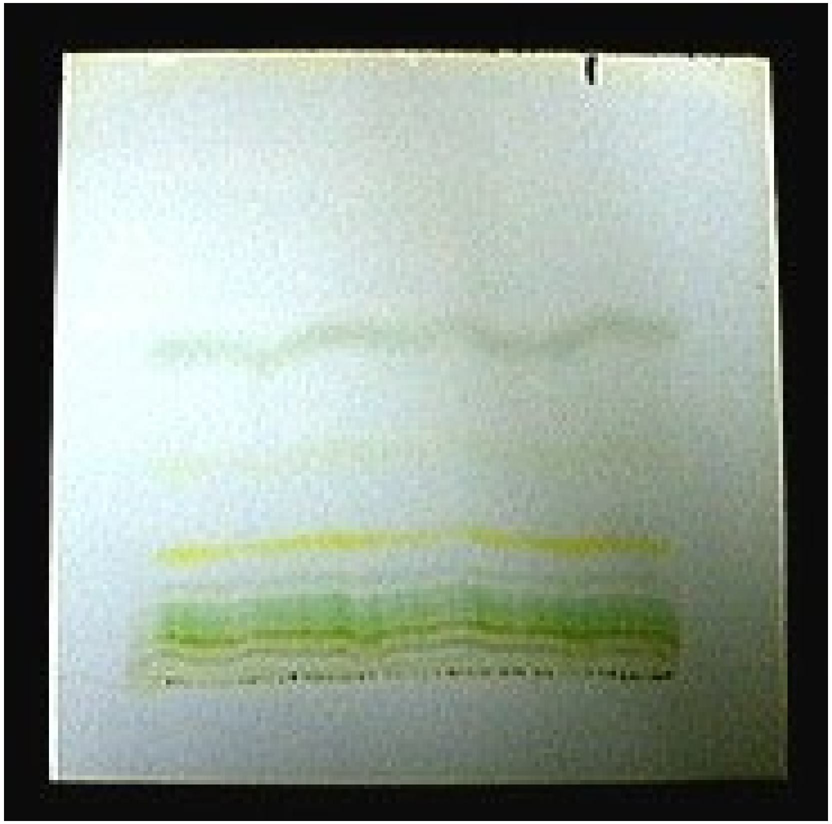
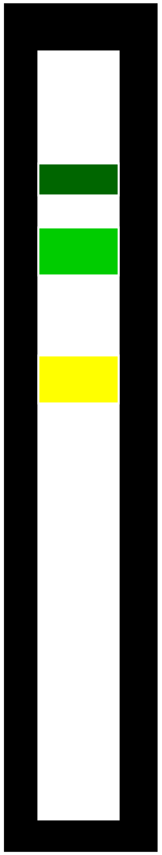
Chromatography and Structure Elucidation

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

1931:
Richard Kuhn and **Edgar Lederer** demonstrated the applicability of this method to purify compounds (e.g. vitamine B2).

Development

Richard L. M. Synge

Archer J.P. Martin

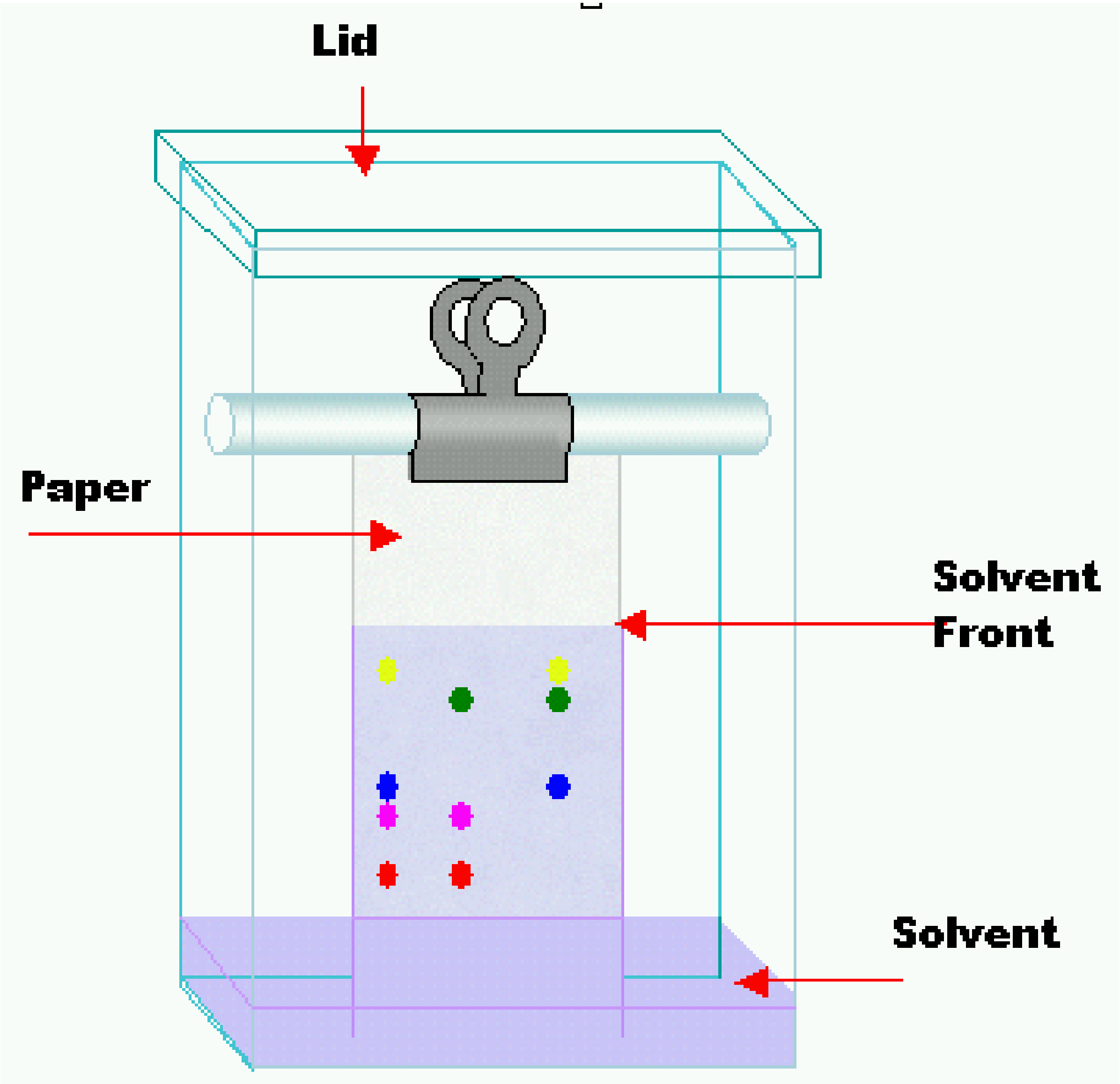
Richard L.M. Synge und **Archer J.P. Martin Kieselgel** standardized silica gel as stationary phase for **column chromatography**.

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

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 Russion chemists **Maria Schraiber** und **Nikolai Izmailov** in **1938**.

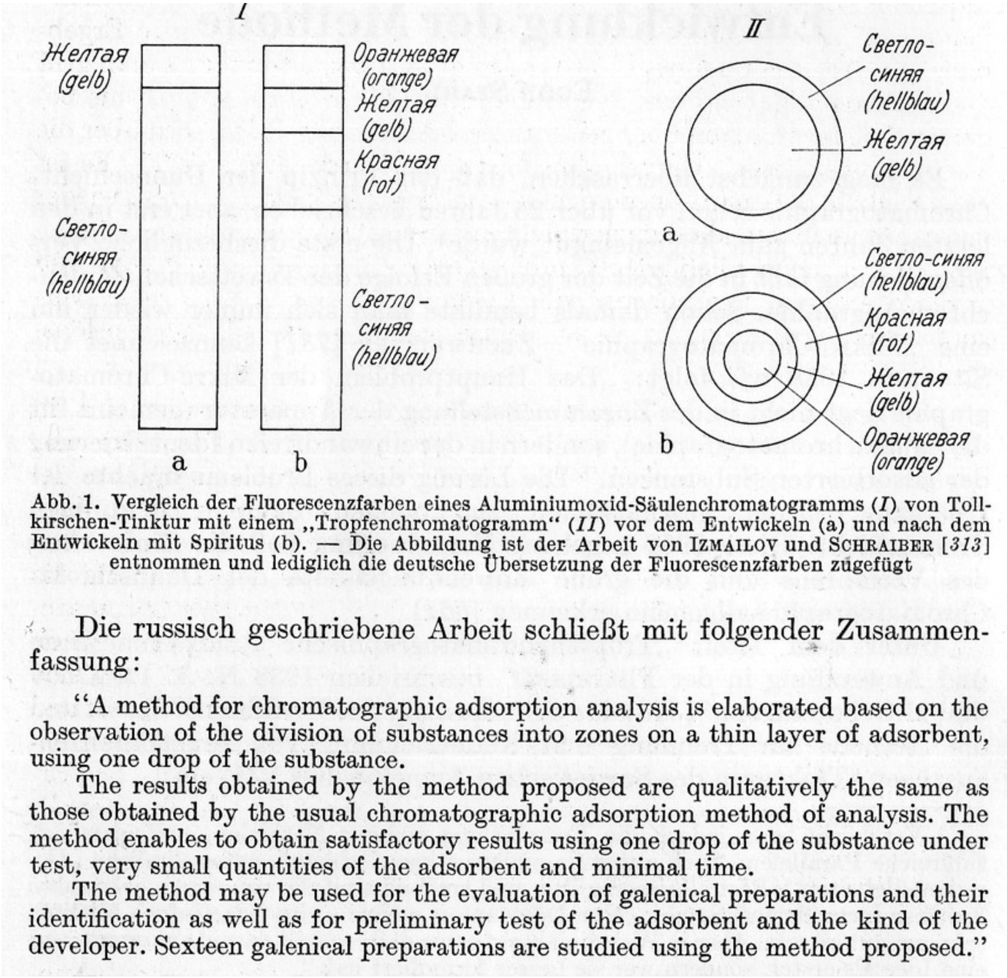


Abb. 1. Vergleich der Fluorescenzfarben eines Aluminiumoxid-Säulenchromatogramms (*I*) von Tollkirschen-Tinktur mit einem „Tropfenchromatogramm" (*II*) vor dem Entwickeln (a) und nach dem Entwickeln mit Spiritus (b). — Die Abbildung ist der Arbeit von IZMAILOV und SCHRAIBER [*313*] entnommen und lediglich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

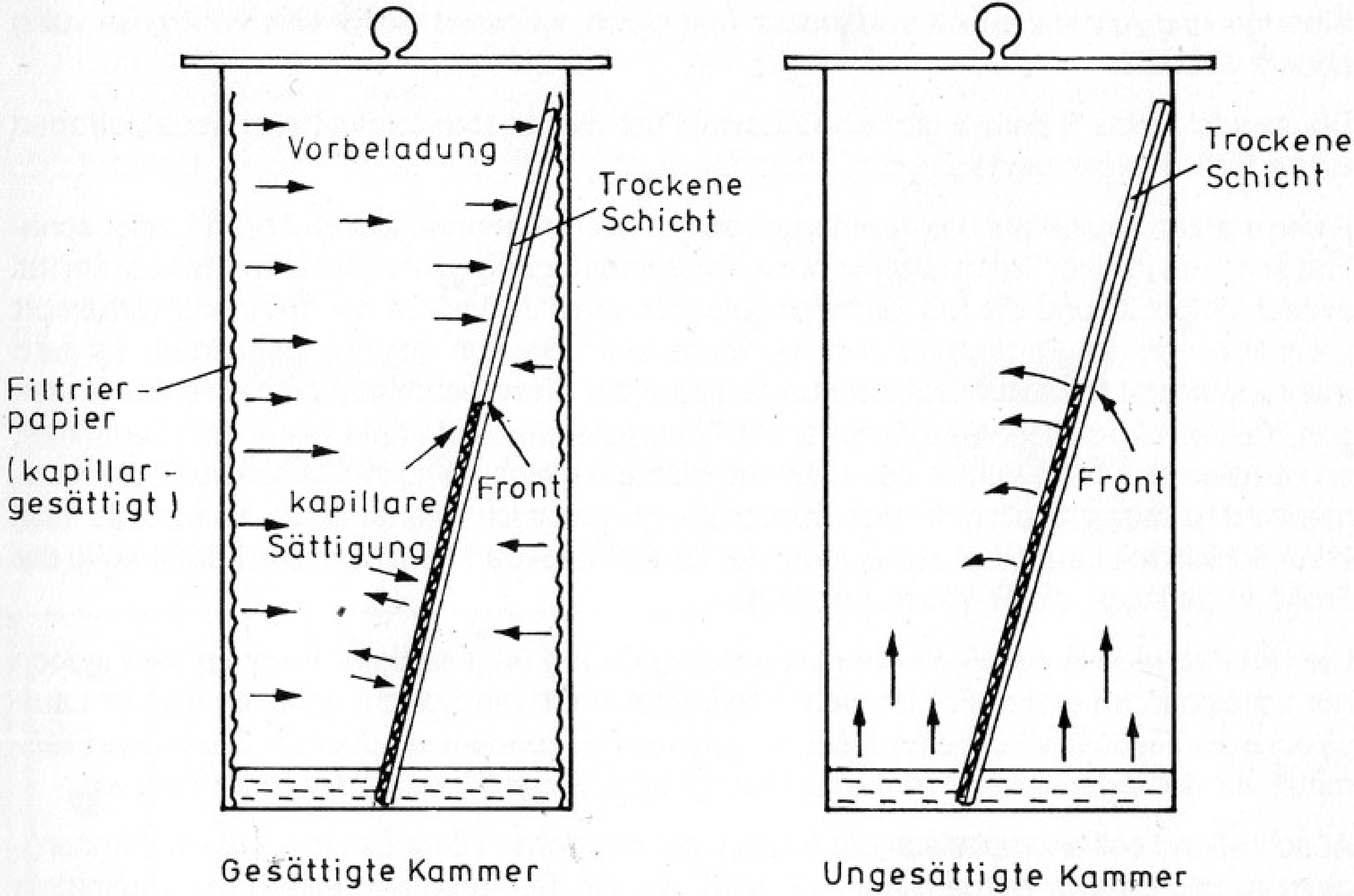
The method may be used for the evaluation of galenical preparations and their identification as well as for preliminary test of the adsorbent and the kind of the developer. Sexteen galenical preparations are studied using the method proposed."

