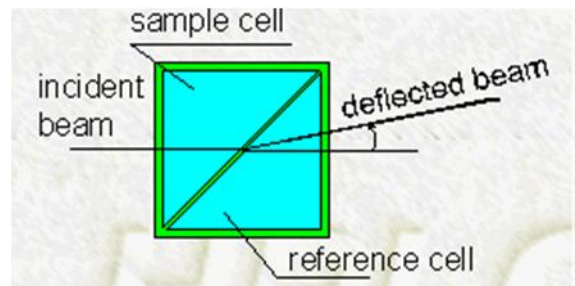


Figure 2. (a) Analytical HPLC of 0.05 mg of the crude extract of the roots of *P. alsaticum*. (For chromatographic protocol see Experimental section—solvent A was water.) (b) Analytical HPLC of 0.05 mg of the crude extract of the roots of *P. alsaticum*. (For chromatographic protocol see Experimental section—solvent A was phosphate buffer.) Peak numbering corresponds to butenolides 1–5.

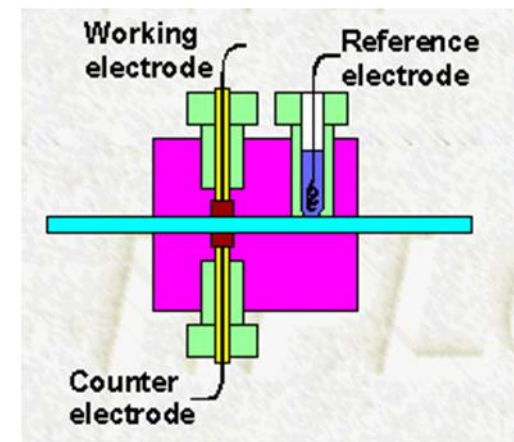
HPLC: various detectors



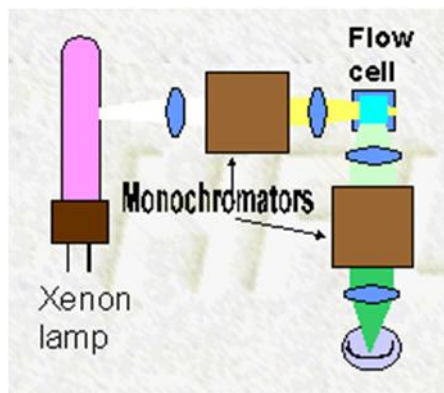
UV-Diode Array (DAD)



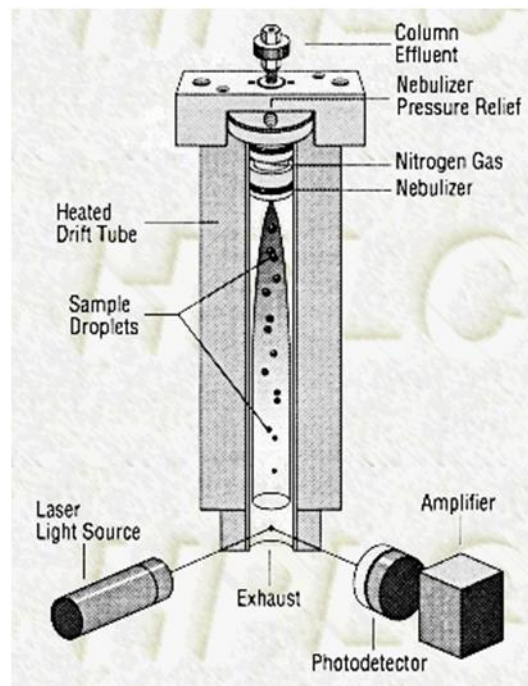
Refractive index



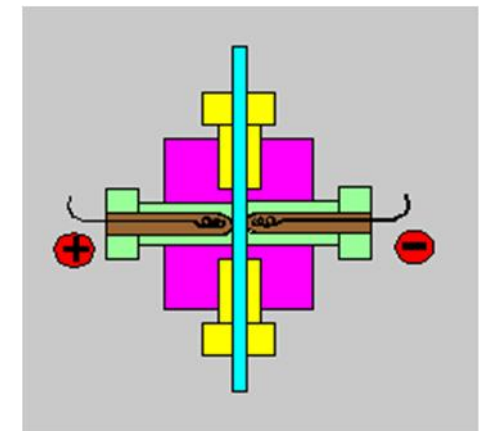
Electrochemical D.



Fluorescence D.

