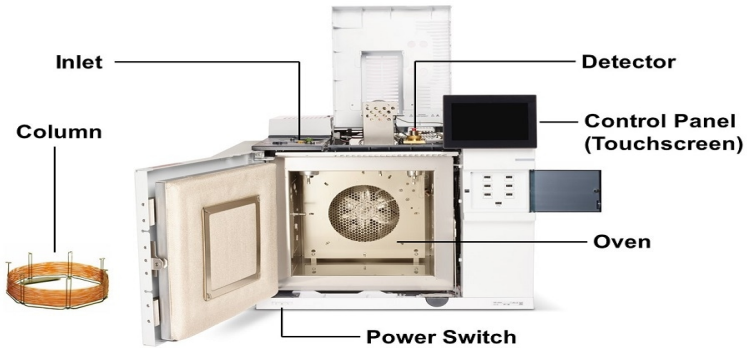
Gas Chromatography

Topics for today's lecture are:



1. Injectors and sampling methods.
2. Detectors.
3. Temperature programming.
4. Qualitative and quantitative analyses, incl. Kovats Index (retention index).
5. Derivatization and applications.
6. Some recent developments in GC.

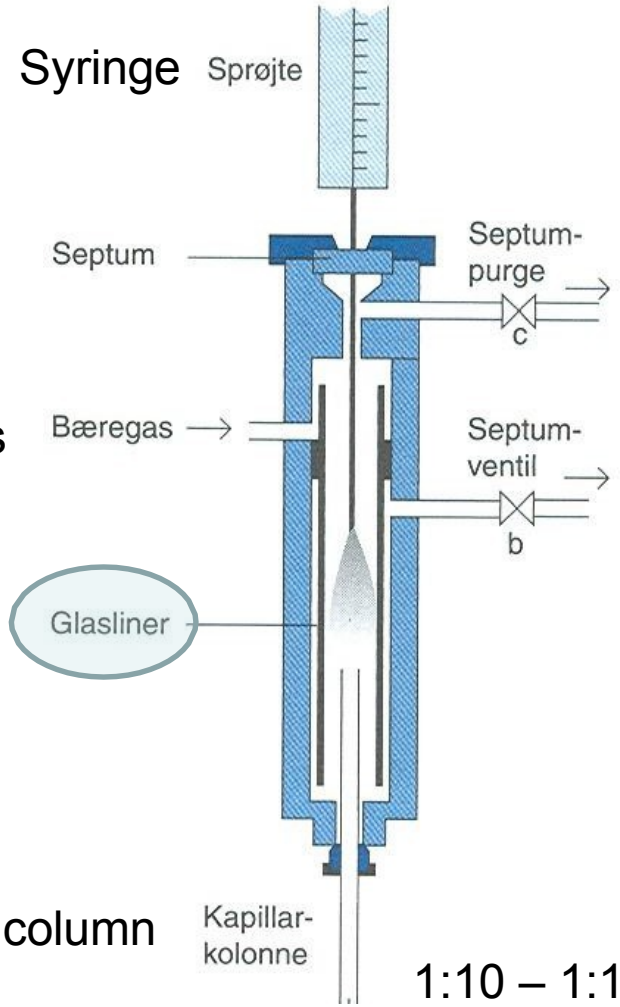
Literature: *Basic Gas Chromatography, Second Edition*, by Harold M. McNair and James M. Miller Copyright © 2009 John Wiley & Sons, Inc., Chapter 4, p. 59–61, Chapter 6, 97–103, Chapter 7, p. 104–128, Chapter 8, p. 138–144, Chapter 9, p. 145–155, Chapter 12, p. 188–195 and Chapter 13, p. 202–205.

Injector

- The capillary column contains very little amount of stationary phase
  - For example, a 25 m column has approx. 10 mg phase
- Inject very small volumes (typically 0,1-1µL)
- **Split/Splitless injector** (most common)
- **Other injectors**
  - For example, it is possible to inject large volumes ('large volume injection or LVI') by using a Programmed Temperature Vaporization (PTV) injector.

Split/Splitless injector (SSL)