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): Eleuotropic Sequence

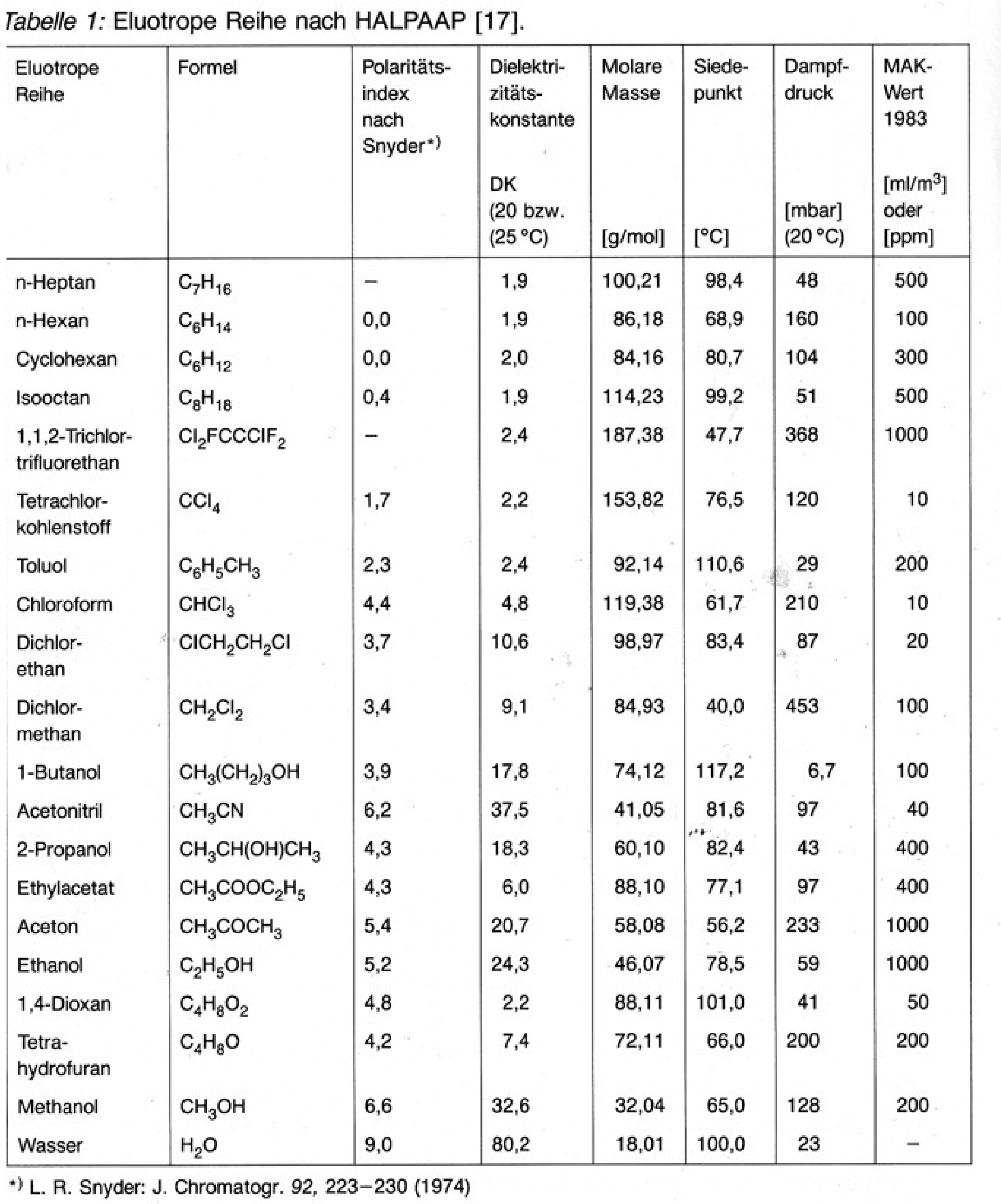


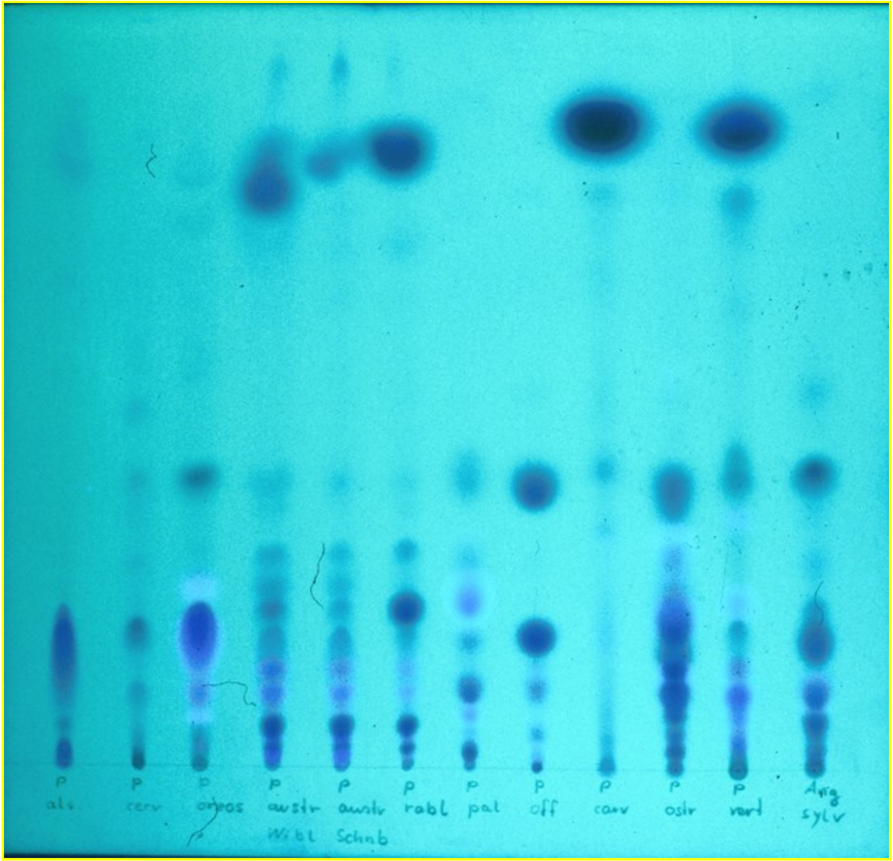
Tabelle 1: Eluotrope Reihe nach HALPAAP [17].

Eluotrope Reihe	Formel	Polaritäts-index nach Snyder*)	Dielektri-zitäts-konstante DK (20 bzw. (25 °C)	Molare Masse [g/mol]	Siede-punkt [°C]	Dampf-druck [mbar] (20 °C)	MAK-Wert 1983 [ml/m³] oder [ppm]
n-Heptan	C_7H_{16}	–	1,9	100,21	98,4	48	500
n-Hexan	C_6H_{14}	0,0	1,9	86,18	68,9	160	100
Cyclohexan	C_6H_{12}	0,0	2,0	84,16	80,7	104	300
Isooctan	C_8H_{18}	0,4	1,9	114,23	99,2	51	500
1,1,2-Trichlor-trifluorethan	$Cl_2FCCClF_2$	–	2,4	187,38	47,7	368	1000
Tetrachlor-kohlenstoff	CCl_4	1,7	2,2	153,82	76,5	120	10
Toluol	$C_6H_5CH_3$	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	$ClCH_2CH_2Cl$	3,7	10,6	98,97	83,4	87	20
Dichlor-methan	CH_2Cl_2	3,4	9,1	84,93	40,0	453	100
1-Butanol	$CH_3(CH_2)_3OH$	3,9	17,8	74,12	117,2	6,7	100
Acetonitril	CH_3CN	6,2	37,5	41,05	81,6	97	40
2-Propanol	$CH_3CH(OH)CH_3$	4,3	18,3	60,10	82,4	43	400
Ethylacetat	$CH_3COOC_2H_5$	4,3	6,0	88,10	77,1	97	400
Aceton	CH_3COCH_3	5,4	20,7	58,08	56,2	233	1000
Ethanol	C_2H_5OH	5,2	24,3	46,07	78,5	59	1000
1,4-Dioxan	$C_4H_8O_2$	4,8	2,2	88,11	101,0	41	50
Tetra-hydrofuran	C_4H_8O	4,2	7,4	72,11	66,0	200	200
Methanol	CH_3OH	6,6	32,6	32,04	65,0	128	200
Wasser	H_2O	9,0	80,2	18,01	100,0	23	–

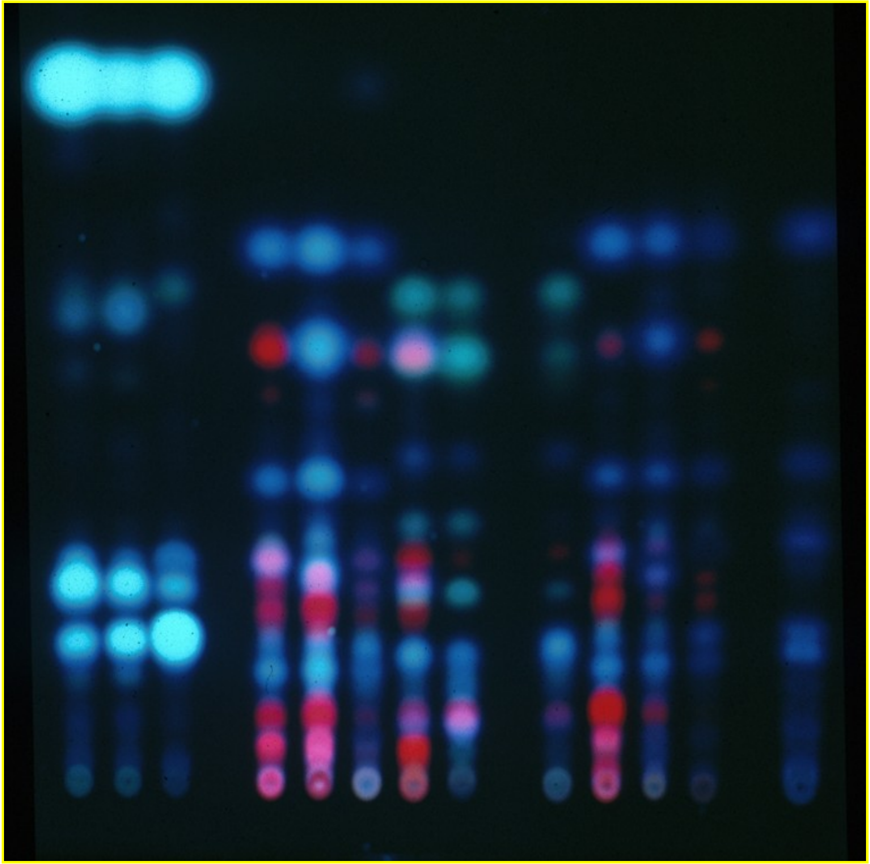
*) L. R. Snyder: J. Chromatogr. *92*, 223–230 (1974)