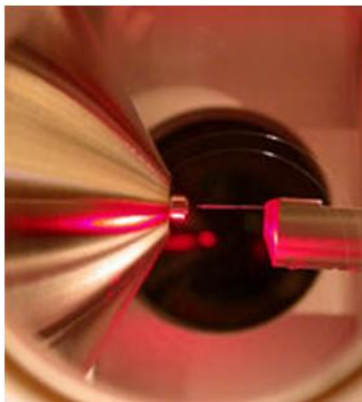


Light scattering

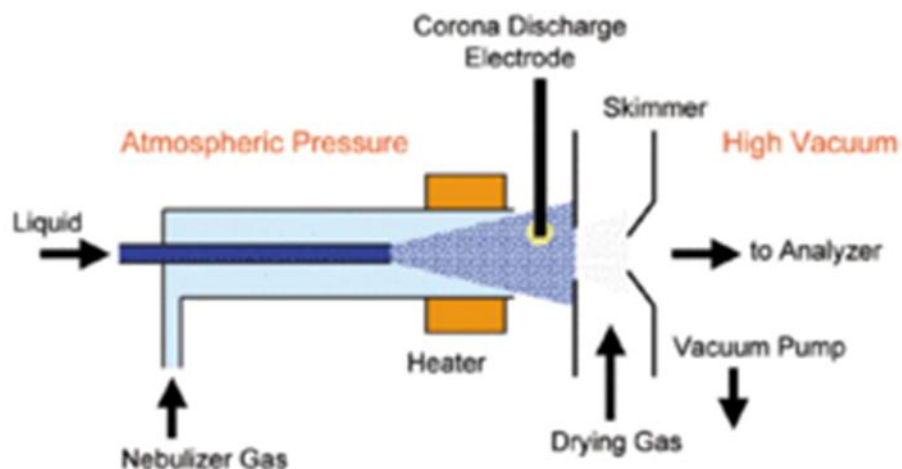


Conductivity

HPLC: mass selective detectors



Electrospray Ionisation (ESI)
Ionisation in the liquid phase



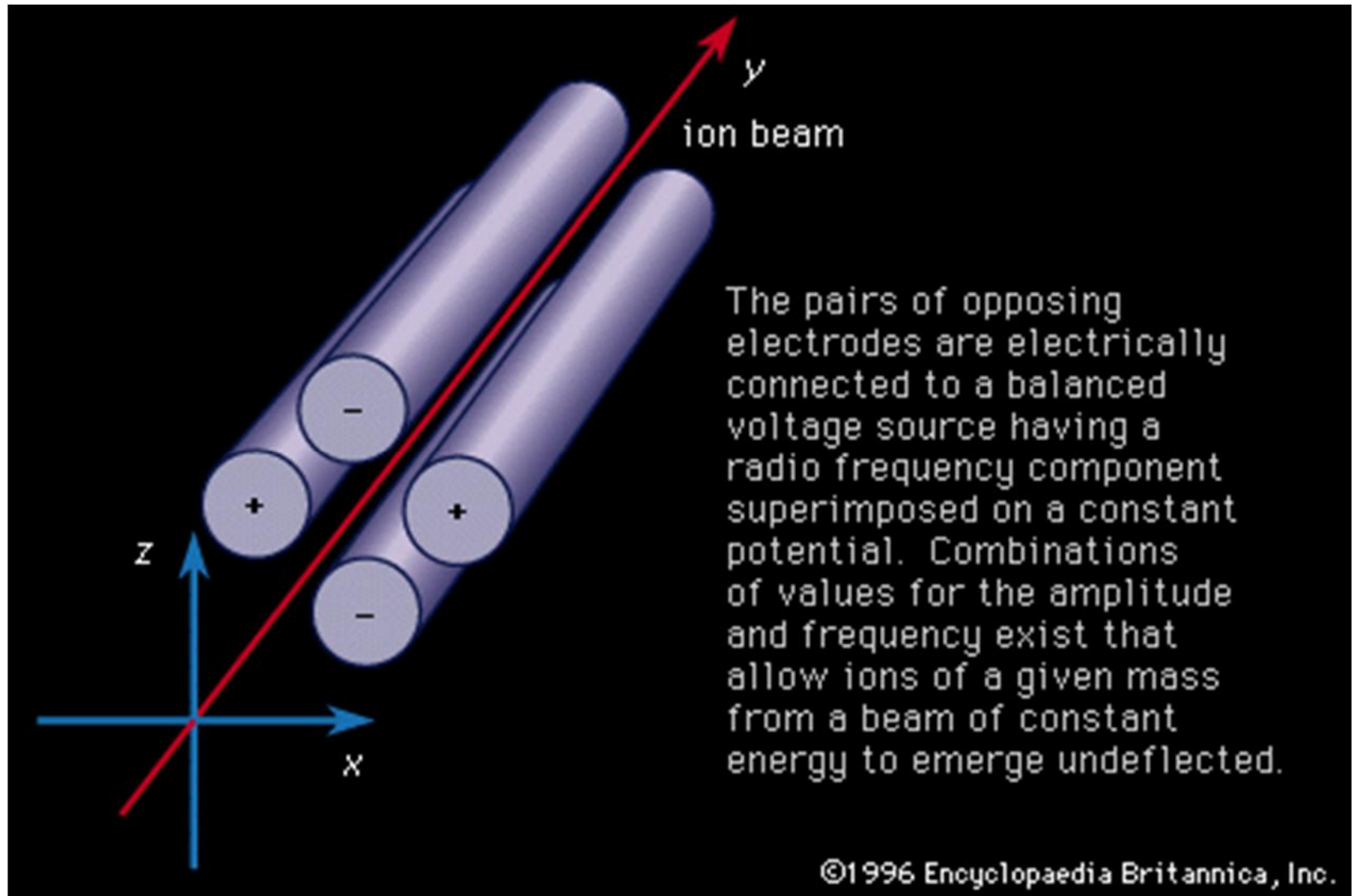
Atmospheric Pressure Chemical Ionisation (APCI)
Ionisation in the gas phase at 400°C