Syringe



T° = 200-250

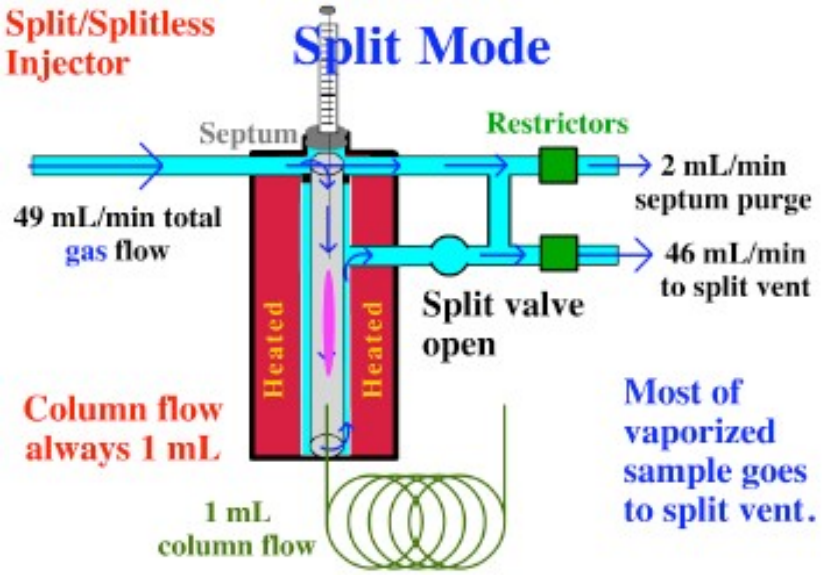
Carrier gas

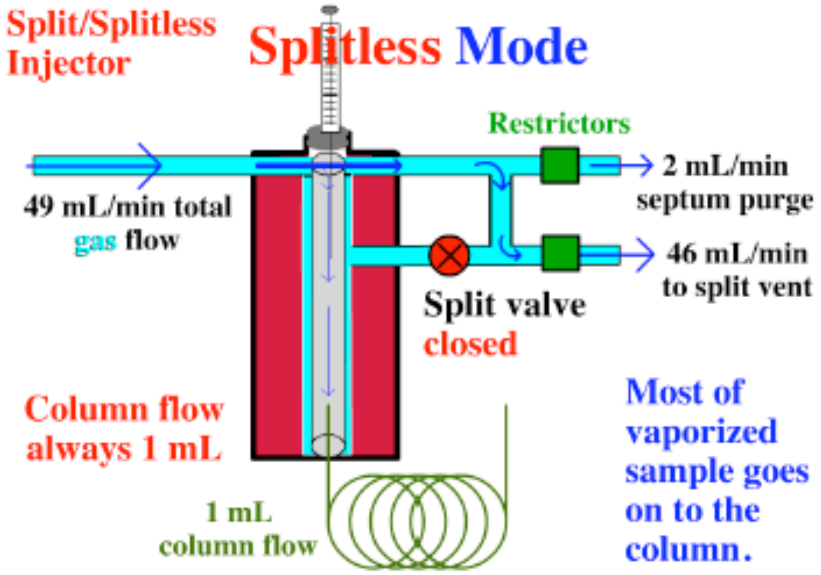
Septum valve

Capillary column

1:10 – 1:1000

Split versus Splitless injector mode

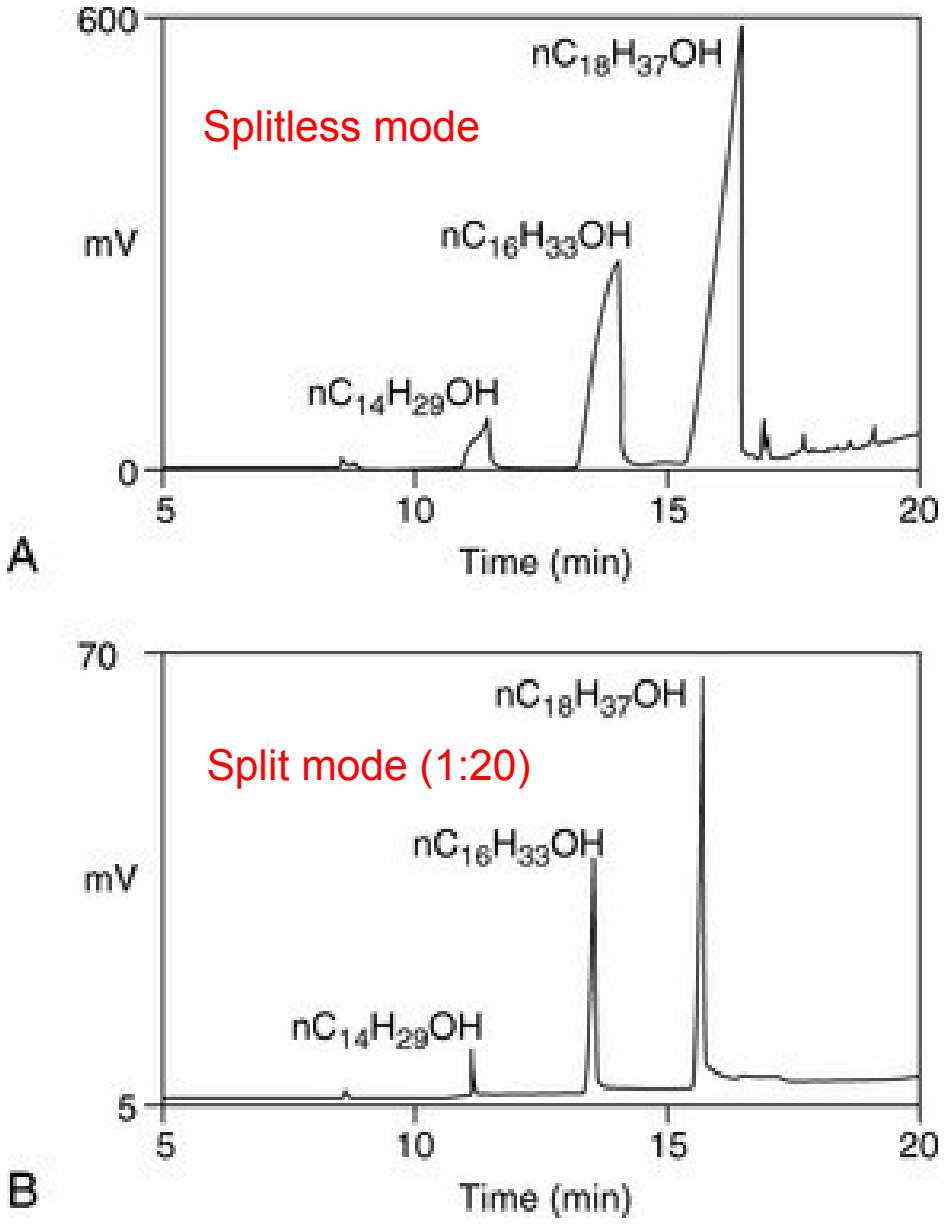




The gas flow passes through the septum purge and the split vent. **This configuration is called the split mode because some of the gas in the injector exits though the split vent.** Some of the sample injected into the injector by the sample syringe will get vaporized and escape through the split vent. **In the above example, the split ratio is 1/49 because 49 parts of the sample is injected, and 1 part goes on column**.

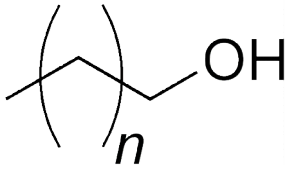
In the splitless mode all the analyte sample vaporized in the injector goes onto the column because the split valve is closed (not split). Splitless mode increases the sensitivity and is commonly used for trace analysis, i.e., trace amounts of analytes.

Analysis in split and splitless mode



Chromatograms of the pharmaceutical excipient cetostearyl alcohol (4 mg/ml) injected in a GC splitless mode (**A**) and split mode (**B**). In the splitless mode, all the sample is introduced onto the column and the injector purge valve remains closed for 0.5–1 min after injection. In splitless mode the peak ***shapes are poor due to overloading*** of the column with too much sample.

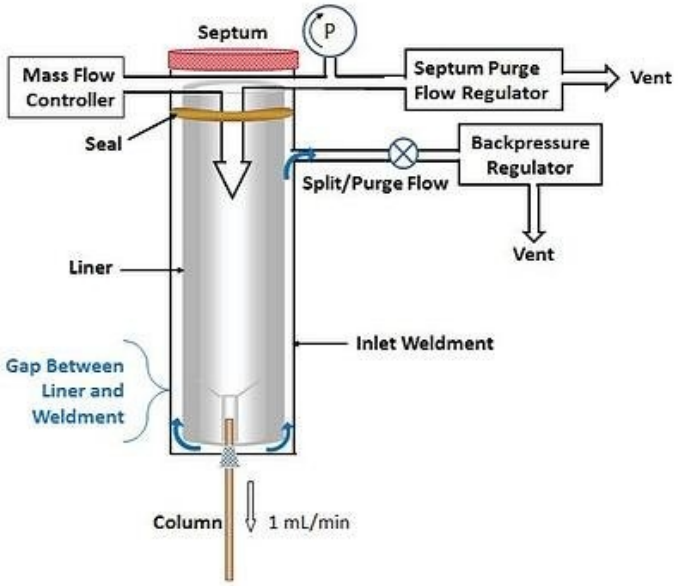
GC-conditions: Flow through column 1 ml/mi, flow out of split vent 20 ml/min. Column Rtx1 15 m x 0.32 mm i.d. x 0.5 µm film, programmed 100 °C (1 min) then 10 °C/min to 290 °C.



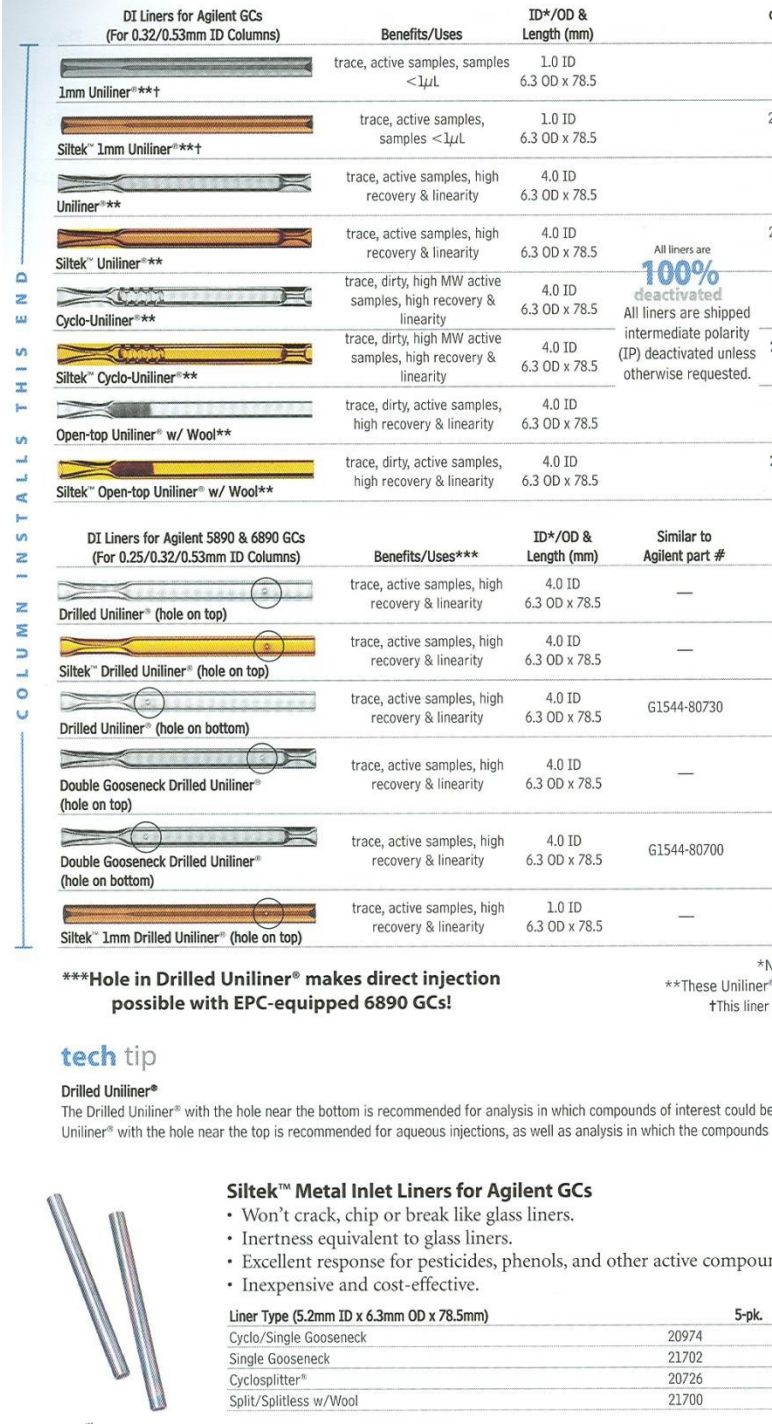
n = 12, 14, 16

Cetostearyl alcohol
(mixture of fatty acid alcohols)

Liner



- The inlet liner prevents catalytic reactions between the sample and the surface of the injector.
- Different types.
- How to choose the right inlet liner?
  - Many inlet liners are available for use in GC, (differ in geometric configuration/design, volume, base material (borosilicate, quartz, or metal) etc.
  - Different styles of liners are used for different types of samples (liquid or gaseous) and injections (split, splitless, on-column, or direct). The choice of GC inlet liner can be greatly simplified by basing the decision on the type of injection that will be used.