Thin Layer Chromatography (TLC): Detection 1

UV



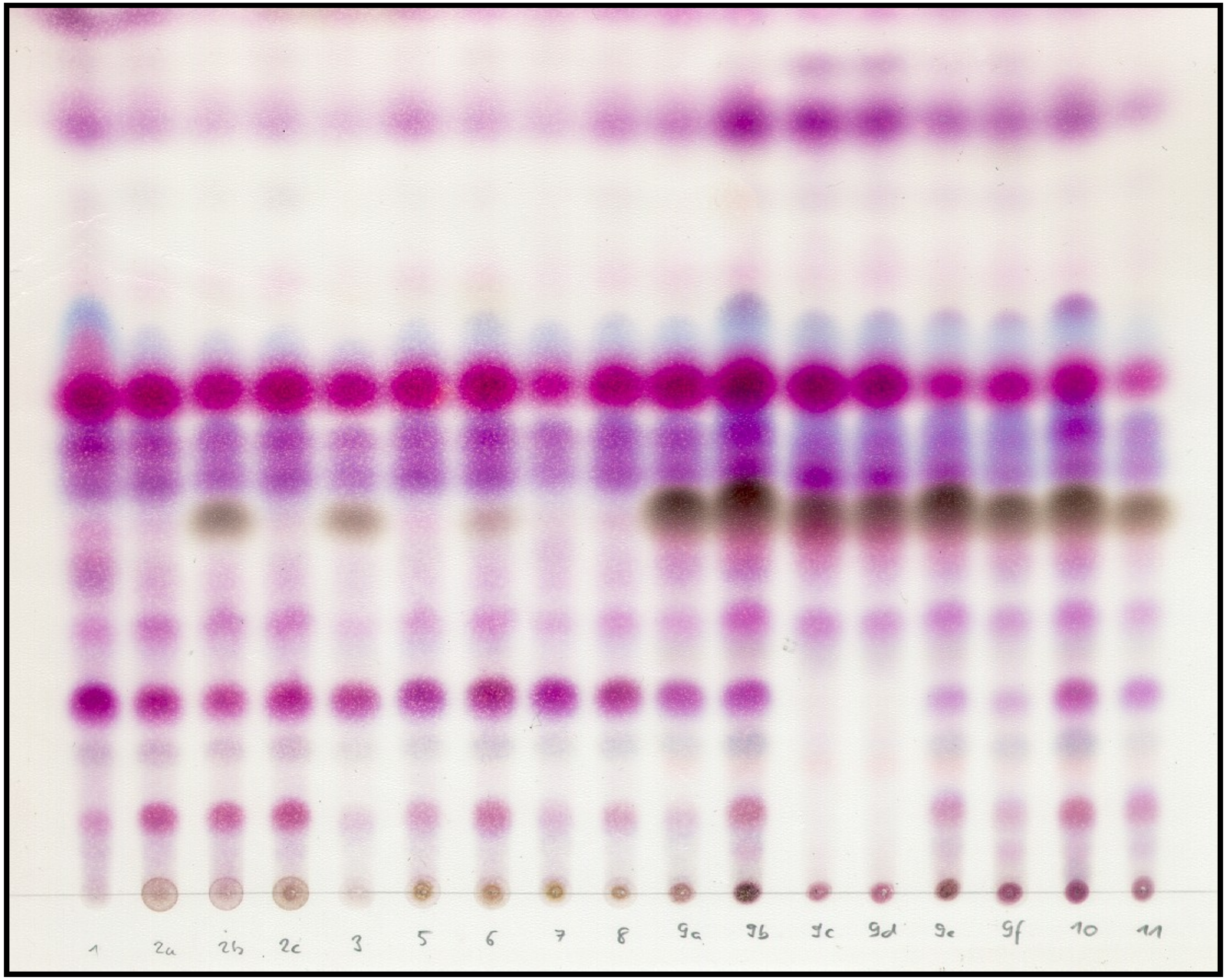
254 nm



366 nm

Thin Layer Chromatography (TLC): Detection 2

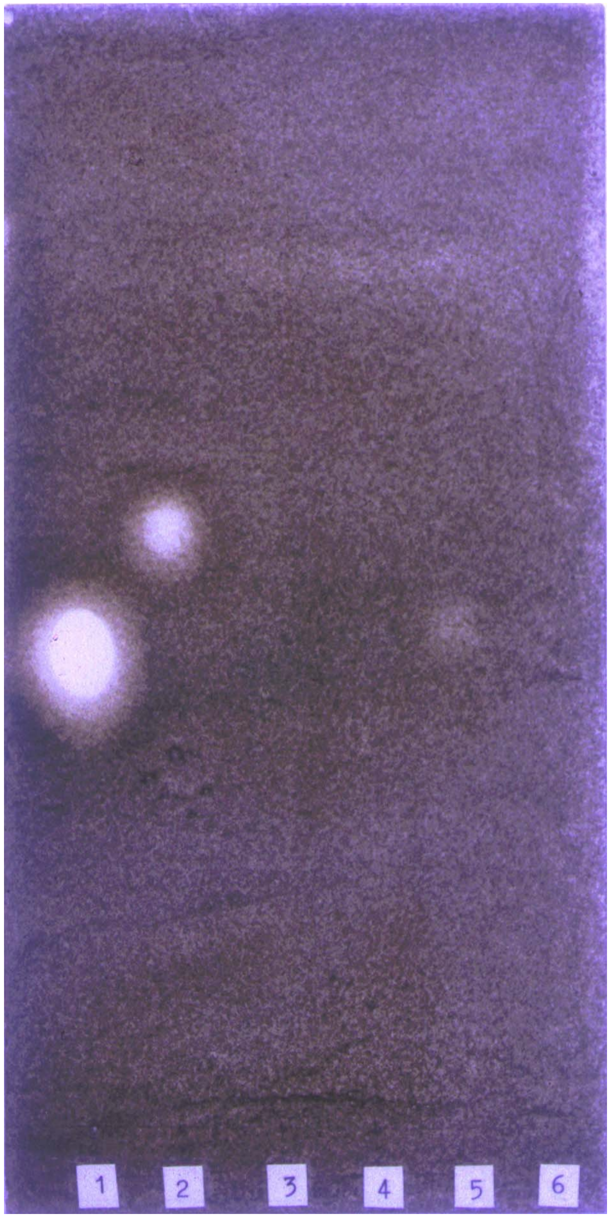
Chemcal derivatisation



Anis aldehyd-sulphuric acid

Thin Layer Chromatography (TLC): Detection 3

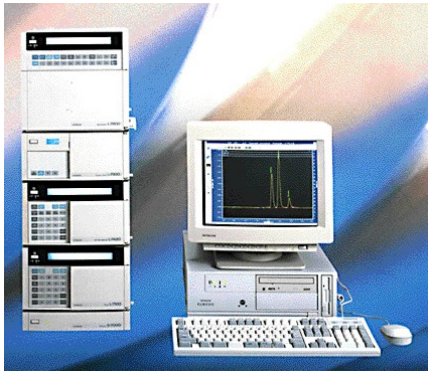
Bioautography

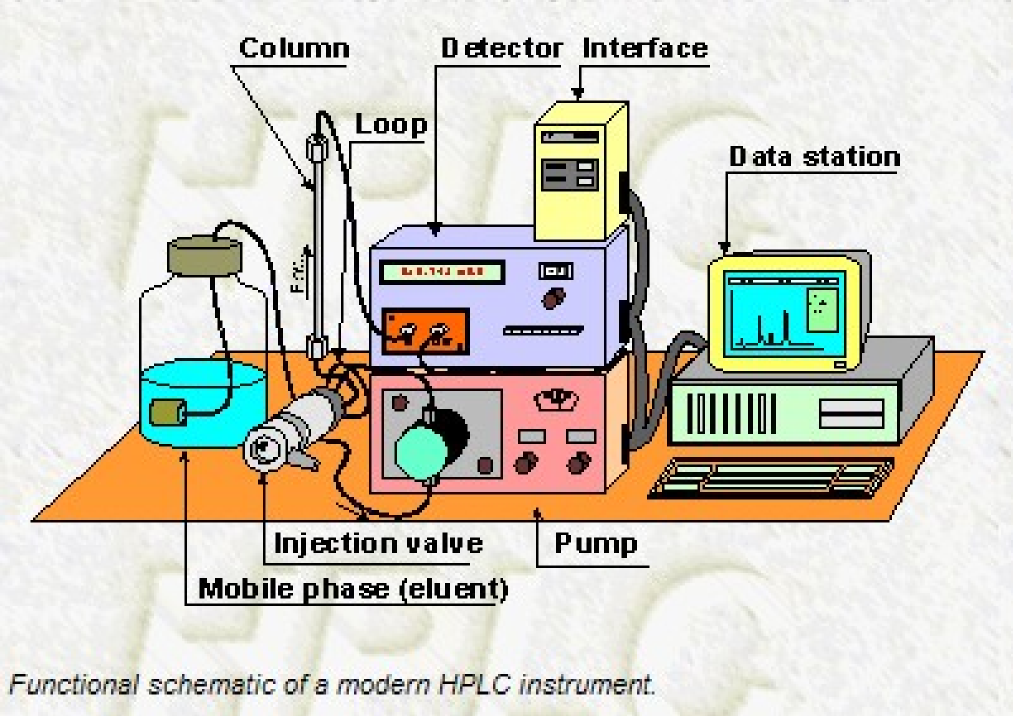


Separation on silica

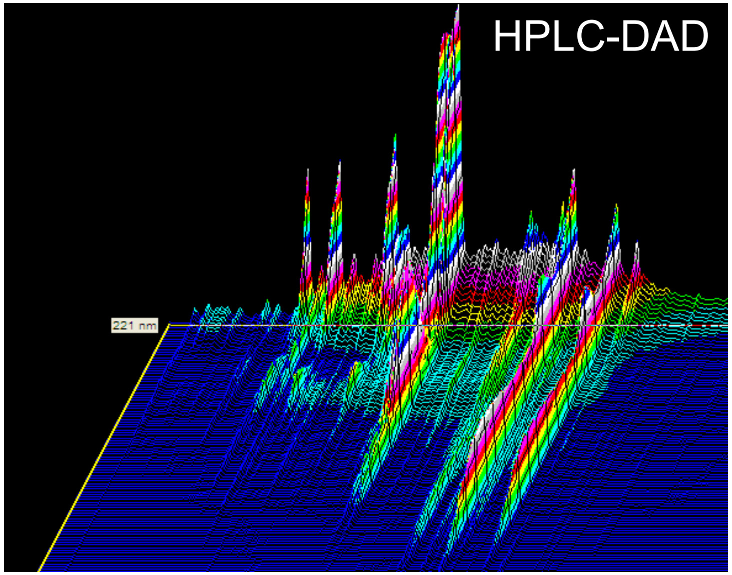
- Absorption
  Pore structure of stationary phase
- Partition
  between mobile and stationary liquid

HPLC (High Performance Liquid Chromatography)





Functional schematic of a modern HPLC instrument.



Stationary phases: partice size

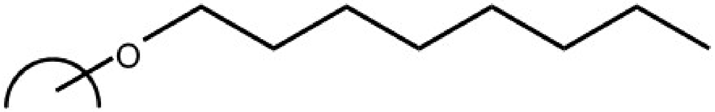
Column chromatography	Gravity	200-500 µm
Thin layer chromatography	Capillary force	20 µm
Middle pressure liquid chromatography	1-10 bar	15-60 µm
High pressure liquid chromatography	100-300 bar	1-5 µm