Positive CI: $[M + H]^+$
 $[M + Na]^+$
 $[M + 2H]^{2+}$
 $[M + 25H]^{25+}$

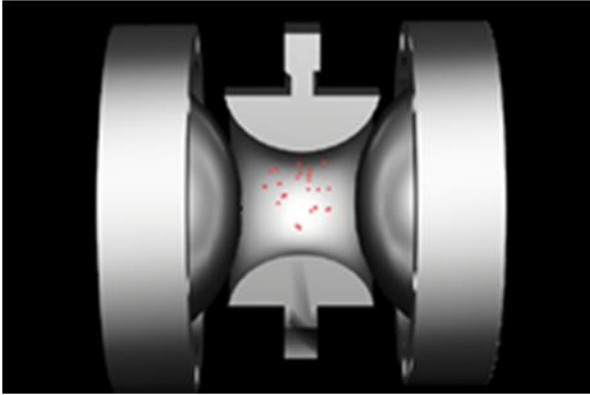
Negative CI: $[M - H]^-$

CI: Chemical Ionisation

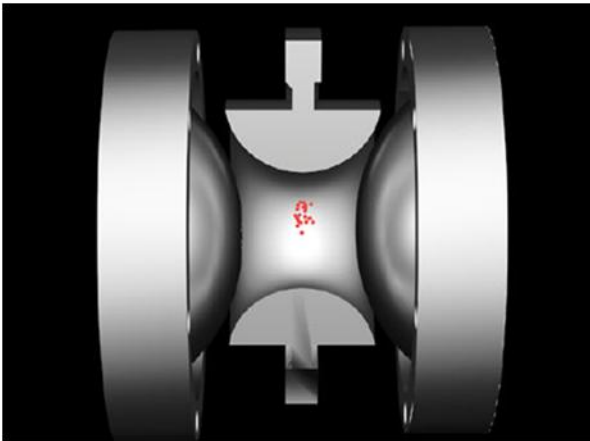
Mass spectrometers: Quadrupol



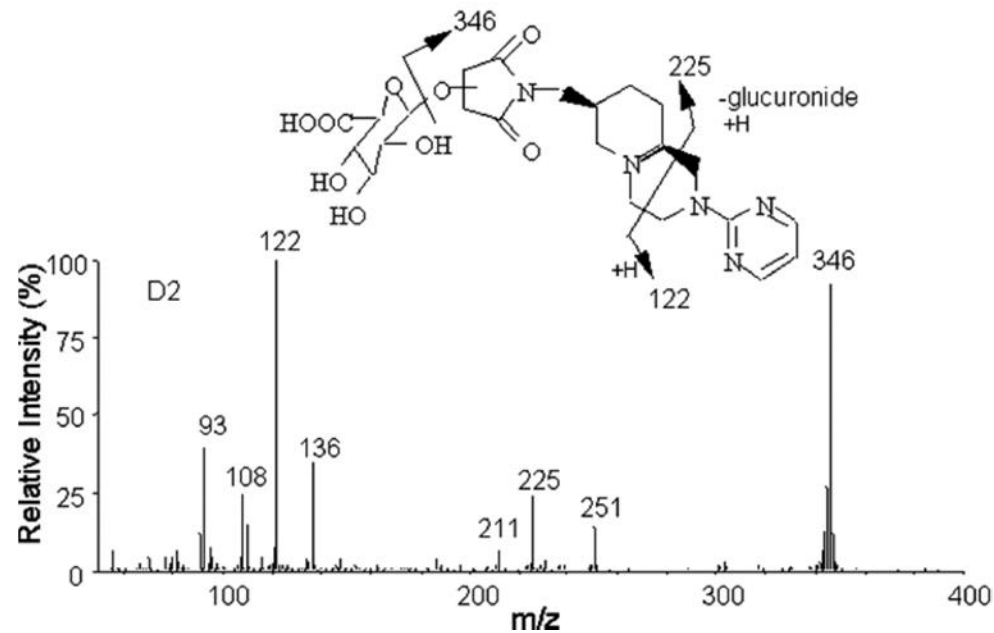
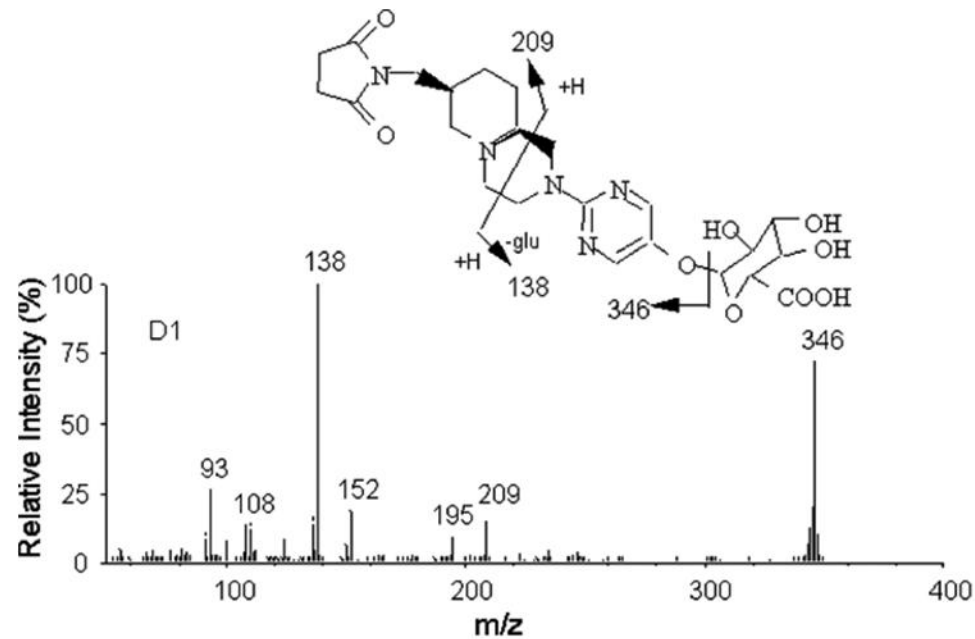
Mass spectrometers: Ion trap



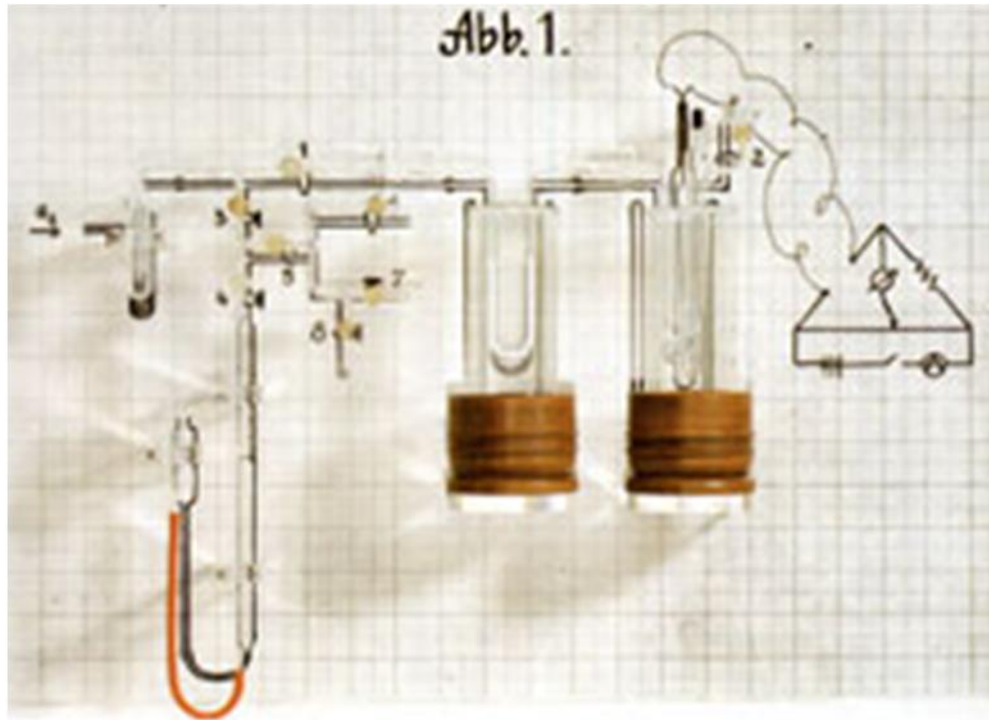
Variable electric fields allow to focus ions of variable m/z ratio.



Mass spectrometers: Ion trap, MSⁿ

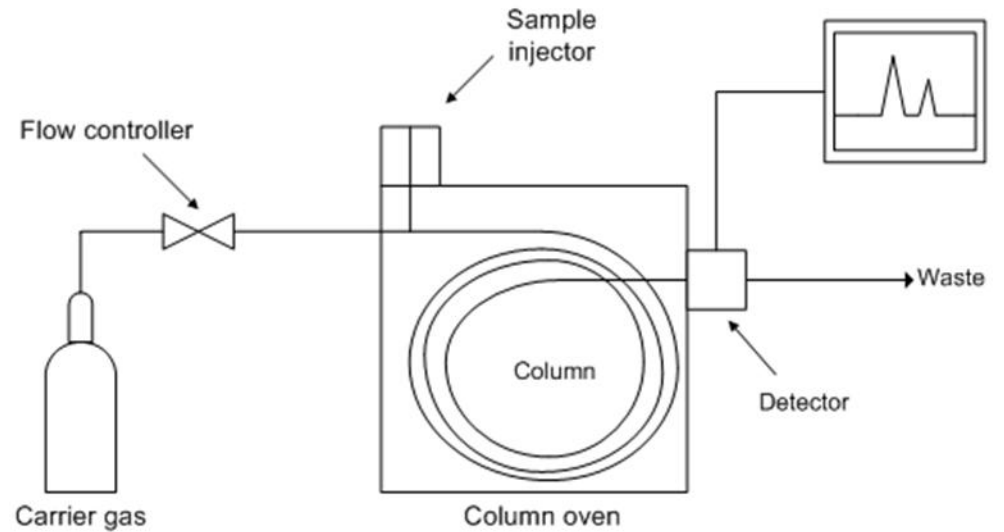


Gas Chromatography (GC)