COLUMN INSTALLS THIS END

DI Liners for Agilent GCs (For 0.32/0.53mm ID Columns)	Benefits/Uses	ID*/OD & Length (mm)
1mm Uniliner®**†	trace, active samples, samples <1µL	1.0 ID 6.3 OD x 78.5
Siltek™ 1mm Uniliner®**†	trace, active samples, samples <1µL	1.0 ID 6.3 OD x 78.5
Uniliner®**	trace, active samples, high recovery & linearity	4.0 ID 6.3 OD x 78.5
Siltek™ Uniliner®**	trace, active samples, high recovery & linearity	4.0 ID 6.3 OD x 78.5
Cyclo-Uniliner®**	trace, dirty, high MW active samples, high recovery & linearity	4.0 ID 6.3 OD x 78.5
Siltek™ Cyclo-Uniliner®**	trace, dirty, high MW active samples, high recovery & linearity	4.0 ID 6.3 OD x 78.5
Open-top Uniliner® w/ Wool**	trace, dirty, active samples, high recovery & linearity	4.0 ID 6.3 OD x 78.5
Siltek™ Open-top Uniliner® w/ Wool**	trace, dirty, active samples, high recovery & linearity	4.0 ID 6.3 OD x 78.5

All liners are 100% deactivated. All liners are shipped intermediate polarity (IP) deactivated unless otherwise requested.

DI Liners for Agilent 5890 & 6890 GCs (For 0.25/0.32/0.53mm ID Columns)	Benefits/Uses***	ID*/OD & Length (mm)	Similar to Agilent part #
Drilled Uniliner® (hole on top)	trace, active samples, high recovery & linearity	4.0 ID 6.3 OD x 78.5	—
Siltek™ Drilled Uniliner® (hole on top)	trace, active samples, high recovery & linearity	4.0 ID 6.3 OD x 78.5	—
Drilled Uniliner® (hole on bottom)	trace, active samples, high recovery & linearity	4.0 ID 6.3 OD x 78.5	G1544-80730
Double Gooseneck Drilled Uniliner® (hole on top)	trace, active samples, high recovery & linearity	4.0 ID 6.3 OD x 78.5	—
Double Gooseneck Drilled Uniliner® (hole on bottom)	trace, active samples, high recovery & linearity	4.0 ID 6.3 OD x 78.5	G1544-80700
Siltek™ 1mm Drilled Uniliner® (hole on top)	trace, active samples, high recovery & linearity	1.0 ID 6.3 OD x 78.5	—

***Hole in Drilled Uniliner® makes direct injection possible with EPC-equipped 6890 GCs!

*N
**These Uniliner®
†This liner

tech tip

Drilled Uniliner®

The Drilled Uniliner® with the hole near the bottom is recommended for analysis in which compounds of interest could be
Uniliner® with the hole near the top is recommended for aqueous injections, as well as analysis in which the compounds

Siltek™ Metal Inlet Liners for Agilent GCs

- Won't crack, chip or break like glass liners.
- Inertness equivalent to glass liners.
- Excellent response for pesticides, phenols, and other active compoun
- Inexpensive and cost-effective.

Liner Type (5.2mm ID x 6.3mm OD x 78.5mm)	5-pk.
Cyclo/Single Gooseneck	20974
Single Gooseneck	21702
Cyclosplitter®	20726
Split/Splitless w/Wool	21700

Other injection methods including specific sampling methods

Other Injection methods

- PTV (Programmed Temperature Vaporisation) injection.
- On-column.

Sampling methods/techniques

- Sample solubilized in solvent incl. solvent extraction, extraction of essential oils, elution of headspace samples.
- Samples collected by a fibre (SPME: Solid Phase Micro Extraction) - ***static headspace***
- Samples collected by ***dynamic headspace*** and ***purge & trap***.

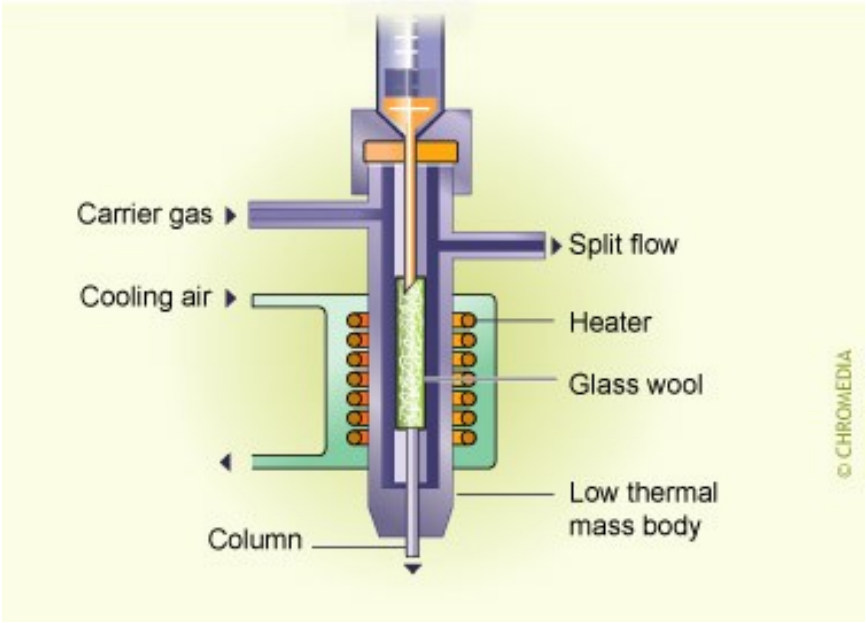
Programmed Temperature Vaporization (PTV) injection

- A PTV, is a complete injector, which can be rapidly heated and cooled allowing the sample to be introduced in liquid form at cool temperatures and then to be vaporized rapidly.

- Numerous PVT injectors commercially available can operate in split and splitless modes, and in some cases, on-column injection, depending on the type of liner chosen.

- Injector designs for the PTV are usually similar to non-programmable versions **except for the inclusion of rapid heating and cooling facilities**. These facilities may include the ability to use different programming ramps and cryogenic cooling. PTV injectors are often operated with packed liners depending on the type of application.

PTV injector



Advantages and disadvantages of PTV injectors

General advantages of PTV are:

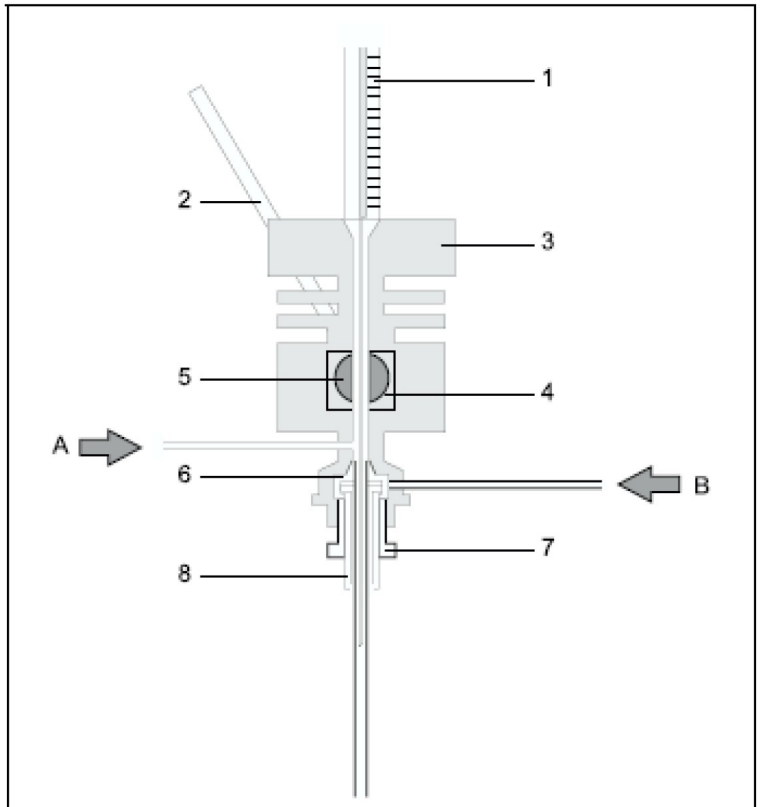
- Reduced needle – and injector discrimination (cold needle injection).
- Large volume injection (LVI) and on-column injection possible.
- Removal of the sample solvent prior to injection.
- Holding back of non-volatile sample components in the liner.
- Good accuracy and reproducibility.
- Cold trapping of samples in the injector and the top of the (pre) column.