HPLC columns

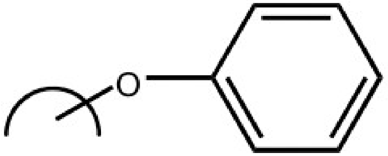
HPLC columns: stationary phases

Straight phase

Reversed phase C18

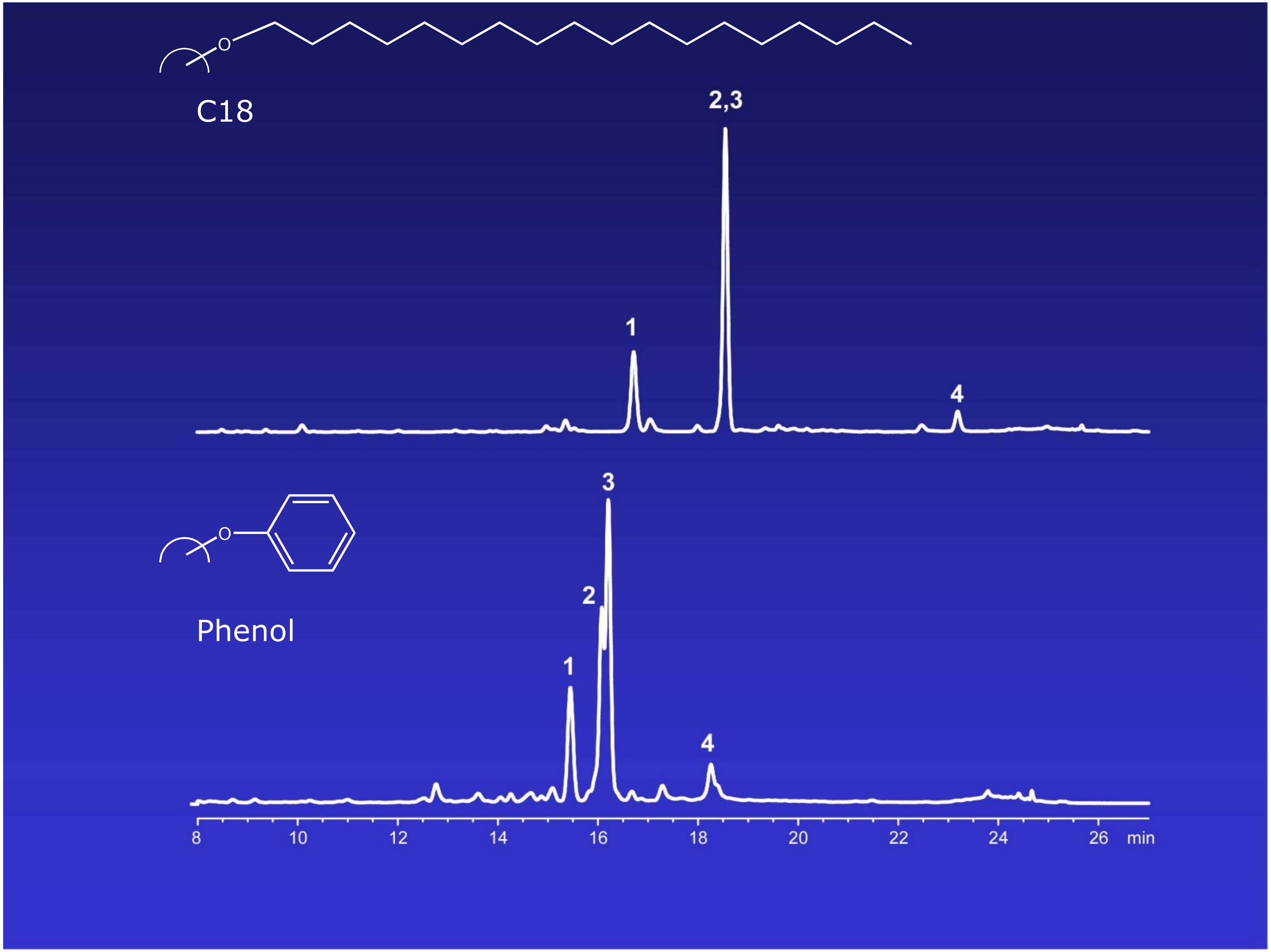


Reversed phase C8

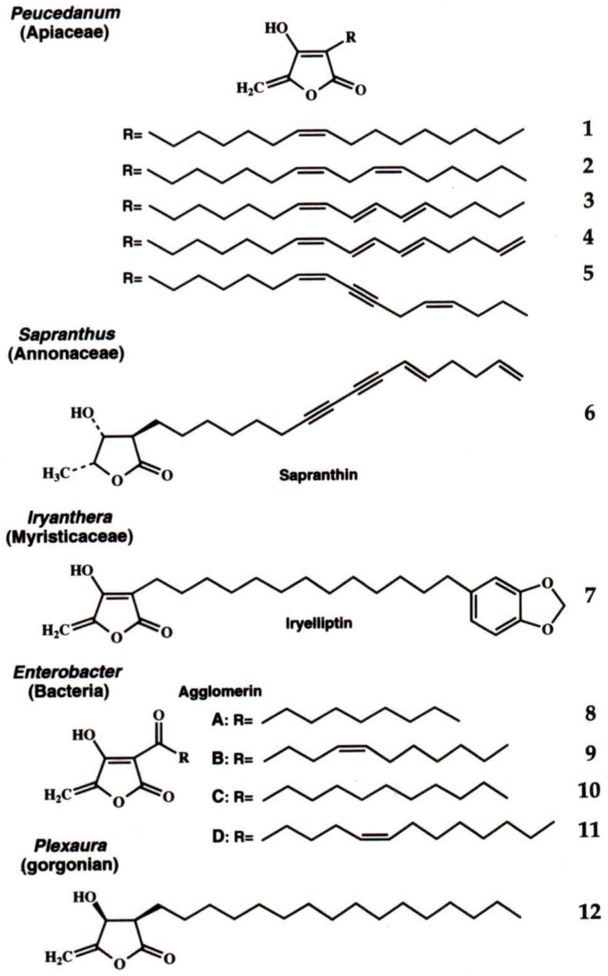


Phenol phase

HPLC columns: selectivity



HPLC columns: effect of pH



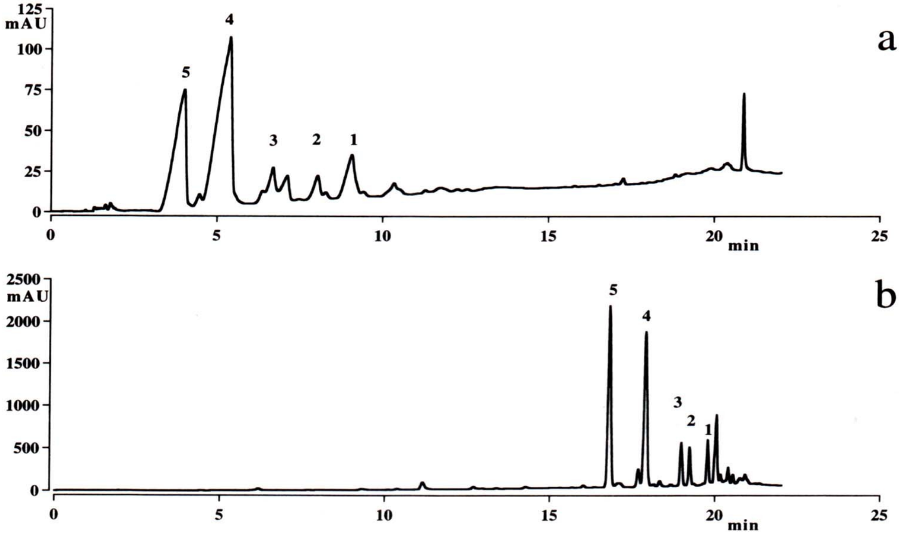
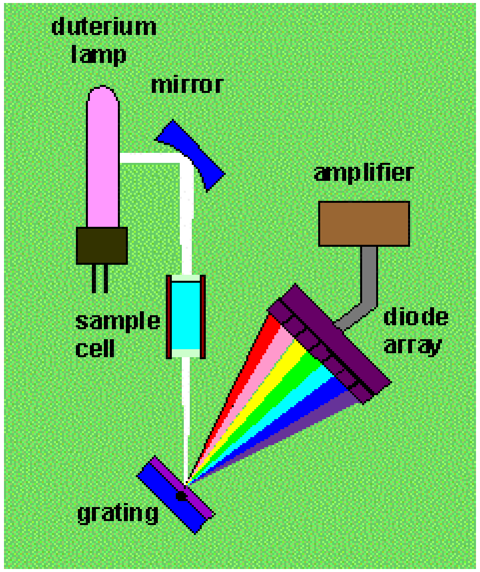
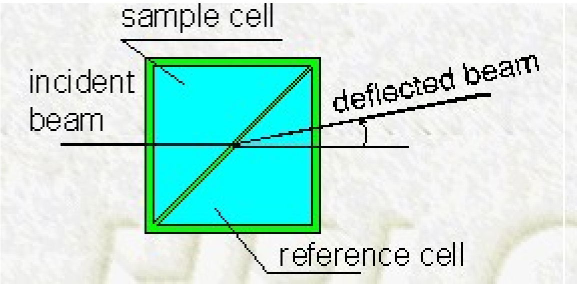


Figure 2. (a) Analytical HPLC of 0.05 mg of the crude extract of the roots of *P. alsaticum*. (For chromatographic protocol see Experimental section—solvent A was water.) (b) Analytical HPLC of 0.05 mg of the crude extract of the roots of *P. alsaticum*. (For chromatographic protocol see Experimental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

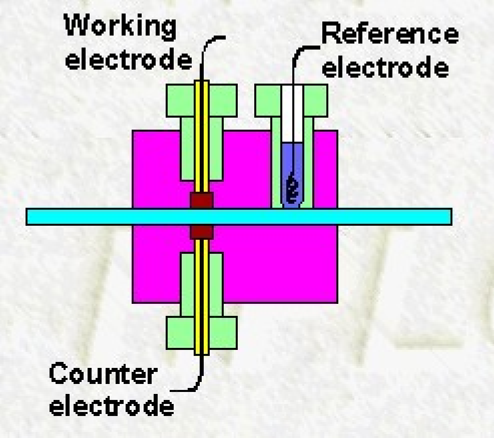
HPLC: various detectors



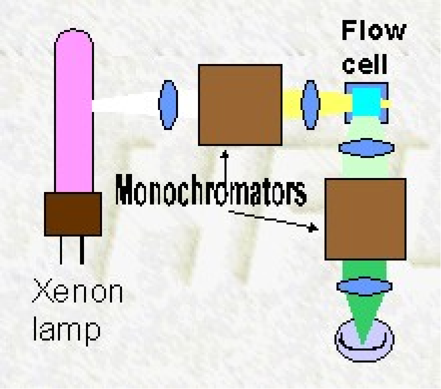
UV-Diode Array (DAD)



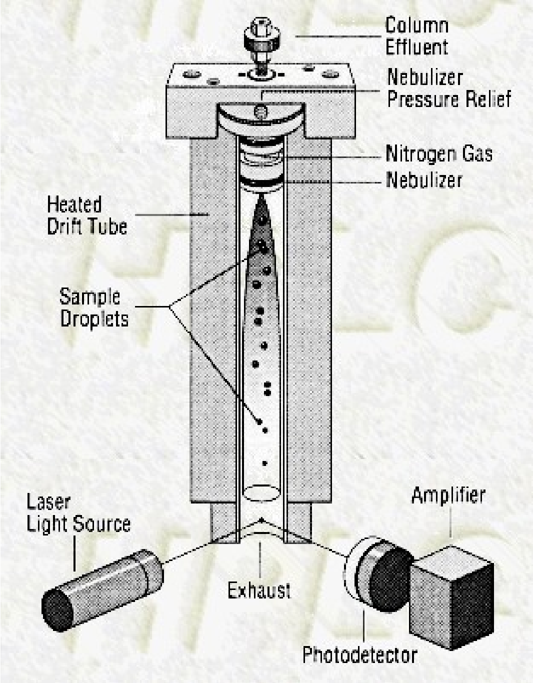
Refractive index



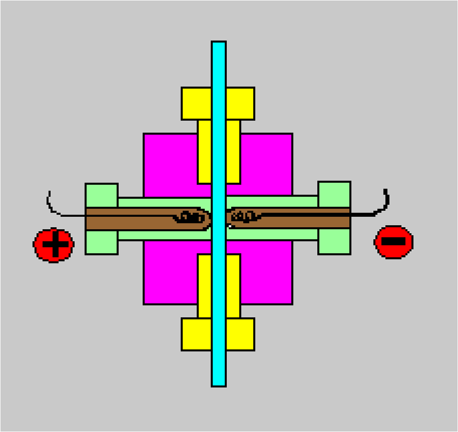
Electrochemical D.



Fluorescence D.