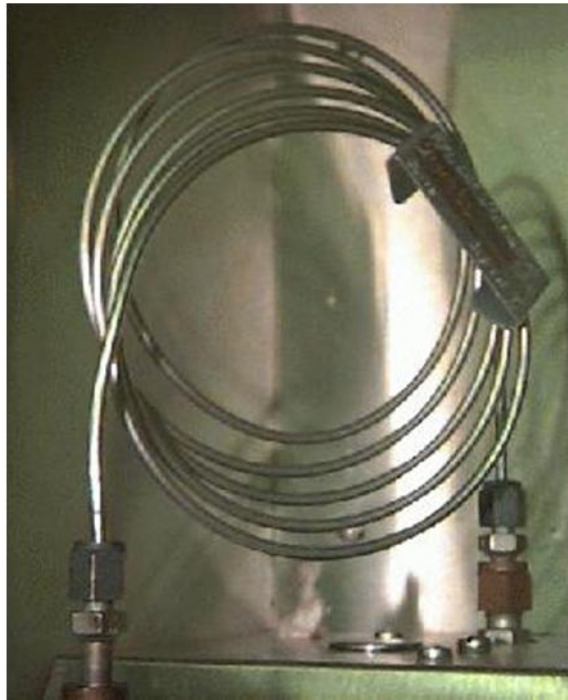


1944: Fritz Prior und Erika Cremer

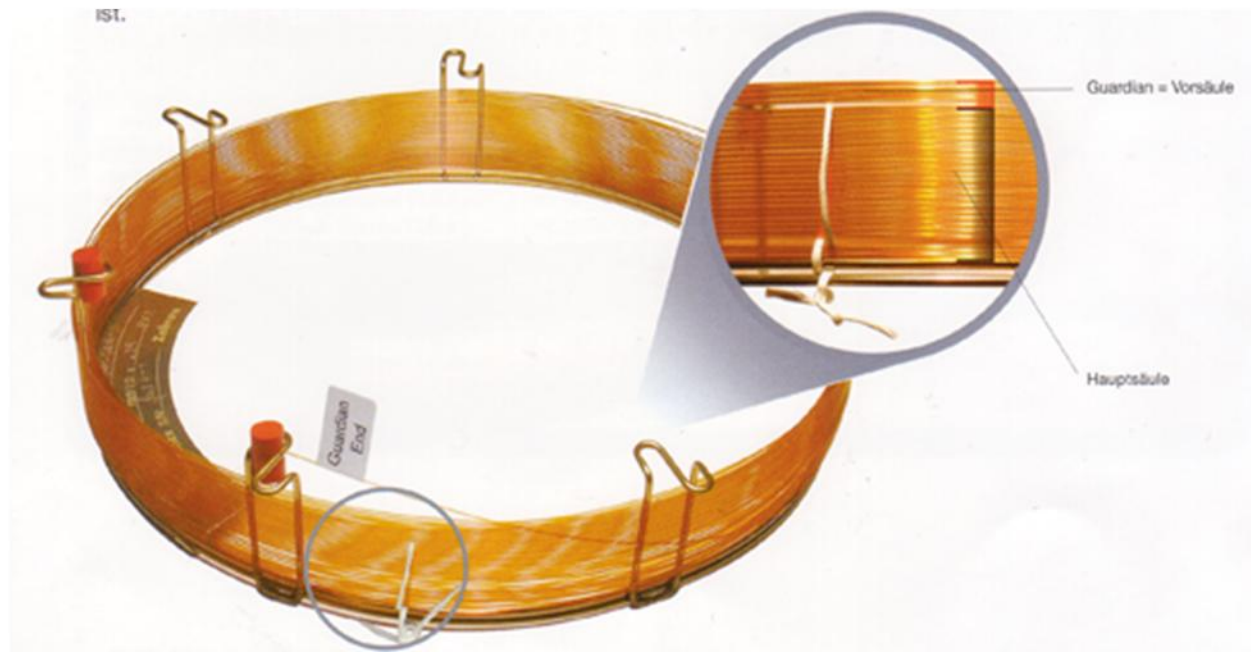
Gas Chromatography (GC)