A **disadvantage** with PTV injector is that it requires special hardware and a control unit, which makes the system relatively expensive.

On-column injection

- In on-column injection, a liquid sample is introduced directly into the column with a thin injection needle.
- During the temperature program, the vapour pressure of the solutes increases, and the chromatographic process begins. With this injection technique no evaporation in a heated space takes place.
- By using an ***initial temperature below the boiling point of the solvent***, ***selective evaporation and, hence, discrimination is precluded***. This makes on-column injection the ***method of choice for all samples containing high-boiling components*** that would not be quantitatively transferred to the column in split- and splitless injection.

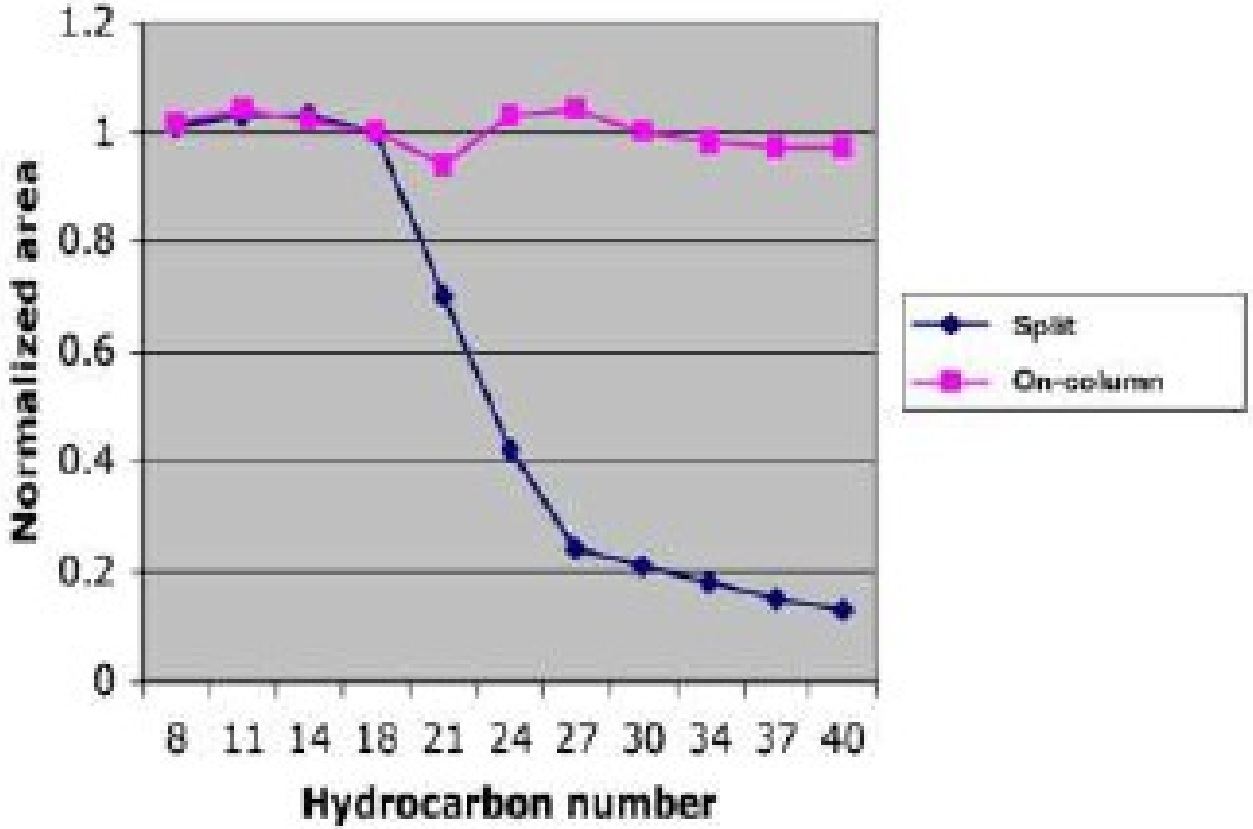
On-column injector
(schematic view)



1. Syringe	6. Seal
2. Valve Lever	7. M8x1 Fixing Nut
3. Upper Block	8. Cooling Sleeve
4. PTFE Valve Seal	A. Carrier Gas Inlet
5. Stainless Steel Rotary Valve	B. Secondary Cooling Inlet

On-column versus split injection

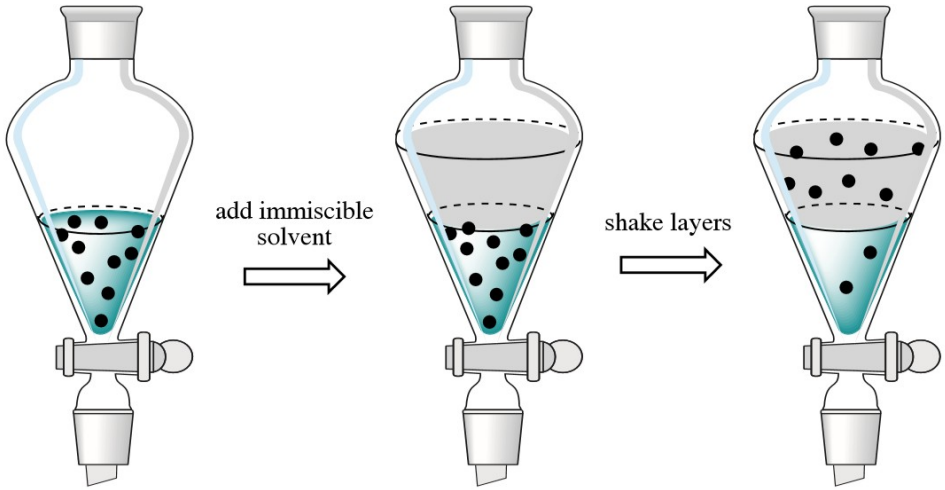
Analysis of an n-alkane standard with on-column and split injection.



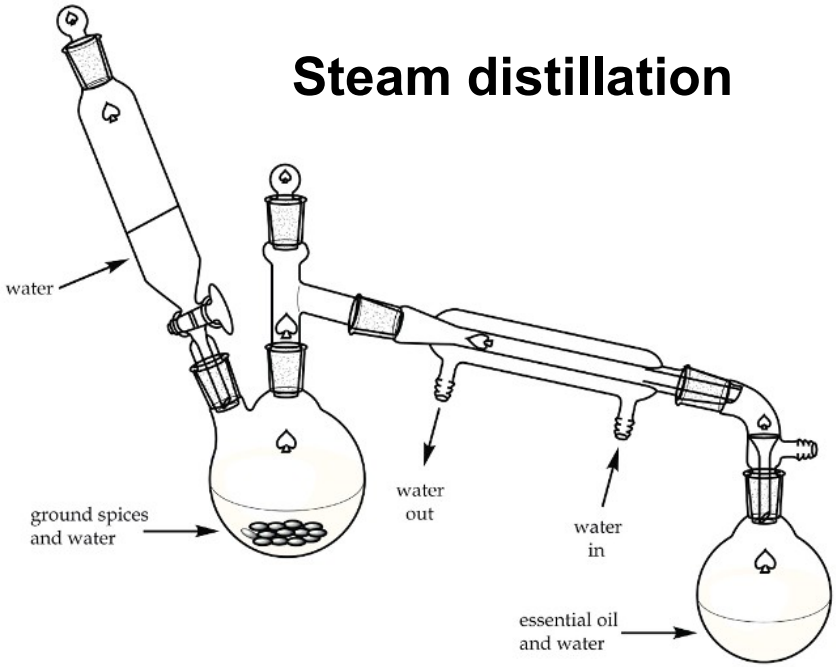
In on-column injection no intermediate evaporation step is used. Consequently, the composition of the sample that is introduced on to the column must be exactly equal to the original composition as also shown in the figure above. .