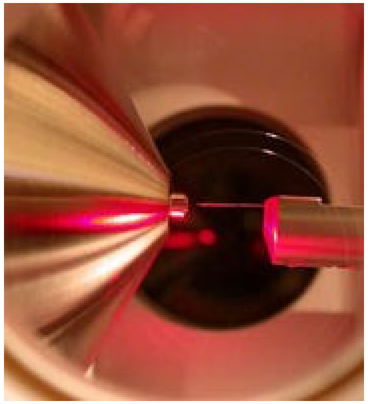


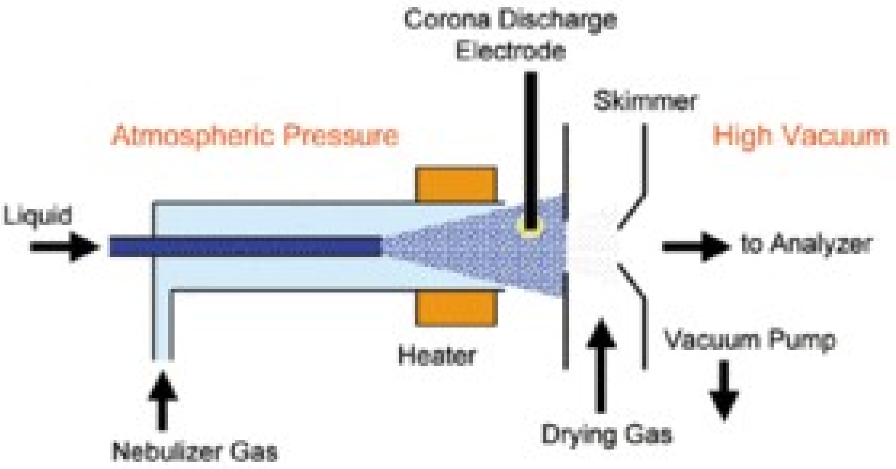
Light scattering



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: $[M+H]^+$

$[M+Na]^+$

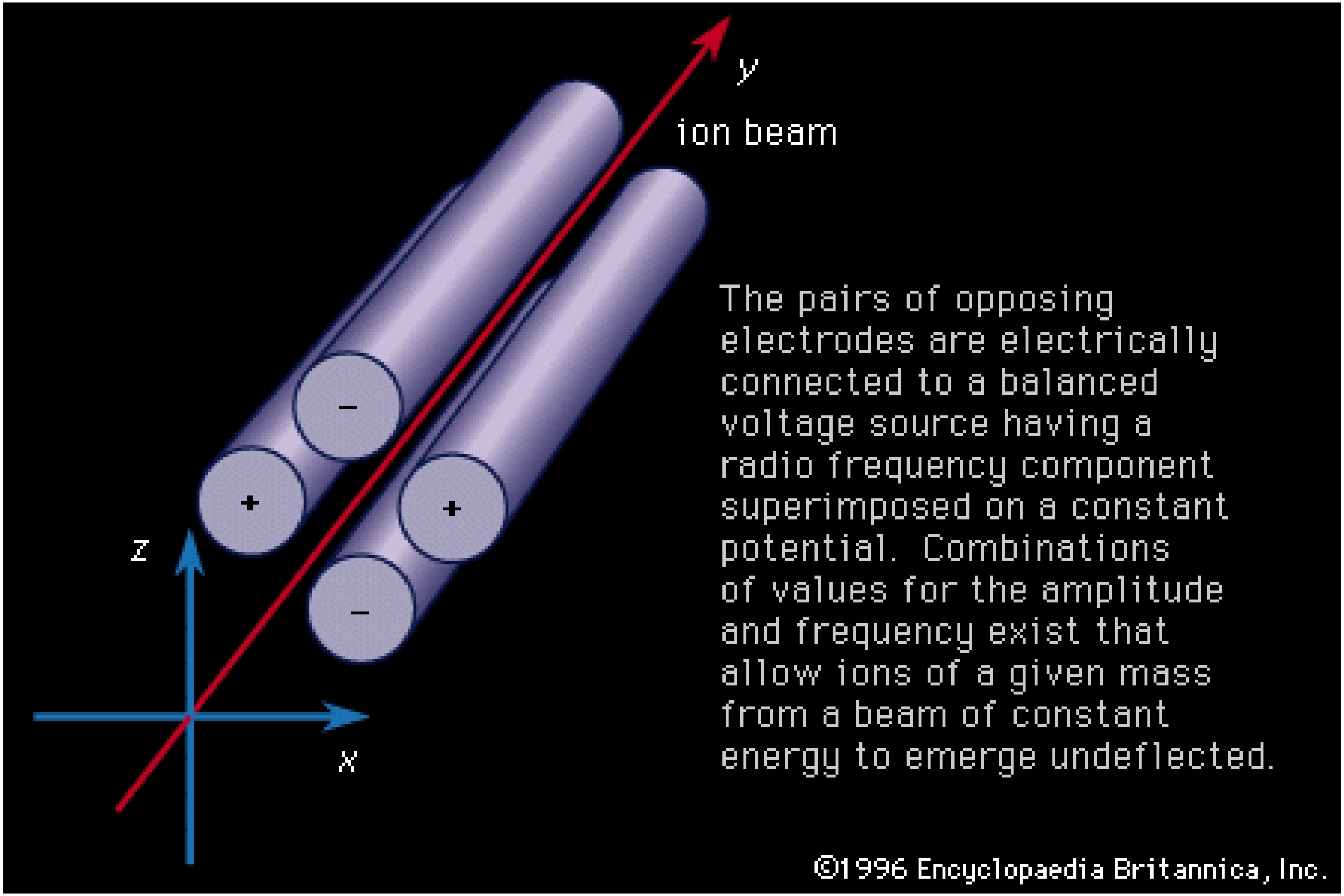
$[M+2H]^{2+}$

$[M+25H]^{25+}$

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

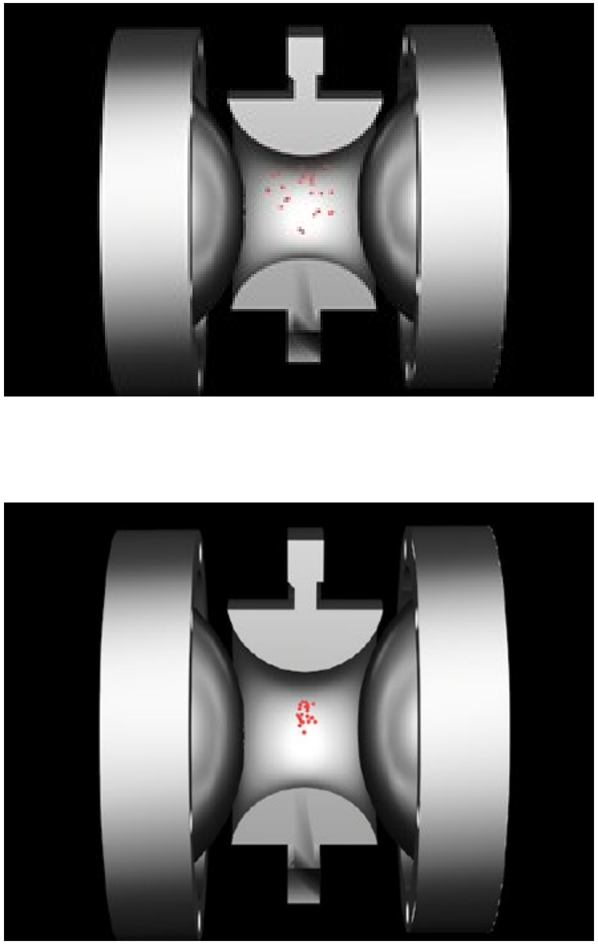
CI: Chemical Ionisation

Mass spectrometers: Quadrupol



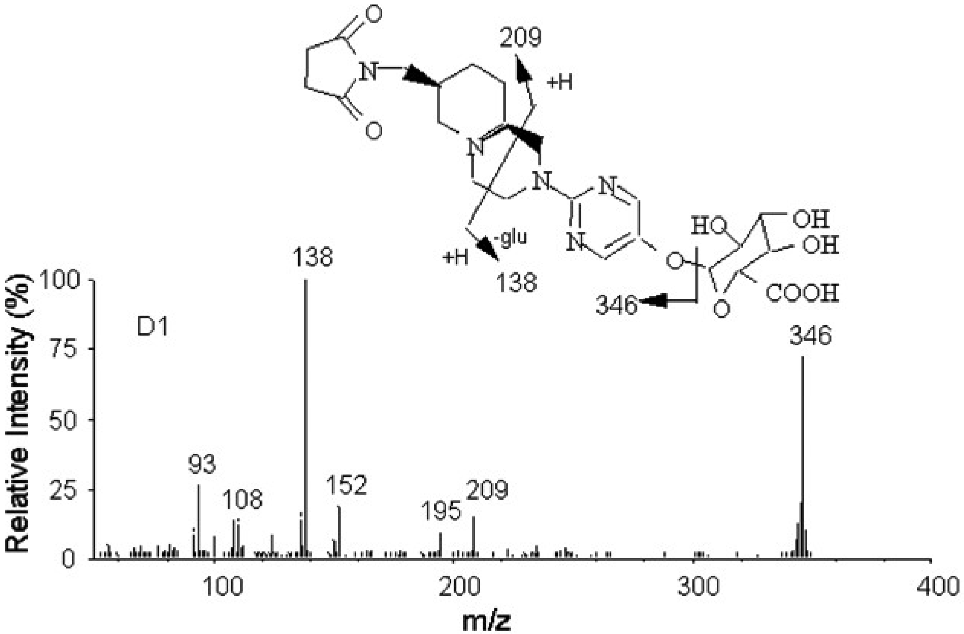
The pairs of opposing electrodes are electrically connected to a balanced voltage source having a radio frequency component superimposed on a constant potential. Combinations of values for the amplitude and frequency exist that allow ions of a given mass from a beam of constant energy to emerge undeflected.

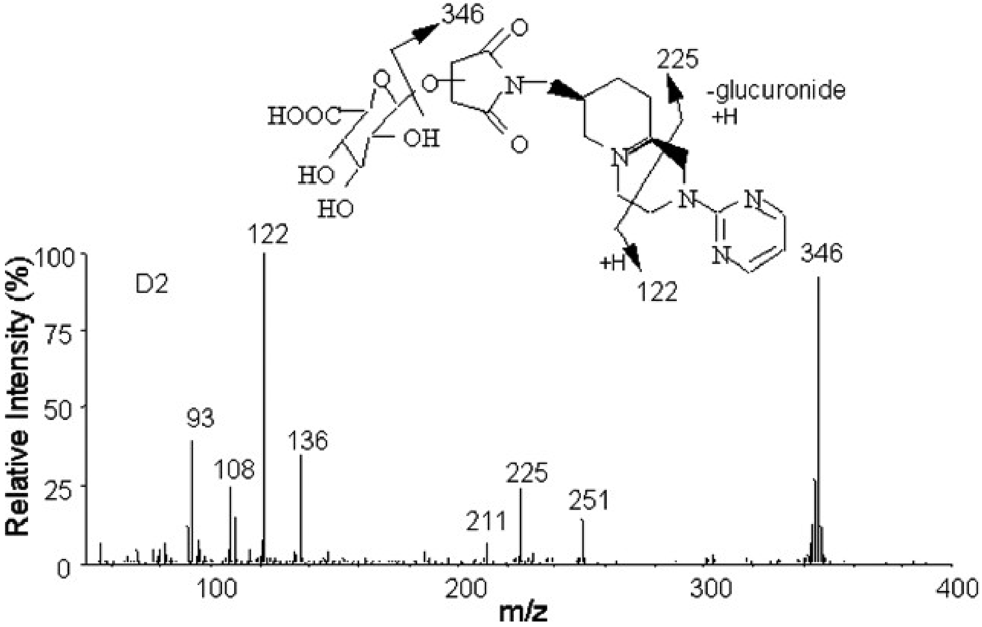
Mass spectrometers: Ion trap



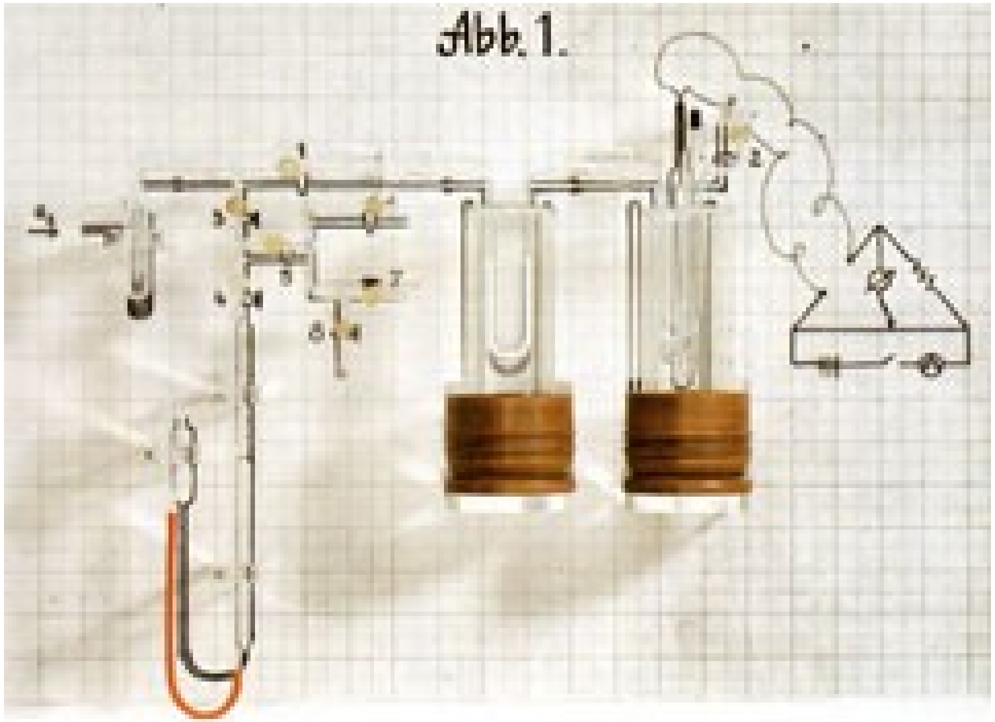
Variable electric fields allow to focus ions of variable *m/z* ratio.