Gas Chromatography (GC): Columns



packed

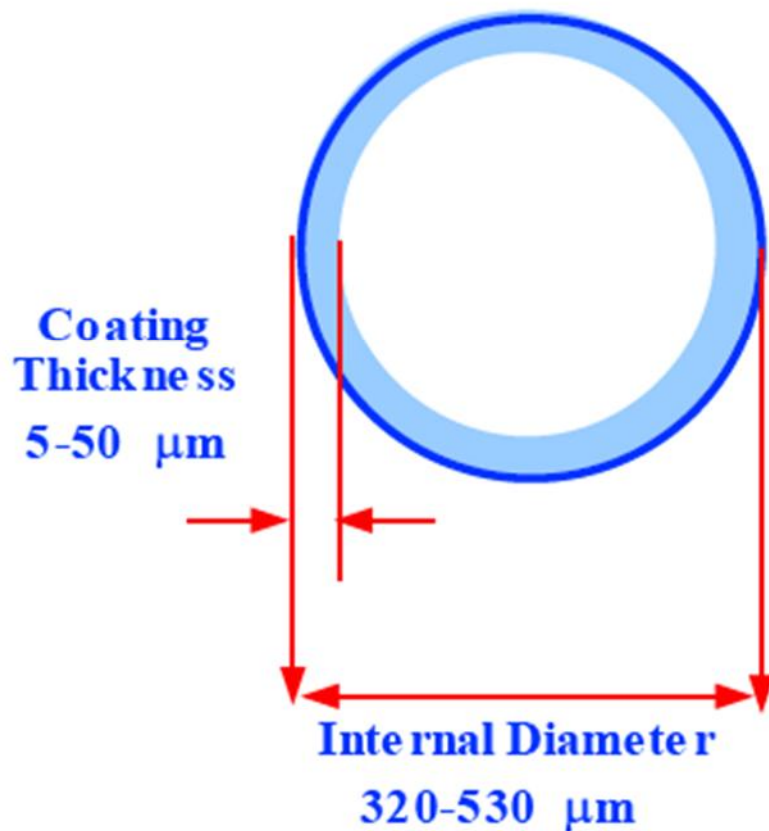


capillary

Capillary columns

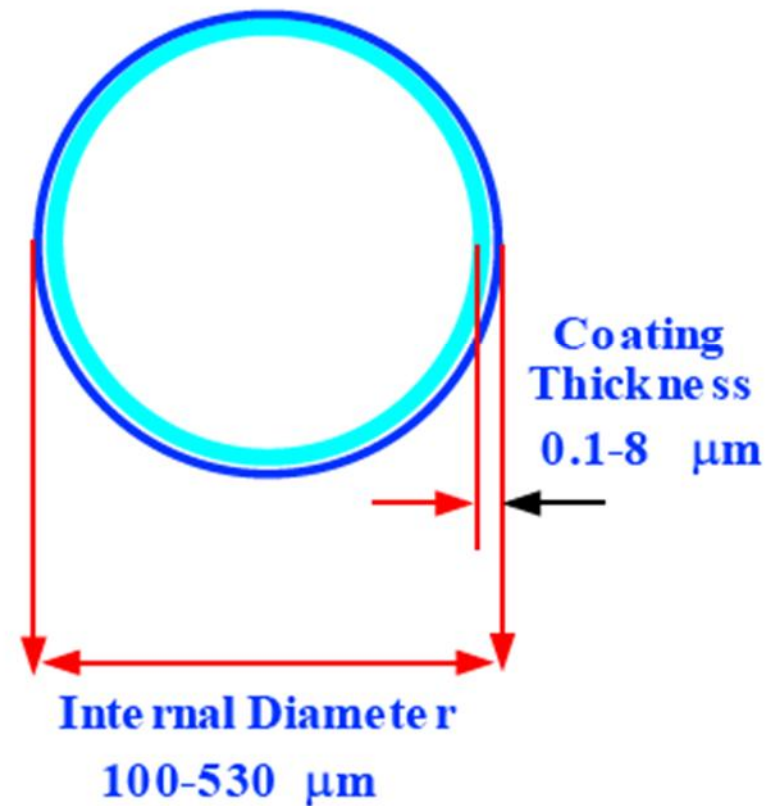
PLOT Columns

Porous Layered Open Tubes



WCOT Columns

Wall Coated Open Tubes



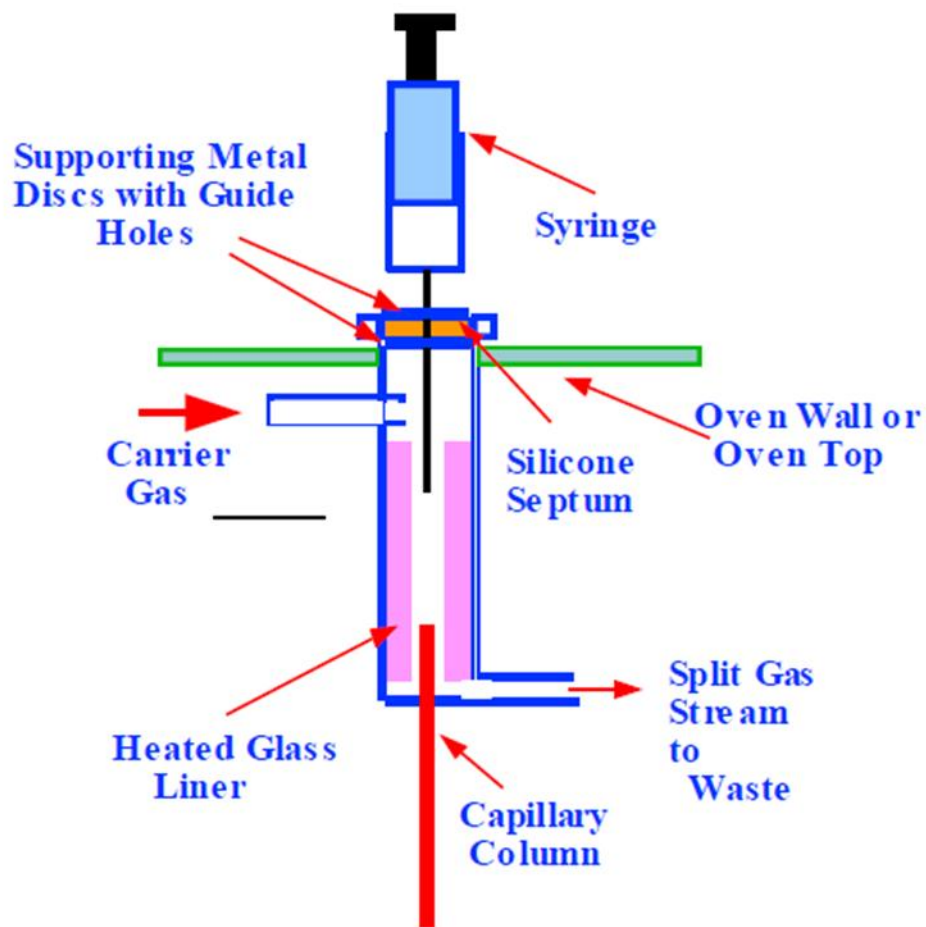
Column types for gas chromatography

Composition	Phenomenex	Restek	J&W	Supelco	Agilent Technologies (HP)	Alltech	SGE	Varian (Chrompack)	OV
100% dimethyl-polysiloxane	ZEBRON ZB-1	Rtx-1, Rtx-1ms, Rtx-1PONA, Rtx-1 F&F	DB-1, DB-1ms DB-1ht, SE-30, Ultra-1, DB-2887, DB-1EVDX	SPB-1, MDN-1, SPB-1 TG, Equity-1, SPB-1 Sulfur, SPB-HAP	HP-1, HP-1ms, MET-1, HP-101, HP-PONA	AT-1, AT-Sulfur, EC-1	BP1, BP1-PONA, BPX1-SimD	CP-Sil 5 CB, CP-Sil 5 CB MS VF-1ms	OV-1
5%-phenyl-95%-dimethylpoly-siloxane	ZEBRON ZB-5	Rtx-5, Rtx-5ms	DB-5, DB-5ms, DB-5ht, Ultra-2, DB-5.625, SE-54, SE-52, DB-5msEVDX	MDN-5, SPB-5, PTE-5, MDN-5S, HT-5, MDN-12, PTA-5, SAC-5, Equity-5	HP-5, HP-5ms, HP-PAS-5, HP-5 Trace Analysis	AT-5, EC-5	BP 5, BPX 5	CP-Sil 8 CB, CP-Sil 8 CB MS VF-5ms	OV-5
35%-phenyl-65%-dimethyl-polysiloxane	ZEBRON ZB-35	Rtx-35 Rtx-35ms	DB-35, DB-35ms	MDN-35, SPB-35, SPB-608	HP-35, HP-35ms	AT-35	BPX35, BPX608		OV-11
50%-phenyl-50%-dimethyl-polysiloxane	ZEBRON ZB-50	Rtx-50	DB-17, DB-17ht, DB-17ms, DB-17EVDX	SP-2250, SPB-17, SPB-50	HP-50+	AT-50	BPX50	CP-Sil 24 CB	OV-17
6%-cyanopropyl-phenyl-94%-dimethyl-polysiloxane	ZEBRON ZB-624	Rtx-1301, Rtx-624	DB-1301, DB-624, DB-VRX	SPB-1301, SPB-624	HP-VOC	AT-624, AT-1301	BP624	CP-1301 CP-Select-624 CB	
14%-cyanopropyl-phenyl-86%-dimethylpolysiloxane	ZEBRON ZB-1701	Rtx-1701	DB-1701, DB-1701P	SPB-1701, Equity-1701		AT-1701	BP10	CP-Sil 19 CB	OV-1701
Polyethylene glycol	ZEBRON ZB-WAX	Stabilwax, Rtx-Wax, Famewax, Stabilwax-DB	DB-Wax, Carbowax-20M, DB-WAXetr, CAM	Supelcowax 10, Met-Wax, Omegawax, Carbowax Amine	HP-20M, HP-Wax, HP-INNOWax	AT-Wax, EC-Wax	BP20	CP-Wax 52 CB, CP-Wax 57 CB	Carbo-wax 20M
Nitroterephthalic acid modified polyethylene glycol	ZEBRON ZB-FFAP	Stabilwax-DA	DB-FFAP	Nukol, SPB-1000	HP-FFAP	AT-1000, EC-1000	BP21	CP-Wax 58 CB	OV-351