Sample solubilized in solvent: *extraction of volatiles*

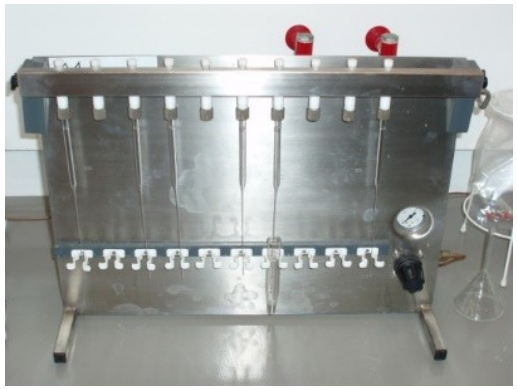
Liquid-liquid extraction



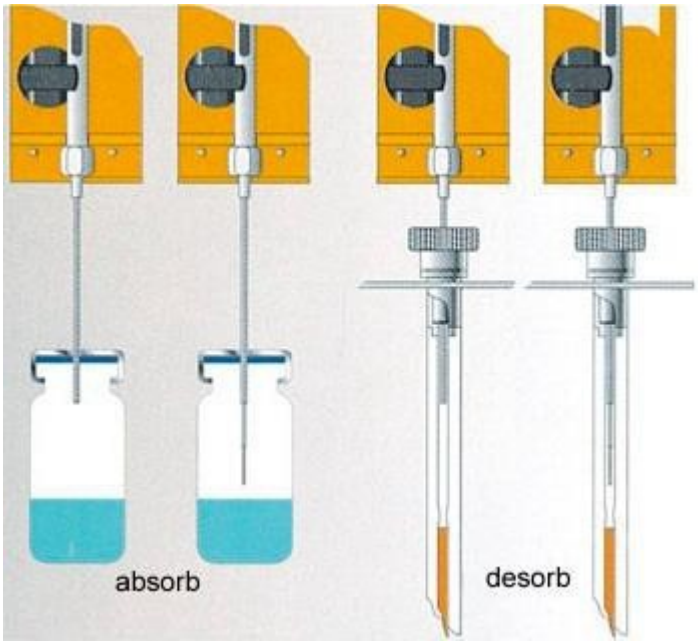
Steam distillation



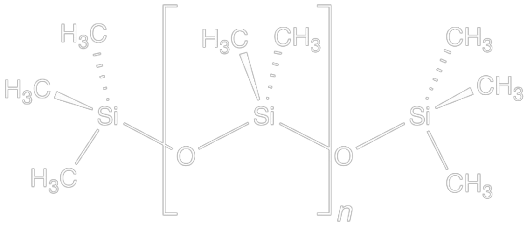
Elution of dynamic headspace samples

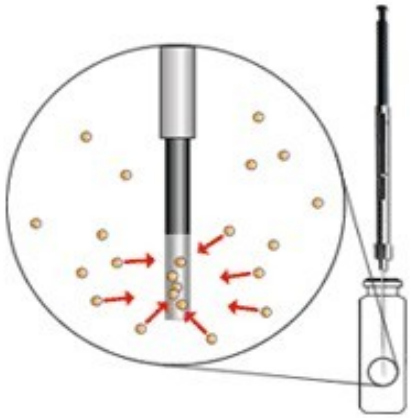


SPME

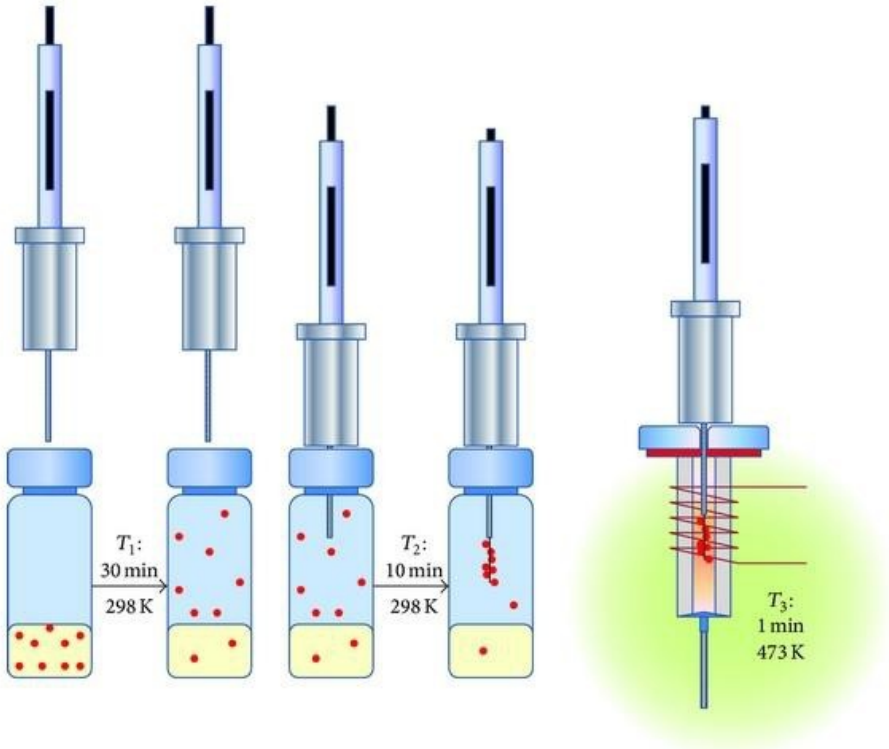


Polydimethylsiloxane (PDMS)