Mass spectrometers: Ion trap, MS^n



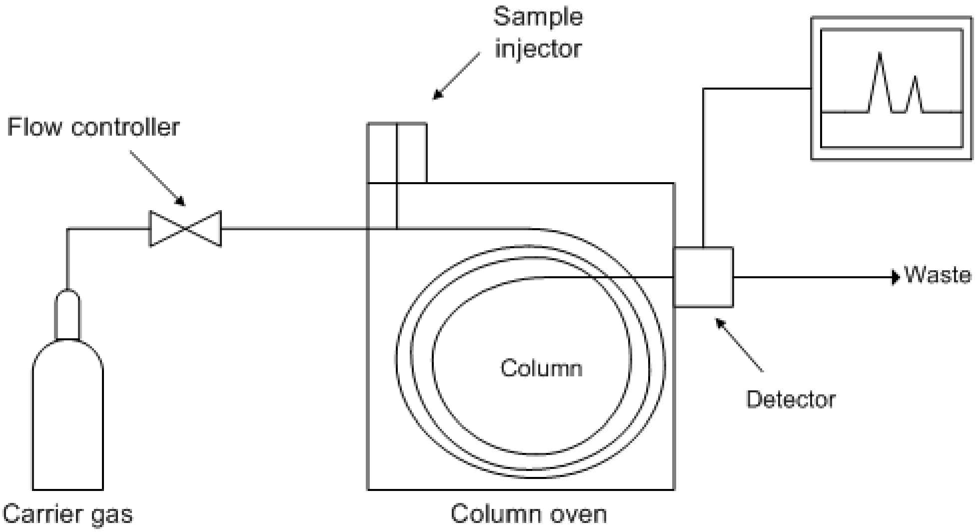


Gas Chromatography (GC)



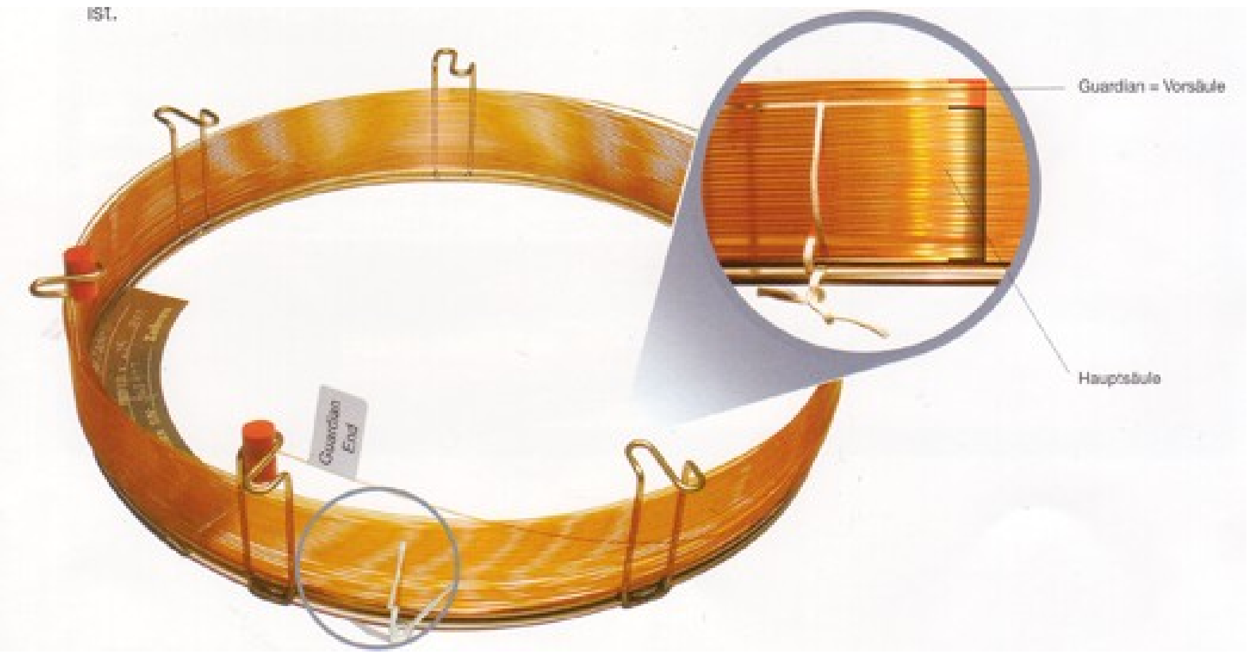
1944: **Fritz Prior** und **Erika Cremer**

Gas Chromatography (GC)