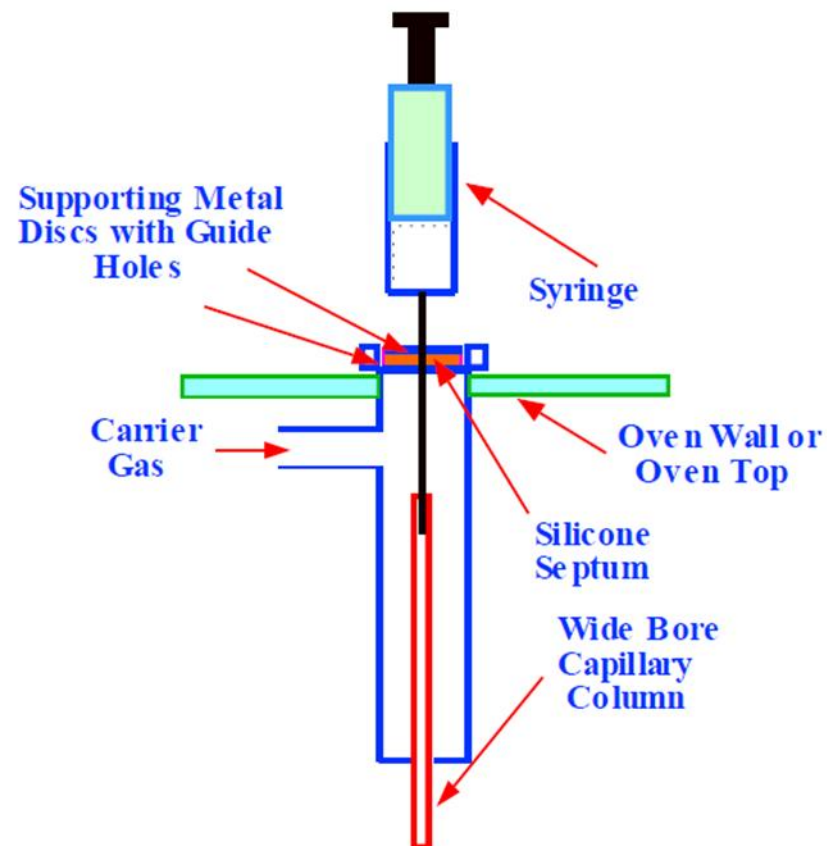
Detectors for gas chromatography

Type	Applicable Samples	Typical Detection Limit
Flame Ionization	Hydrocarbons	1 pg/s
Thermal Conductivity	Universal Detector	500 pg/mL
Electron Capture	Halogenated Compounds	5 fg/s
Mass Spectrometer (MS)	Tunable for any species	0.25 to 100 pg
Thermionic	Nitrogen & Phosphorus compounds	0.1 pg/s (P); 1 pg/s (N)
Electrolytic Conductivity	Compounds containing halogens, sulfur, or nitrogen	0.5 pg Cl/s, 2 pg S/s, 4 pg N/s
Photoionization	Compounds ionized by UV radiation	2 pg C/s
Fourier Transform IR (FTIR)	Organic Compounds	0.2 to 40 ng

Inlets for gas chromatographs

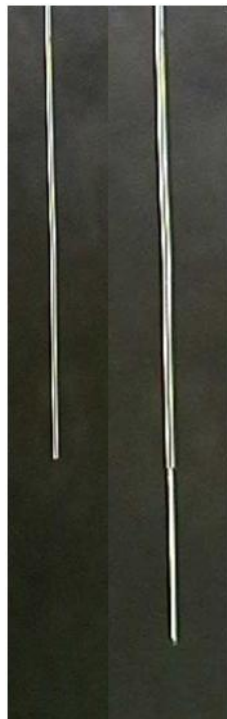


Split-Splitless



On-column

SPME (solid phase microextraction)

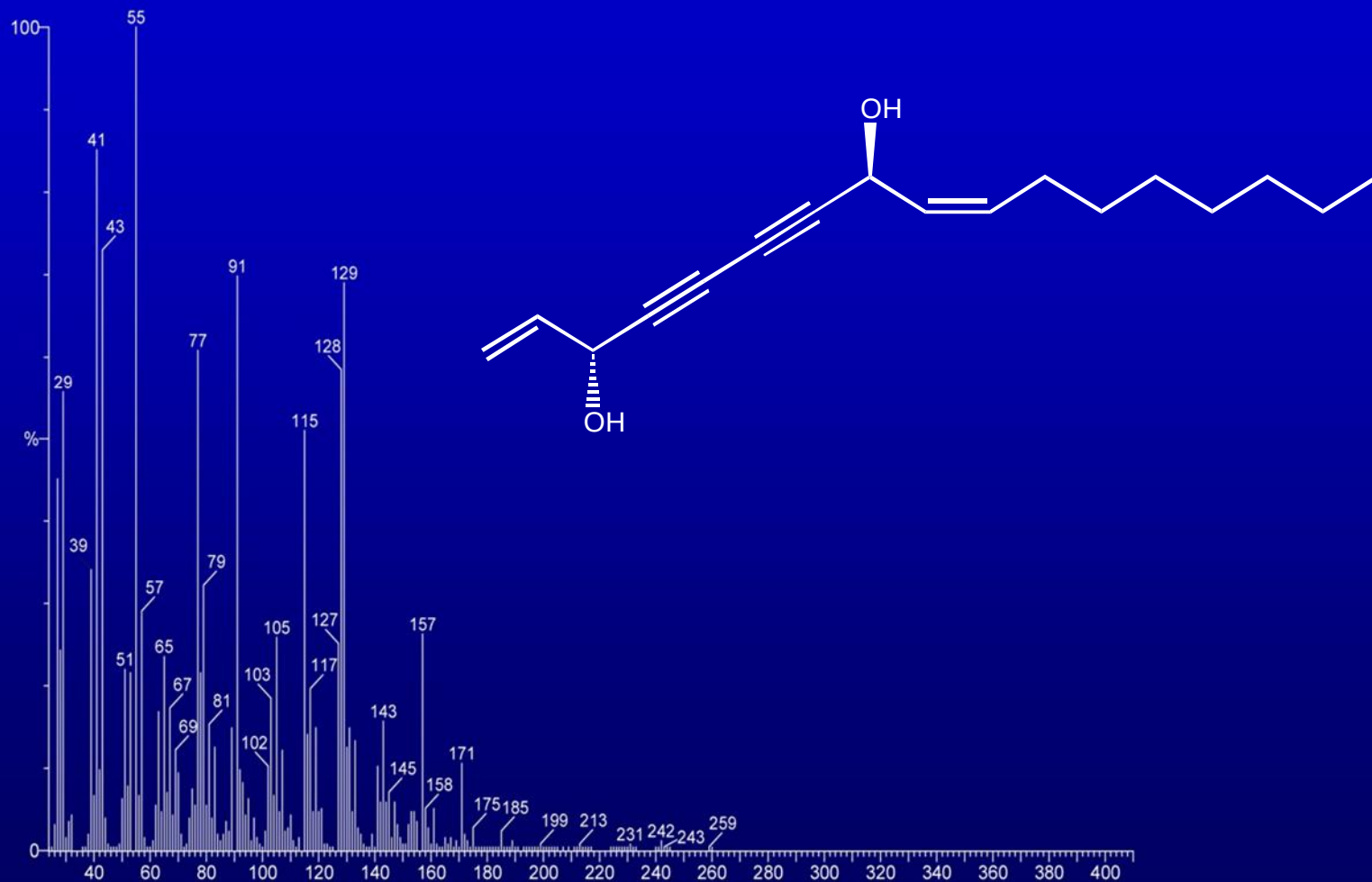


Fibers:

DVB/CAR/PDMS
(Divinylbenzene,
Carboxen,
Polydimethylsiloxan)

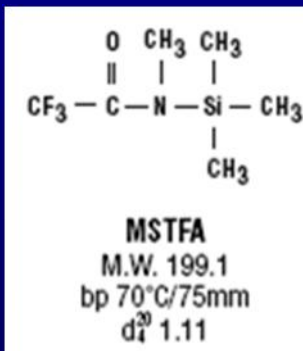
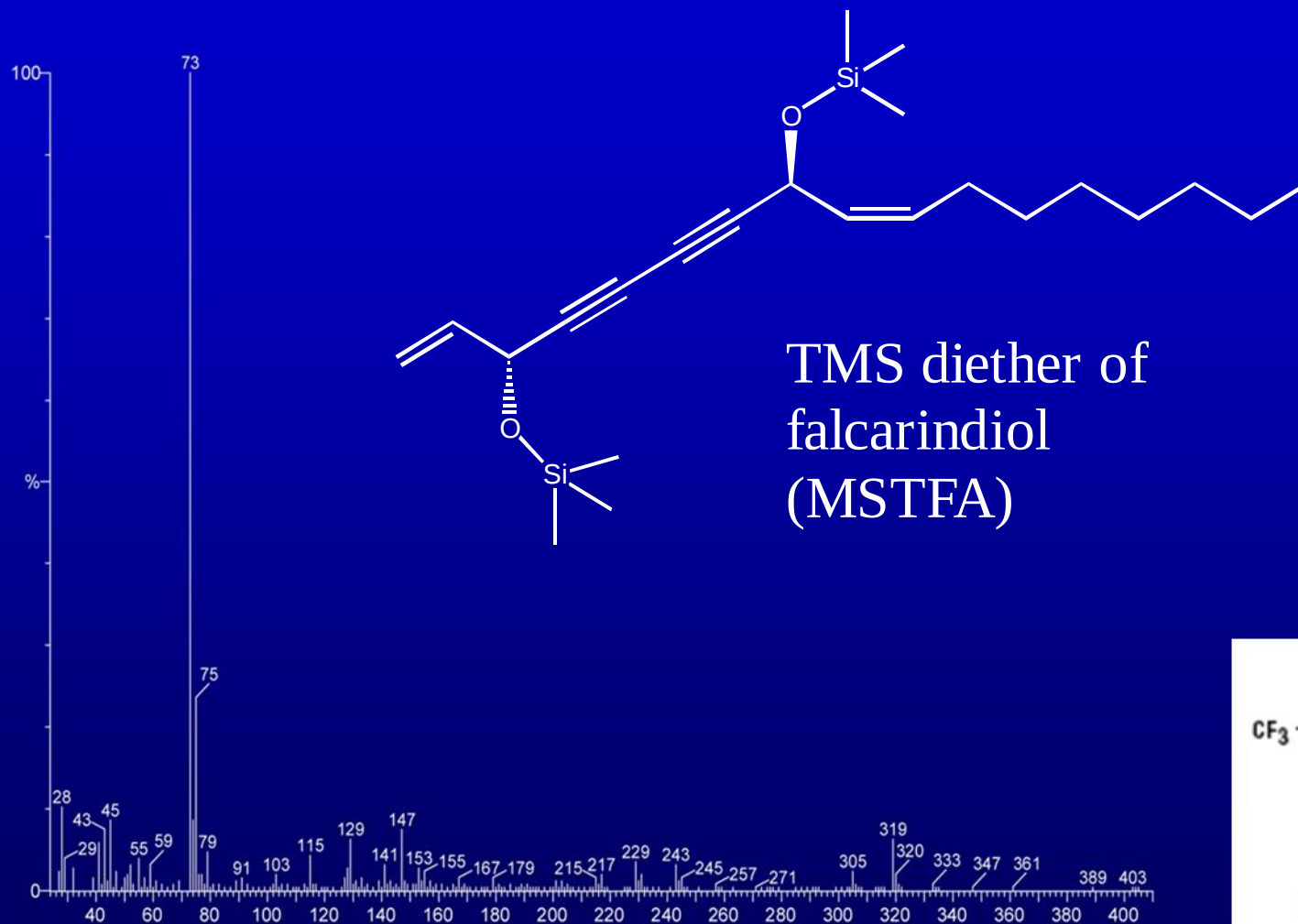
Static Headspace
10–60 min,
Room temperature

Derivatisation of analytes

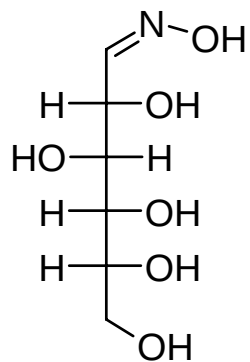
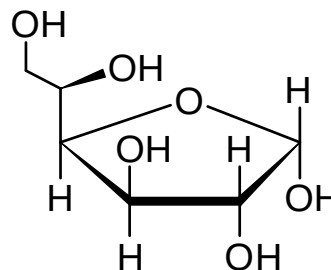
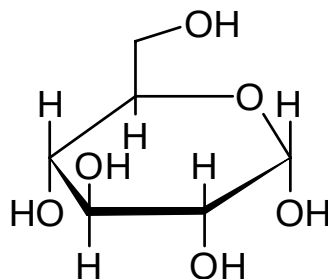
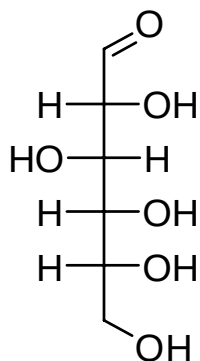