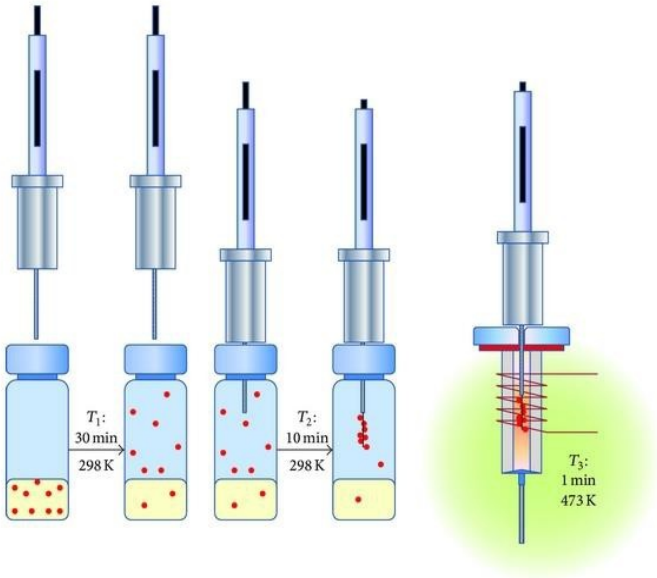


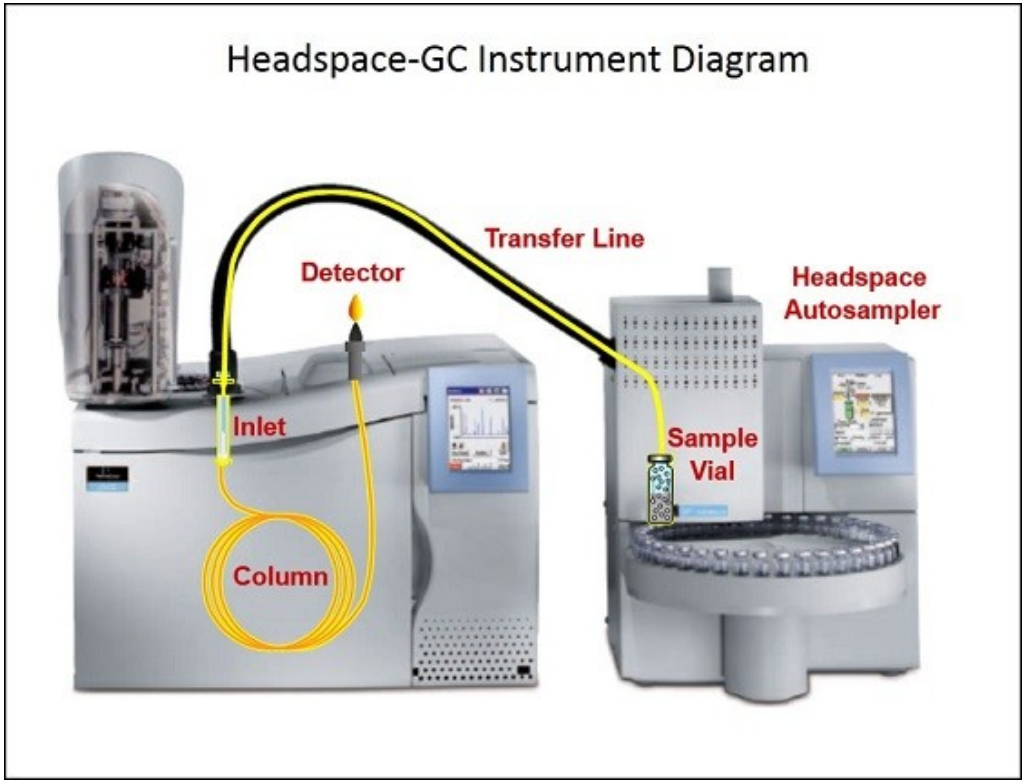


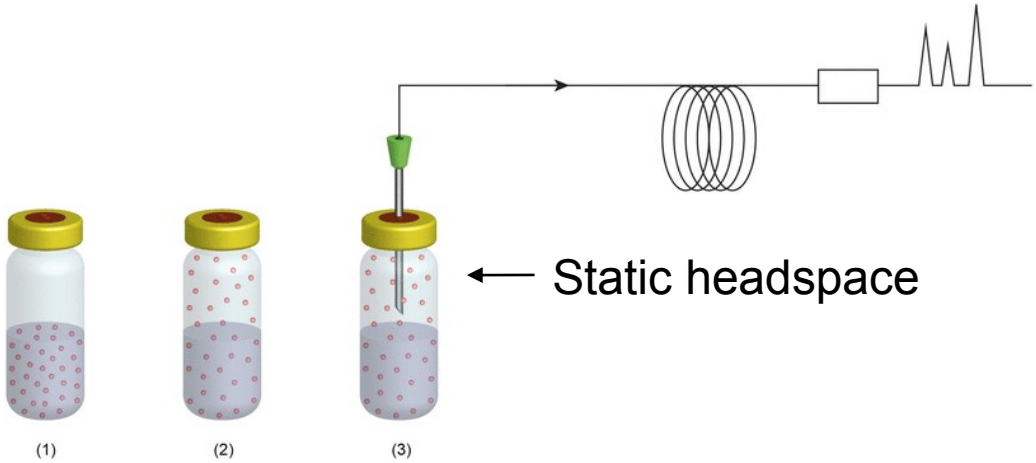
SPME, is a sampling technique that involves the use of a fiber coated with an extracting phase (liquid polymer or a solid sorbent), which extracts different kinds of analytes (volatile and non-volatile) from a liquid or gas phase. The quantity of analyte extracted by the fibre is proportional to its concentration in the sample at equilibriium or in case of short time pre-equilibrium with help of for example agitation.



SPME is a static headspace technique







← Static headspace

SPME: Advantages and disadvantages

Advantages

- Fast (short analysis time)
- Solvent elimination.
- Simple analysis conducting and maintenance
- Low analysis cost
- Easiness of automation.
- On-site sampling and potential for field application.
- SPME samples can be stored without loss of volatiles.
- Excellent for target analytes.

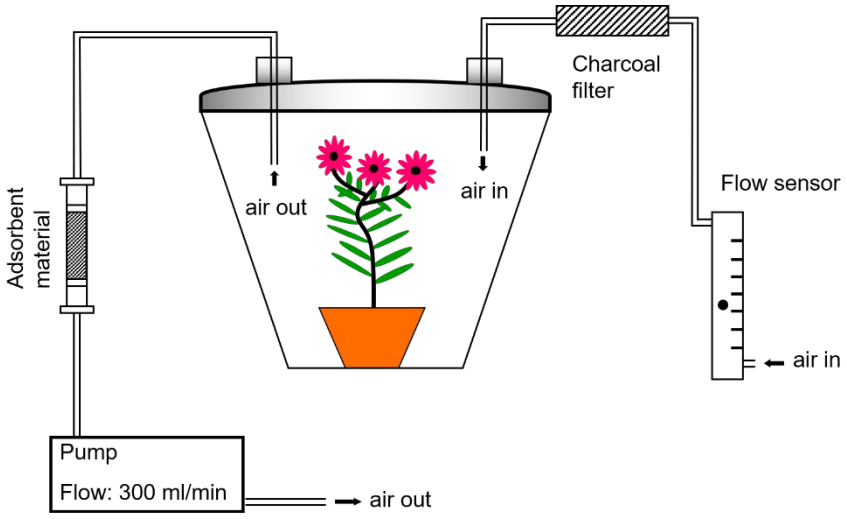
Disadvantages

- Limit number of commercially available stationary phases (fiber materials) only roughly covering the scale of polarity of target compounds and therefore not always suitable for extracting volatiles from complex extracts.
- Analytes may have different affinity to the fiber and therefore are in competition regarding the absorption to the fiber even at equilibrium, thus the composition of analytes on the fiber does not reflect the composition of the sample.
- Low effectiveness due to small amounts of extracting phases (e.g., PDMS).

Dynamic headspace and purge & trap

- **Dynamic headspace** sampling involves purging the headspace with a large known volume of inert gas (typically N_2), which ultimately removes most of the volatile compounds. Exceptions would be those compounds showing a strong affinity for the sample matrix, such as polar compounds in aqueous samples. The volatiles are retained in an **adsorbent trap** (collected) consisting of a polymer such as porapak Q or Tenax TA with high affinity to non-polar compounds.
- **Purge and trap** sampling is a type of dynamic headspace technique where the sample is purged with an inert gas (N_2 or He) and the volatiles are then trapped on the adsorbent trap.
- The volatiles are released from the adsorbent materials by elution with an organic solvent (porapak Q, Tenax TA) or thermal desorption (only Tenax TA).

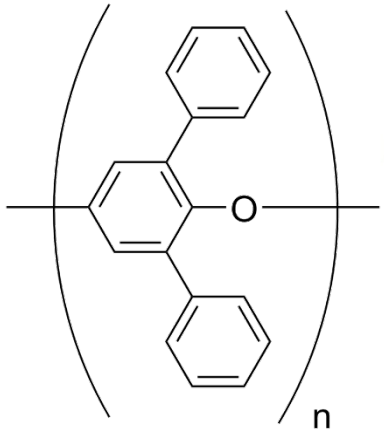
Dynamic headspace a versatile technique for trapping volatiles



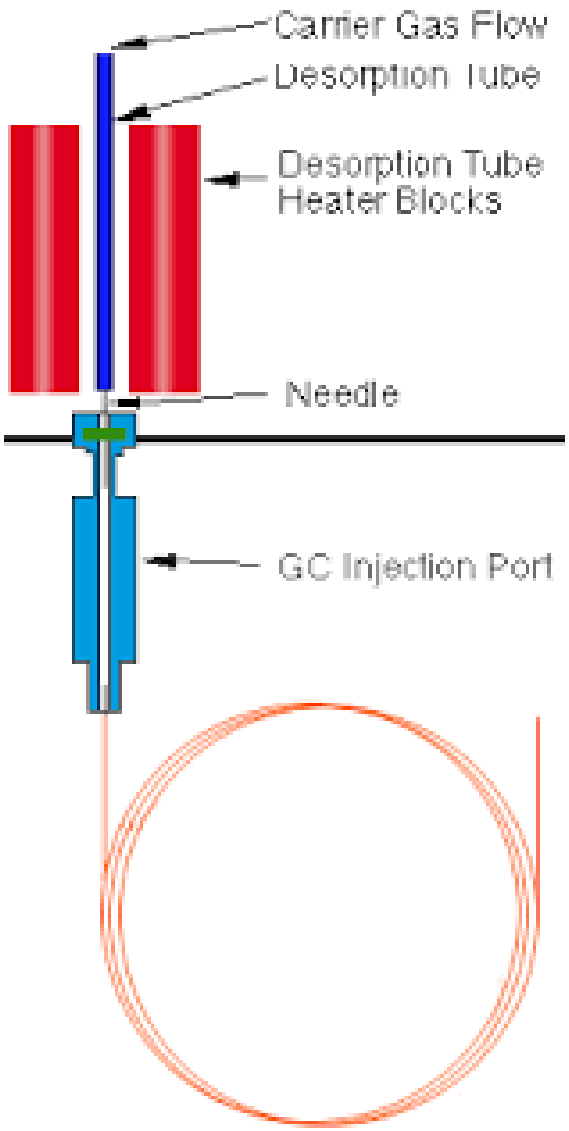
Adsorbents materials for dynamic headspace and purge & trap

Porapak Q is a porous ethylvinylbenzene and divinylbenzene copolymer consisting of spherical beads with consistent particle size, porosity and surface area. The copolymer is not heat resistant but is resistant to all organic solvents and efficienty trap **volatiles with different polarities**.