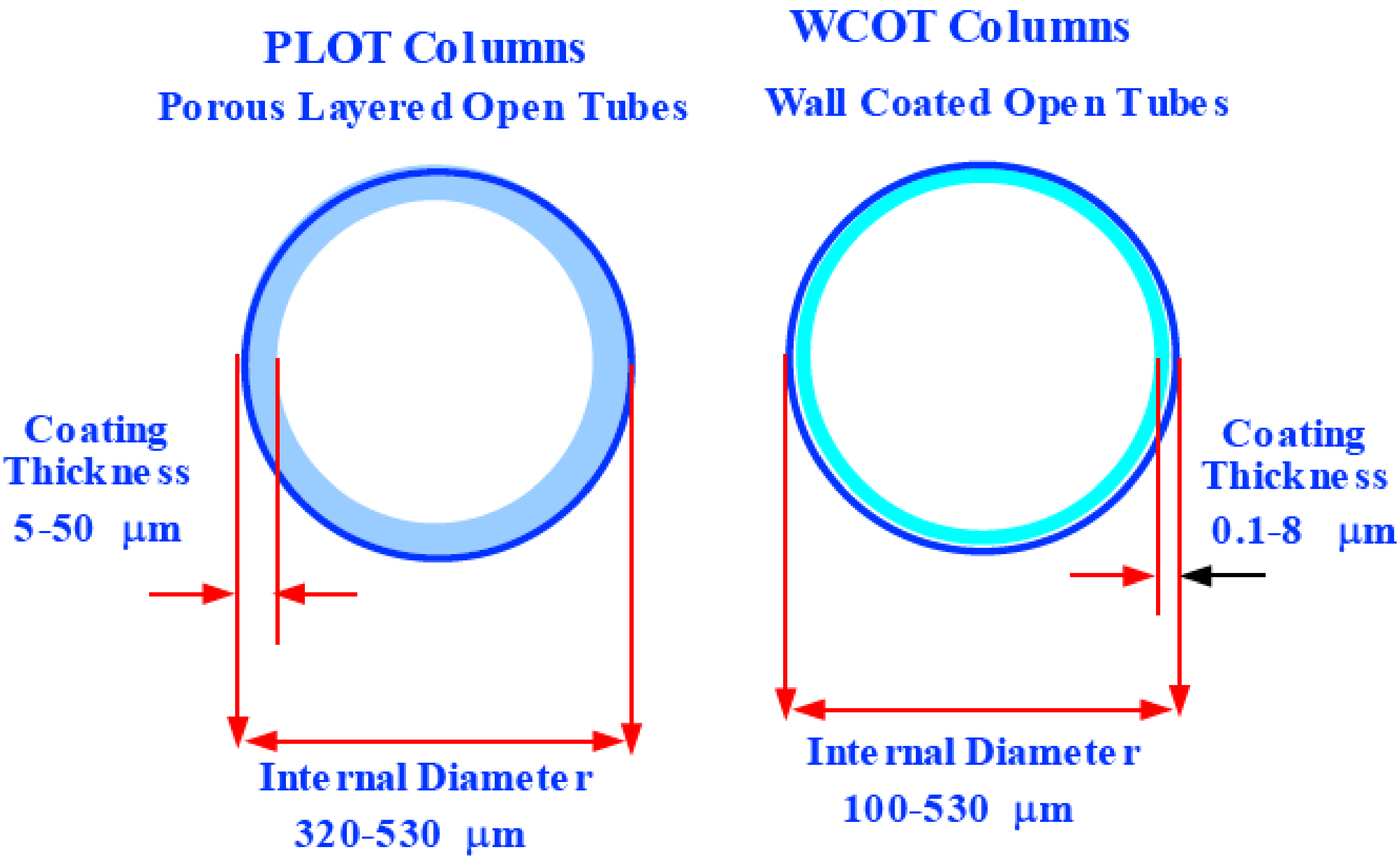
Gas Chromatography (GC): Columns

packed

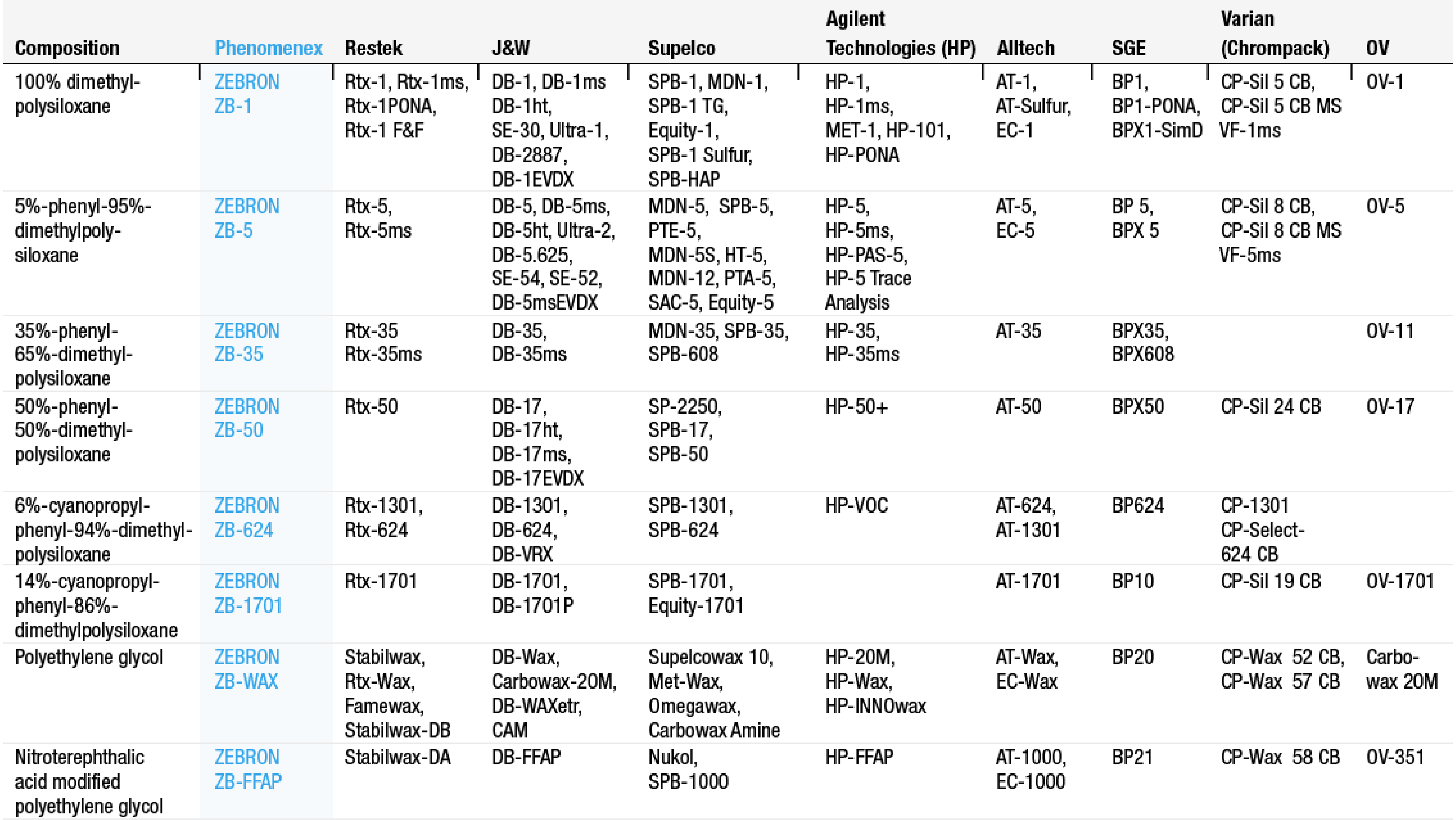


capillary

Capillary columns

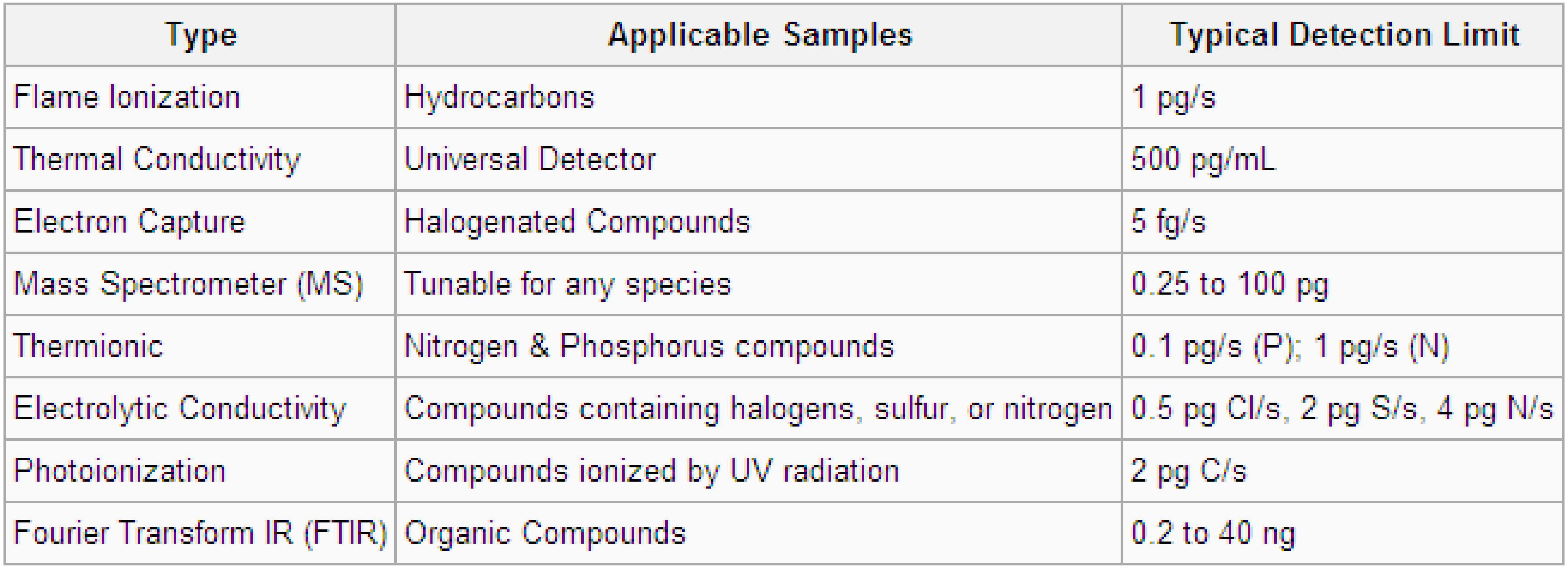


Column types for gas chromatography



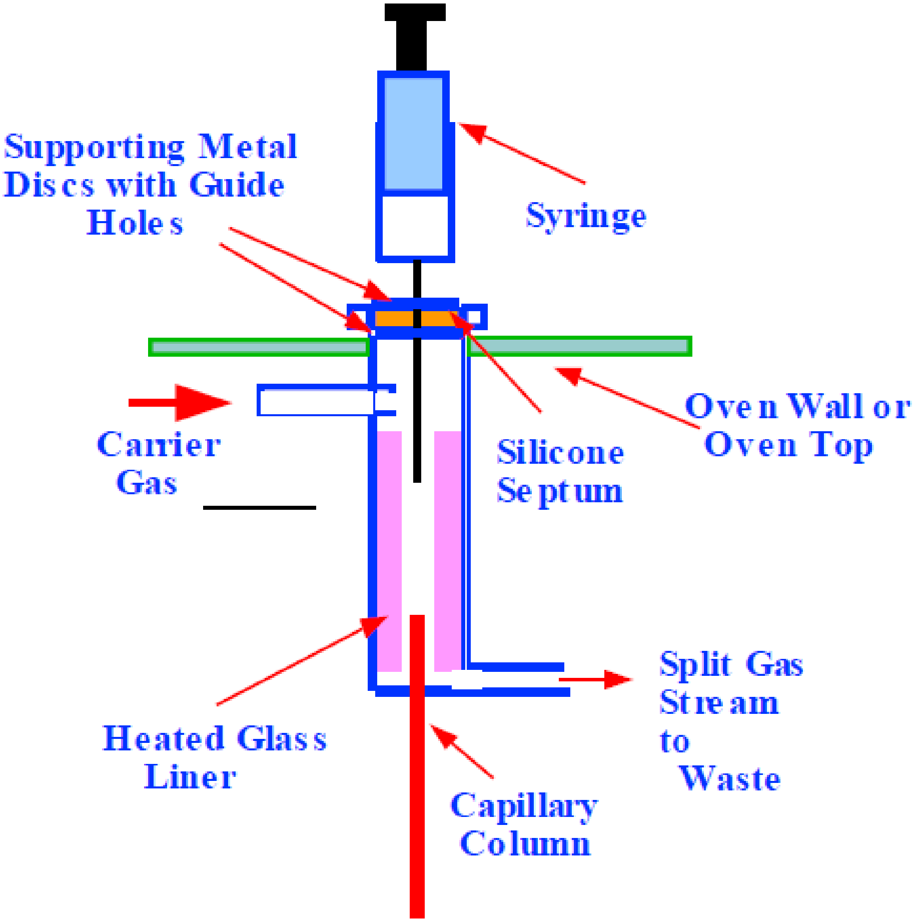
Composition	Phenomenex	Restek	J&W	Supelco	Agilent Technologies (HP)	Alltech	SGE	Varian (Chrompack)	OV
100% dimethyl-polysiloxane	ZEBRON ZB-1	Rtx-1, Rtx-1ms, Rtx-1PONA, Rtx-1 F&F	DB-1, DB-1ms DB-1ht, SE-30, Ultra-1, DB-2887, DB-1EVDX	SPB-1, MDN-1, SPB-1 TG, Equity-1, SPB-1 Sulfur, SPB-HAP	HP-1, HP-1ms, 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	Carbowax 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

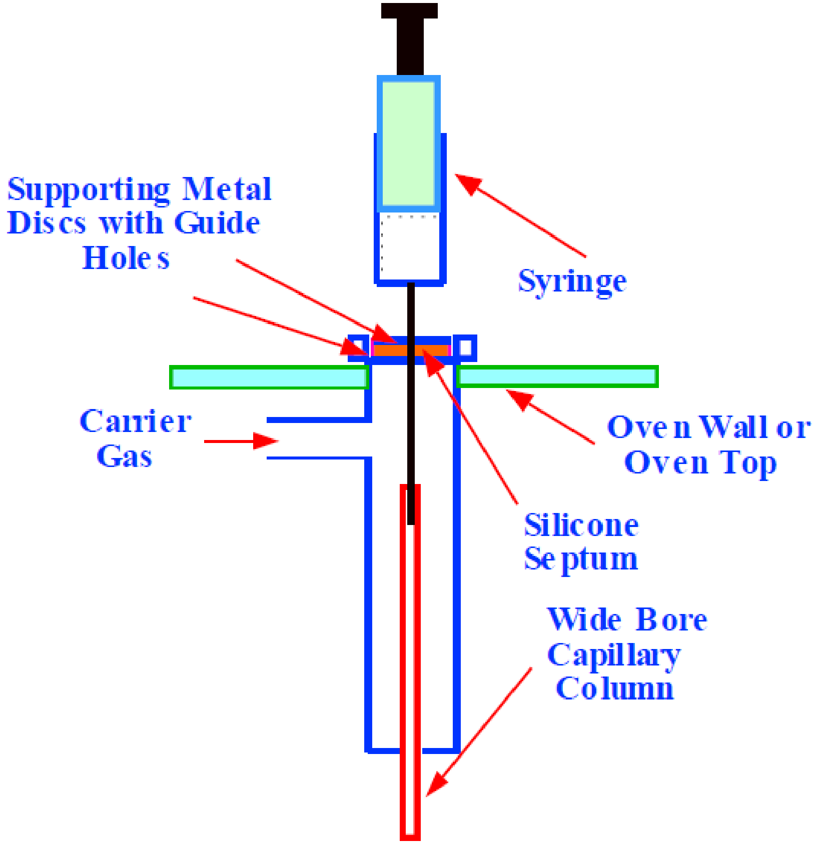


Type	Applicable Samples	Typical Detection Limit
Flame Ionization	Hydrocarbons	1 pg/s
Thermal Conductivity	Universal Detector	500 pg/mL
Electron Capture	Halogenated Compounds	5 fg/s
Mass Spectrometer (MS)	Tunable for any species	0.25 to 100 pg
Thermionic	Nitrogen & Phosphorus compounds	0.1 pg/s (P); 1 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 chromatograps



Split-Splitless



On-column

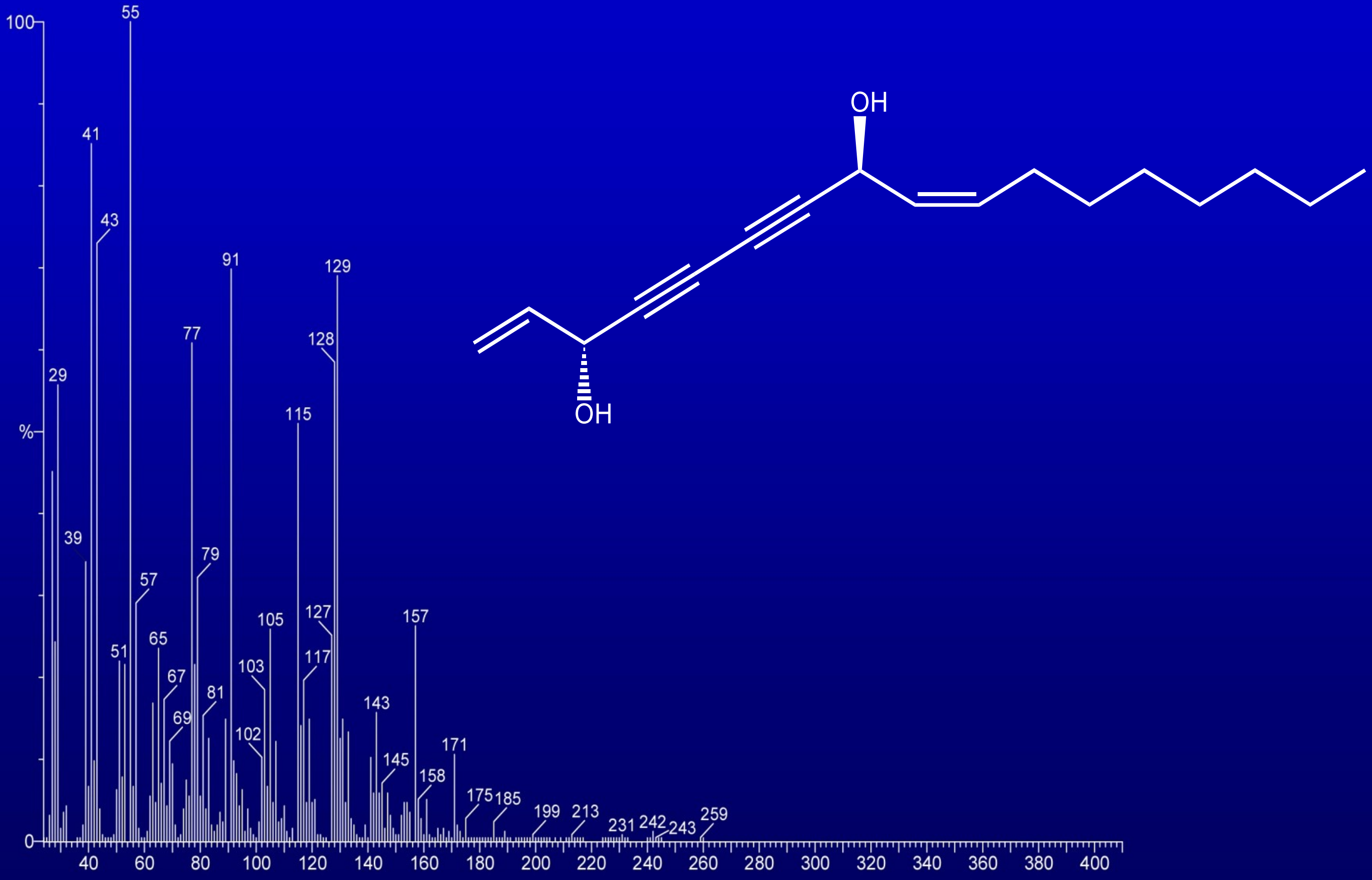
SPME (solid phase microextraction)

Fibers:

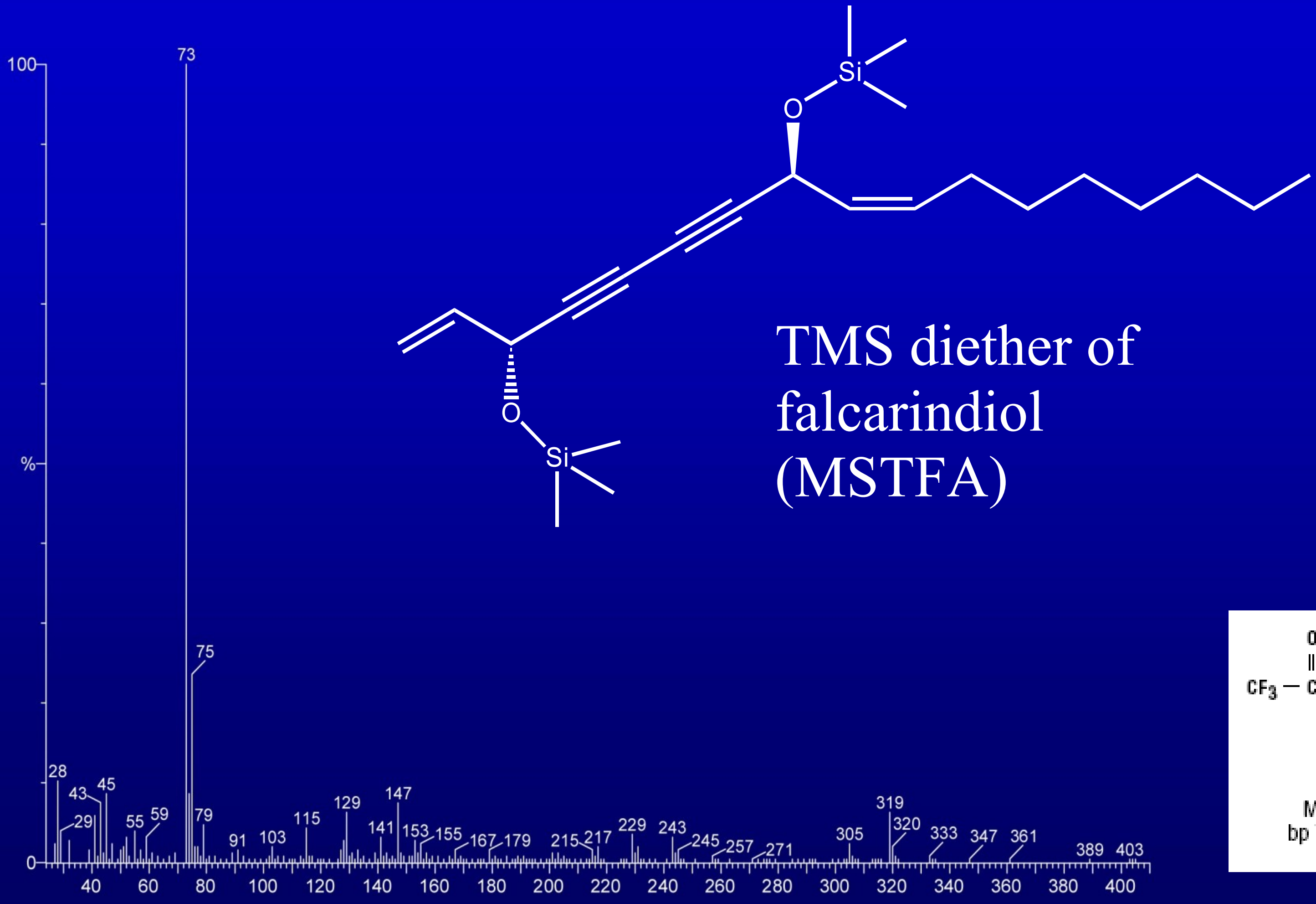
DVB/CAR/PDMS
(Divinylbenzene,
Carboxen,
Polydimethylsiloxan)

Static Headspace
10–60 min,
Room temperature

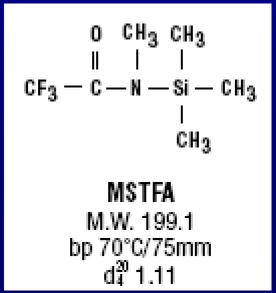
Dervatisation of analytes



Dervatisation of analytes

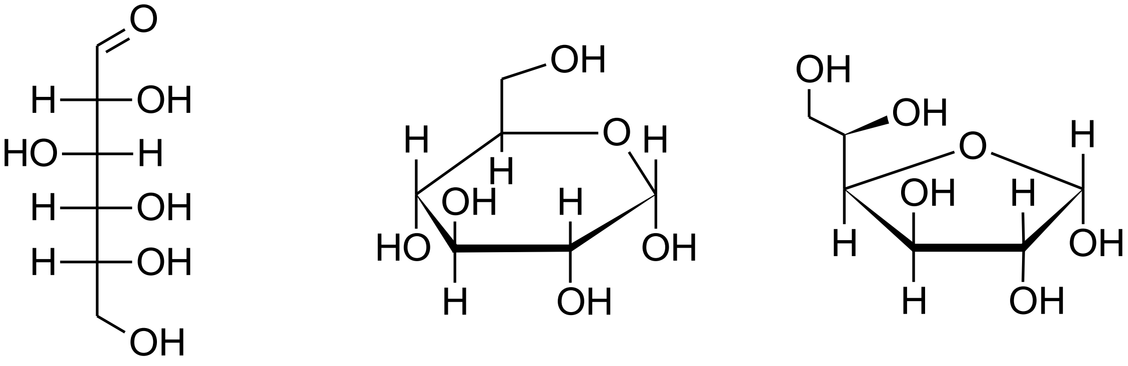


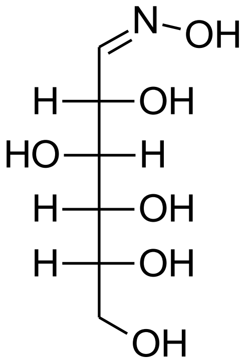
TMS diether of falcarindiol (MSTFA)



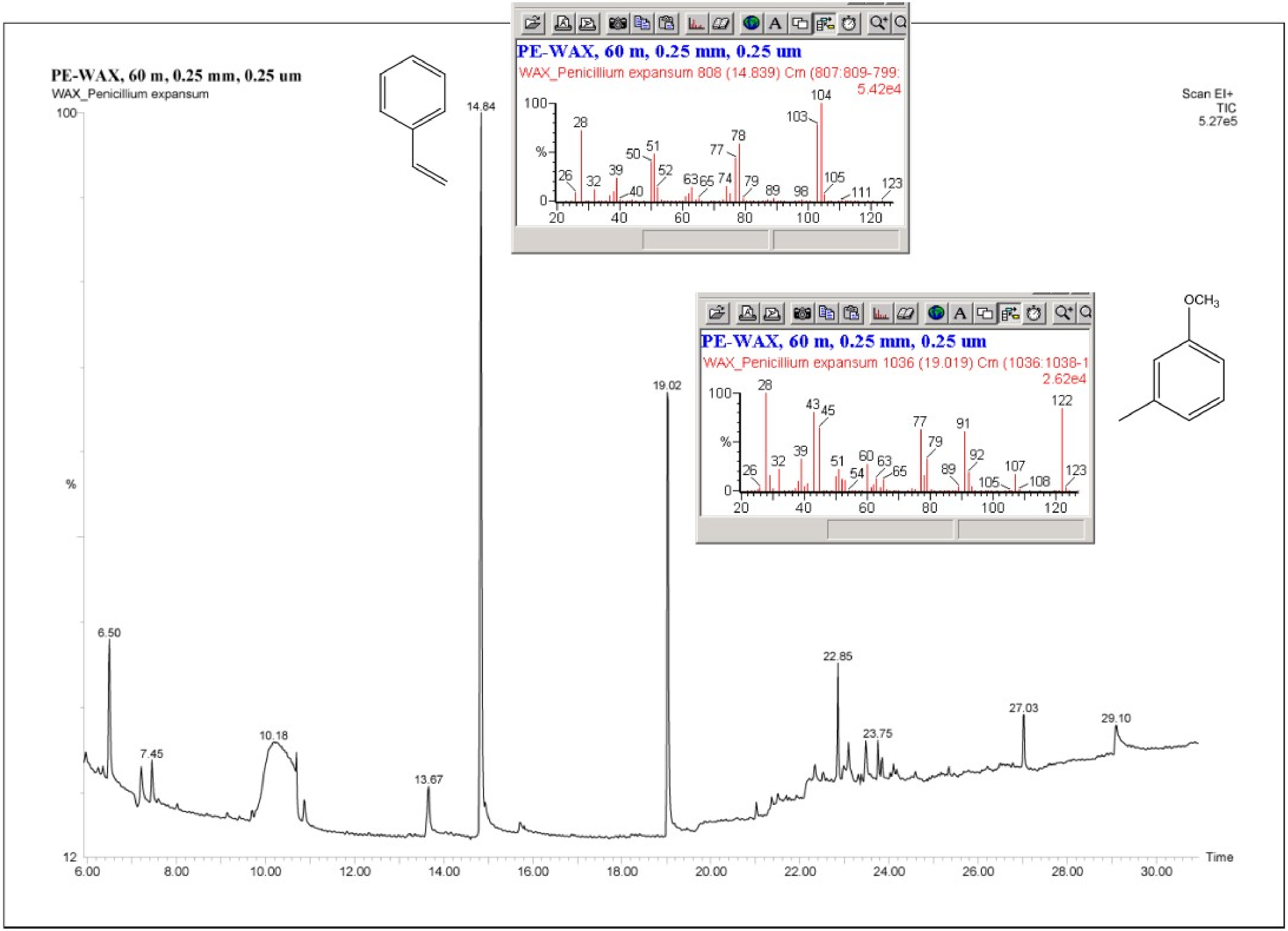
MSTFA
M.W. 199.1
bp 70°C/75mm
d_4^{20} 1.11

Special dervatisation of sugars: Oximation

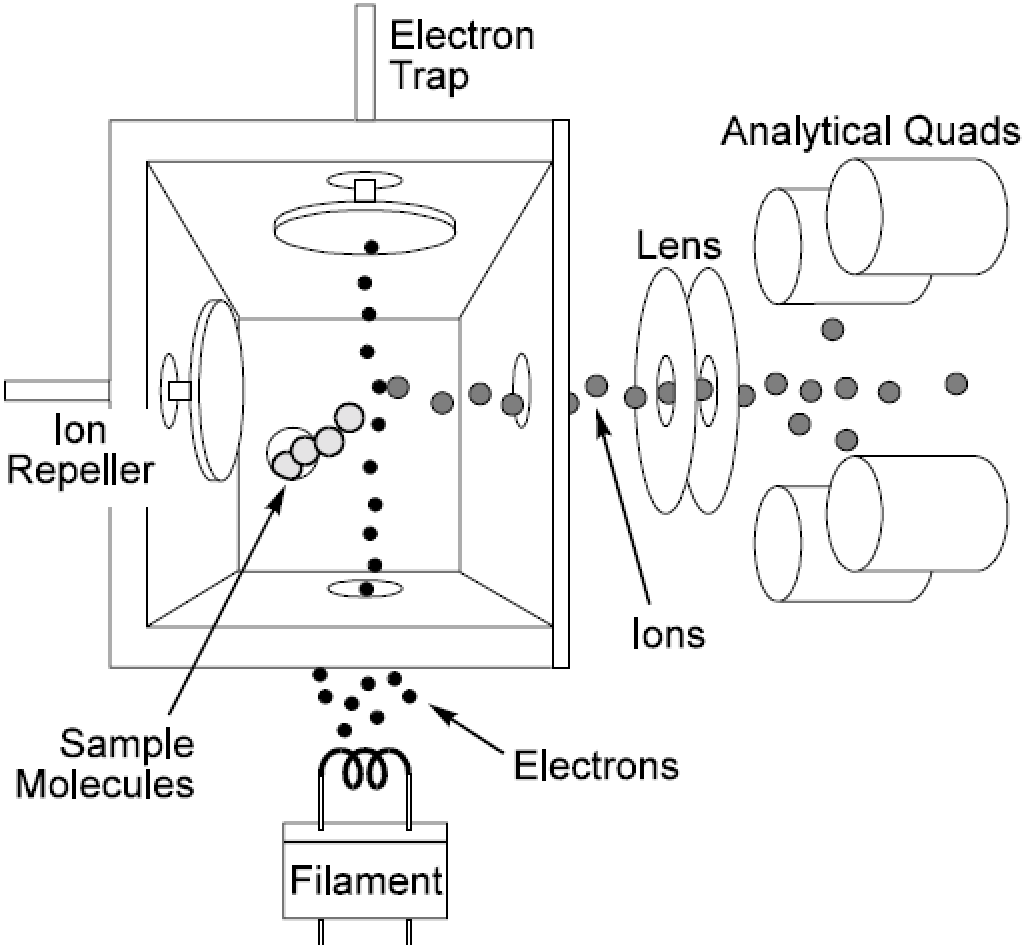


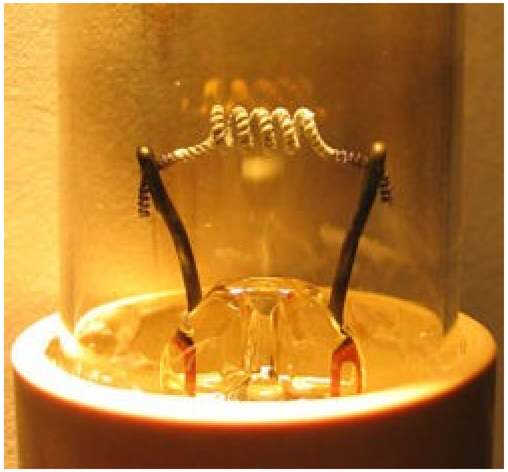


Tentative identification by comparison with MS libraries



Electron Impact (EI) Ionization





$$M + e^- \rightarrow M^{+\bullet} + 2e^-$$

GC – HPLC: Comparison

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