Derivatisation of analytes

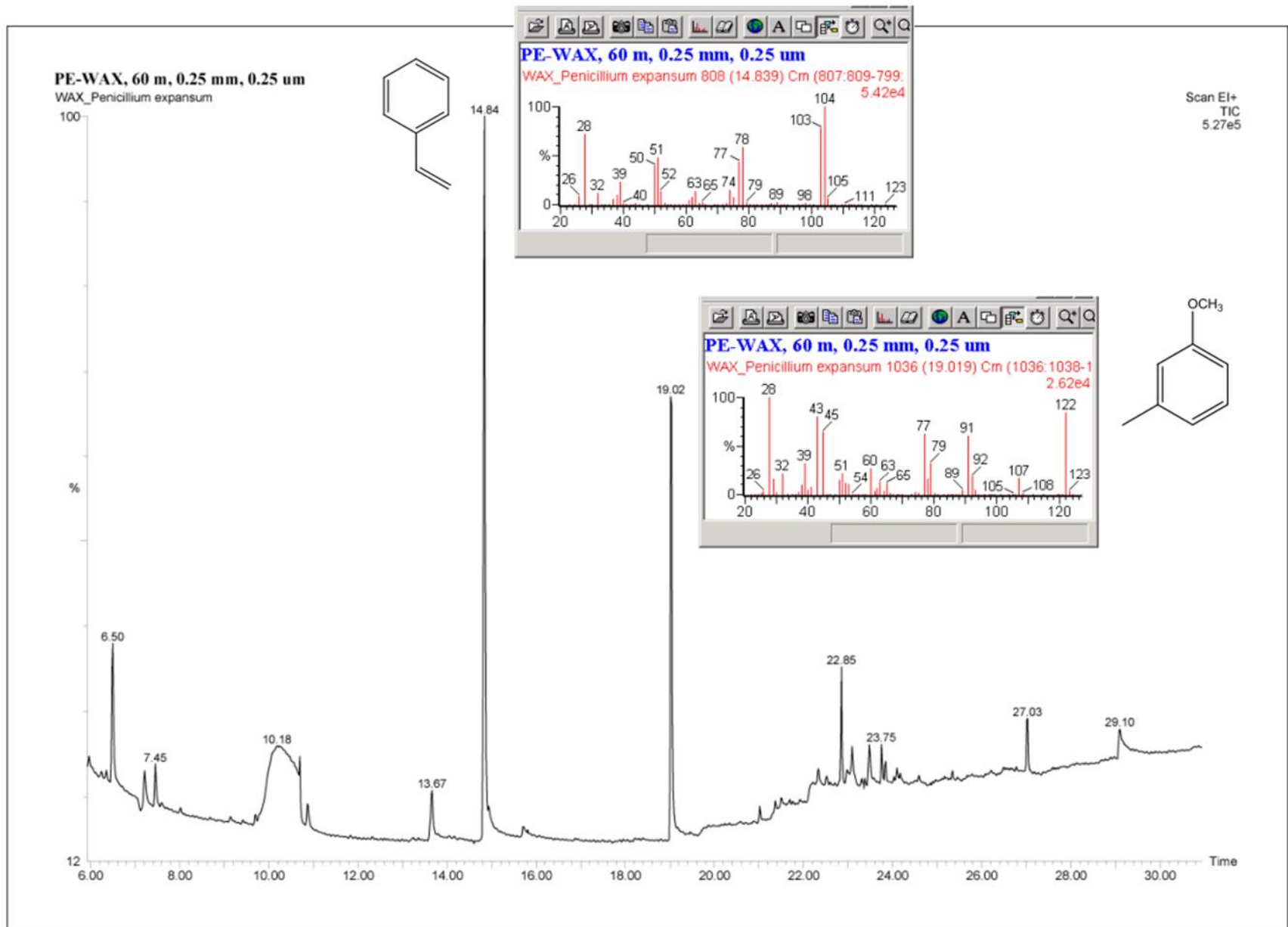
N-Methyl-N-(Trimethylsilyl)-trifluoroacetamid



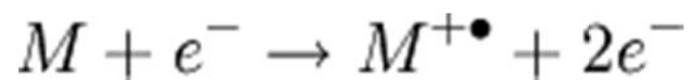
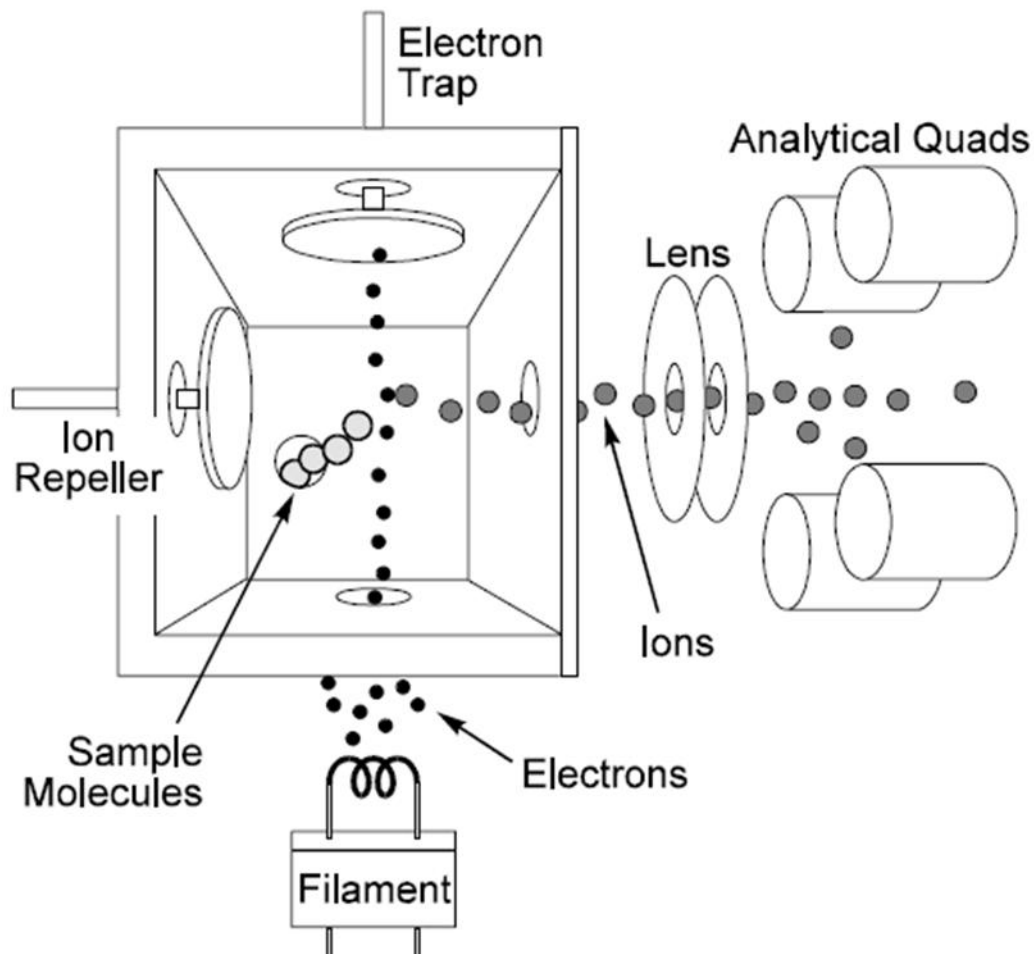
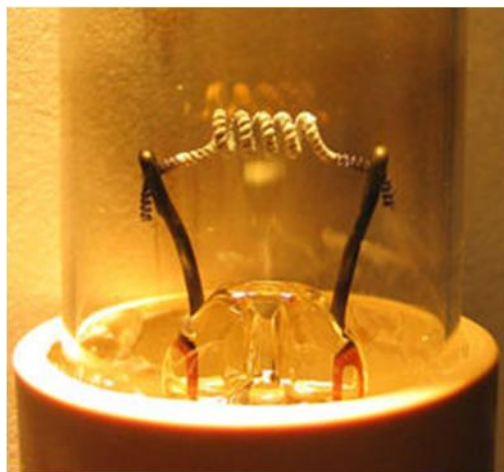
Special derivatisation of sugars: Oximation



Tentative identification by comparison with MS libraries



Electron Impact (EI) Ionization



GC – HPLC: Comparison

	GC	HPLC
Temperature	Gradient	constant
Mobile phase	constant	Gradient
Stationary phase	Film	Column packing