Tenax TA is a poly(2,6-diphenyl-p-phenylene oxide) porous polymer, which is stable to 375 °C and gives insignificant bleed of organics. It is suitable for the efficient trapping of low to medium polarity compounds and these can then be recovered either by solvent extraction (not resistant to chlorinated organic solvents) or thermal desorption.



Thermal desorption (principles)



Common types of GC detectors

but there are many more

Detector type	Detector principle	Selectivity	Detectability (LOD)
FID	Flame ionization	Most organic compounds	100 pg
TCD	Thermal conductivity	Universial	1 ng
ECD	Electron capture	Halides, nitrates, nitriles, organometallics	50 fg
NPD	Nitrogen-phosphorous or thermionic	N- and P- containing compounds	10 pg
FPD	Flame photometric	S- and P- containing compounds and others (e.g, B, Se, Cr)	100 pg
PID	Photoionization	Organic compounds incl. organometallics	2 pg
IRD (FT-IR)	Infrared	Organic compounds	In the ng range
MSD	Mass spectrometric	Organic compounds	in the ng range
GC-O	Olfactometry	Operators nose☺	Depends on nose and odor threshhold

Common commercially available detectors

TABLE 7.1 Common Commercially Available Detectors

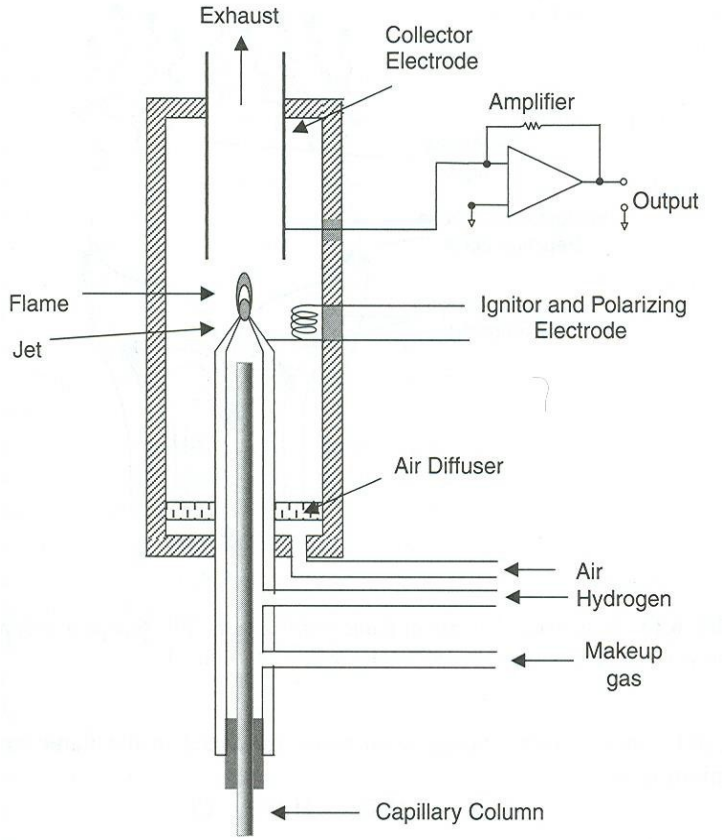
Name	Selective
1. Flame ionization detector (FID)	No
2. Thermal conductivity detector (TCD) (katharometer)	No
3. Electron capture detector (ECD)	X[a]
Other Ionization-Type Detectors	
4. Nitrogen/phosphorous detector (NPD); alkali flame ionization detector (AFID); thermionic ionization detector (TID)	N, P, X
5. Photoionization detector (PID); discharge ionization detector (DID)	Aromatics
6. Helium ionization detector (HID)	No
Emission-Type Detectors	
7. Flame photometric detector (FPD)	S, P
8. Plasma atomic emission (AED)	Metals, X, C, O
Electrochemical Detectors	
9. Hall electrolytic conductivity (HECD)	S, N, X
Other Types of Detector	
10. Chemiluminescent	S
11. Gas density detector (GADE)	No
12. Radioactivity detector	^{3}H, ^{14}C
13. Mass spectrometer (MS or MSD)	Yes
14. Fourier transform infrared (FTIR)	Yes

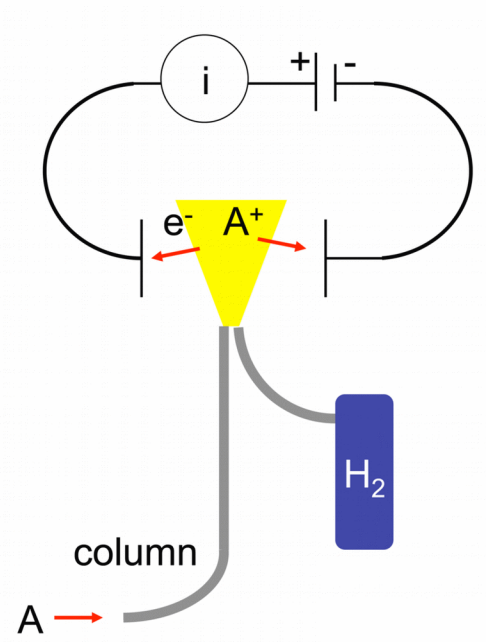
[a]X = halogen.

Flame ionization detector (FID)

- The operation of the FID is based on the detection of ions formed during combustion of organic compounds in a hydrogen flame. The generation of these ions is proportional to the concentration of organic species in the sample gas stream and thus the FID detector is able to detect all organic compounds.

- The FID signals is proportional to the number of carbon atoms in a hydrocarbon molecule; the presence of heteroatoms usually reduces the signal.

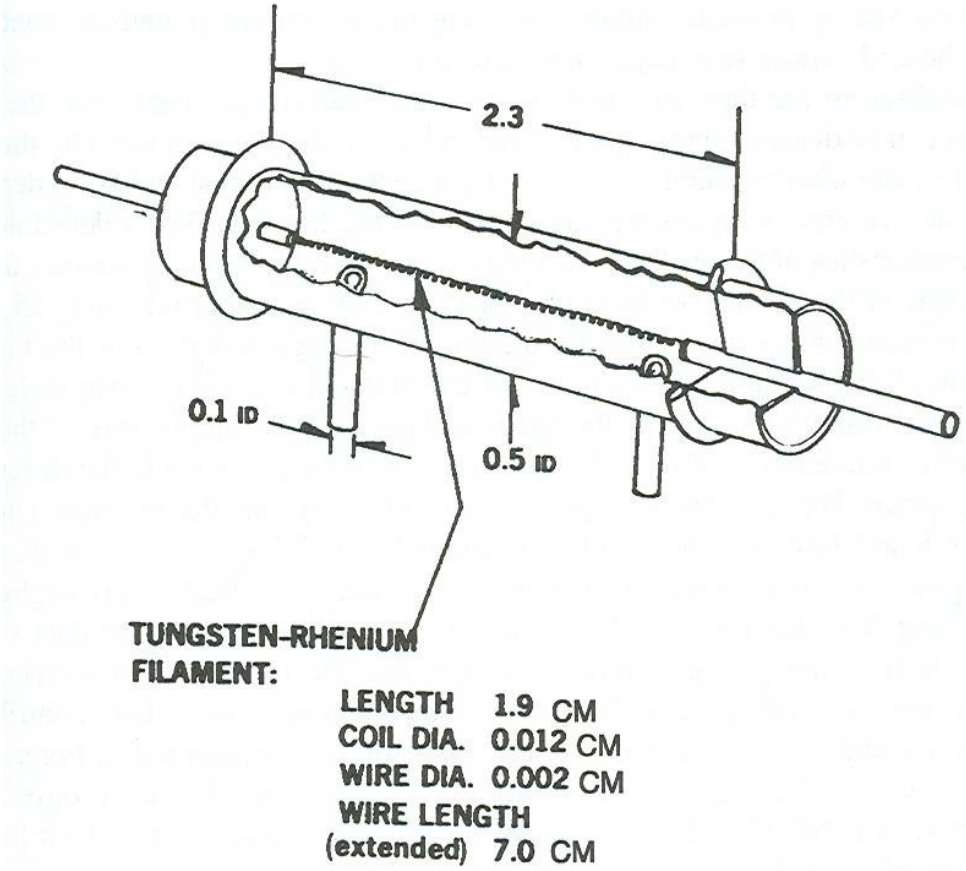




Thermal conductivity detector (TCD)

- The TCD consists of an **electrically heated filament in a temperature-controlled cell** (se figure below). When an analyte elutes and the thermal conductivity of the column effluent is reduced, the filament heats up and changes resistance often sensed by a ***Wheatstone bridge circuit***, which produces a measurable voltage change.

- Universal detector.



The filament's electrical resistance varies with the composition of the surrounding gas.

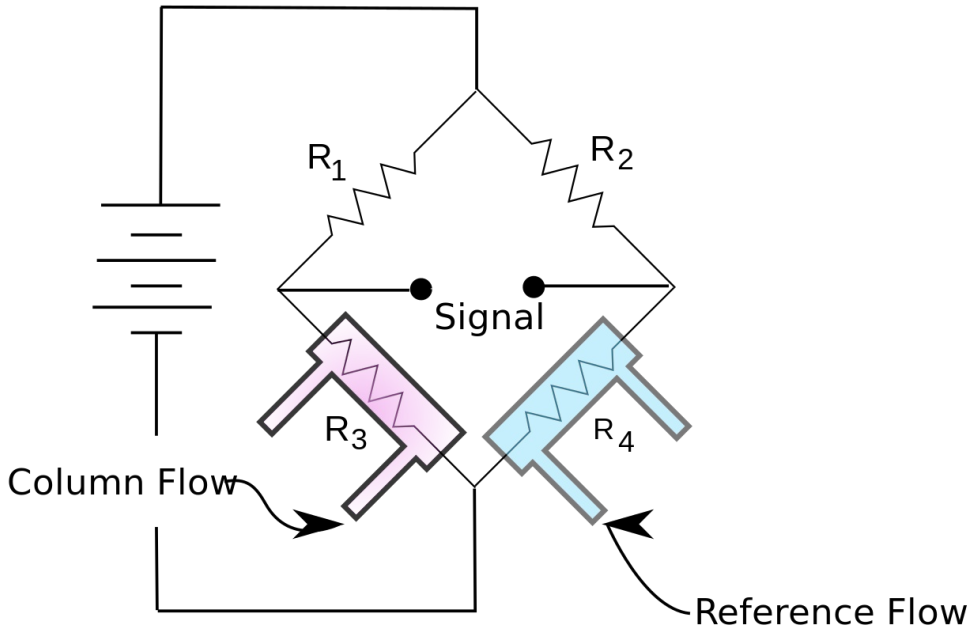
He must be used as the carrier gas because of its low heat conductivity.

The detector in TCD - *Wheatstone bridge circuit*

- TCD senses changes in the thermal conductivity of the column eluent and compares it to a reference flow of carrier gas. Most compounds have a thermal conductivity much less than that of the carrier gases He or H_2, so when an analyte elutes from the column the effluent thermal conductivity is reduced, and a detectable signal (resistance) is produced.

- The resistance is sensed by the *Wheatstone bridge ciurcuit.* The column effluent flows over one of the resistors while the reference flow is over a second resistor in the four-resistor circuit (se figure below).

Schematic view of thermal conductivity detector

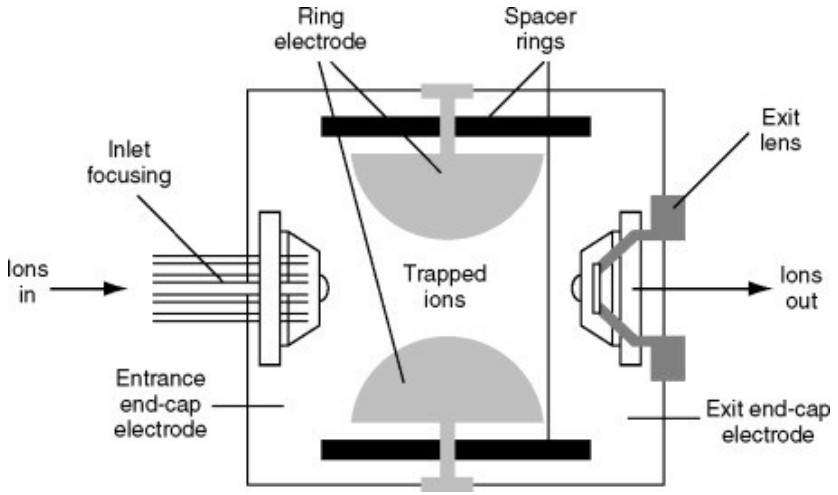


Gas chromatography–mass spectrometry (GC-MS)

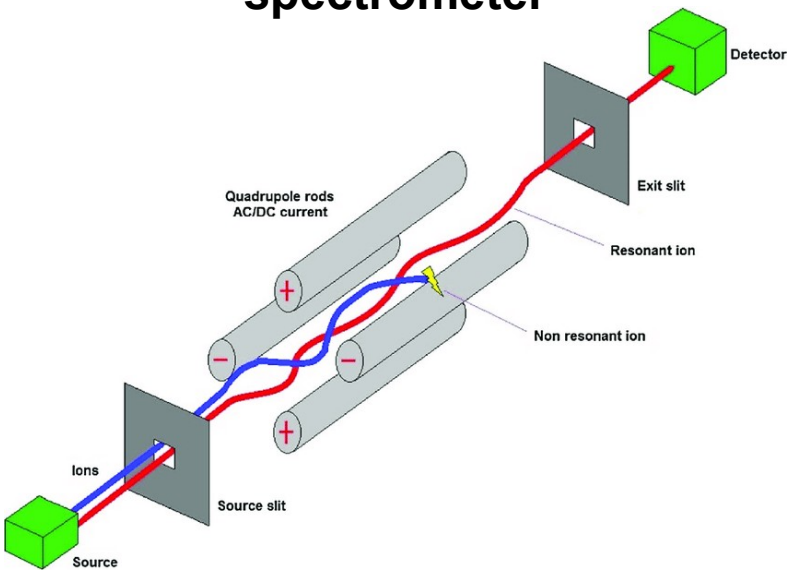
The most common MS mass filters (detectors) in connection with GC-MS today are:

- Quadrupole mass spectrometer
- Ion trap mass spectrometer

Detection of ions in an ion trap mass spectrometer



Detection of ions in a quadrupole mass spectrometer



Mass spectrometry and ionization methods

- In MS the mass-to-charge ratio of ions is measured generating a mass spectrum (a plot of intensity as a function of the mass-to-charge ratio).

- The ions generated depends on the ion source, which is a central part of the mass spectrometer. The ions are transported by magnetic or electric fields to the mass analyzer.

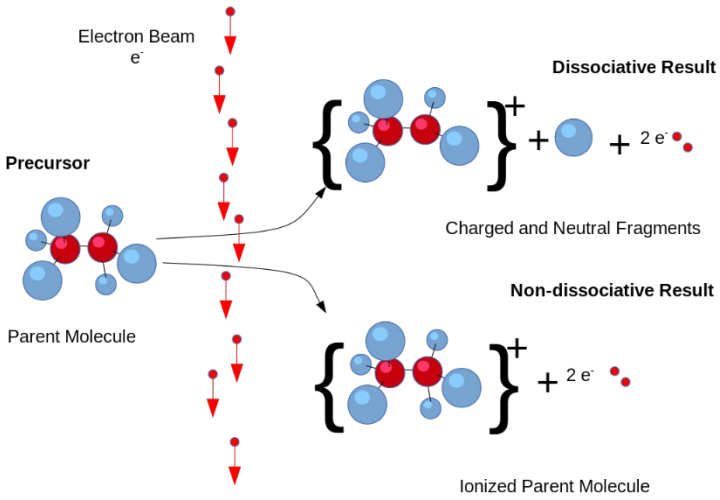
- Many ionization techniques exists and the most common used are:
  - **Electron ionization or electron impact ionization (EI)**
  - Chemical ionization (CI)
  - Electrospray ionization (ESI)
  - Atmospheric-pressure chemical ionization (APCI)

- In connection with GC, the ionization most used is the EI because it gives a high degree of fragmentation, yielding highly detailed mass spectra which can provide important information for structural elucidation/characterization and facilitate identification of unknown compounds by comparison to mass spectral libraries (e.g., The National Institute of Standards and Technology (NIST) database) obtained under identical operating conditions.

- EI is, however, not suitable for coupling to HPLC, i.e. LC-MS, since at atmospheric pressure, the filaments used to generate electrons burn out rapidly. Thus, EI is coupled predominantly with GC, i.e., GC-MS, where the entire system is under high vacuum.

GC-MS spectra

The **electrons** are **accelerated to 70 eV** in the region between the filament and the entrance to the ion source block



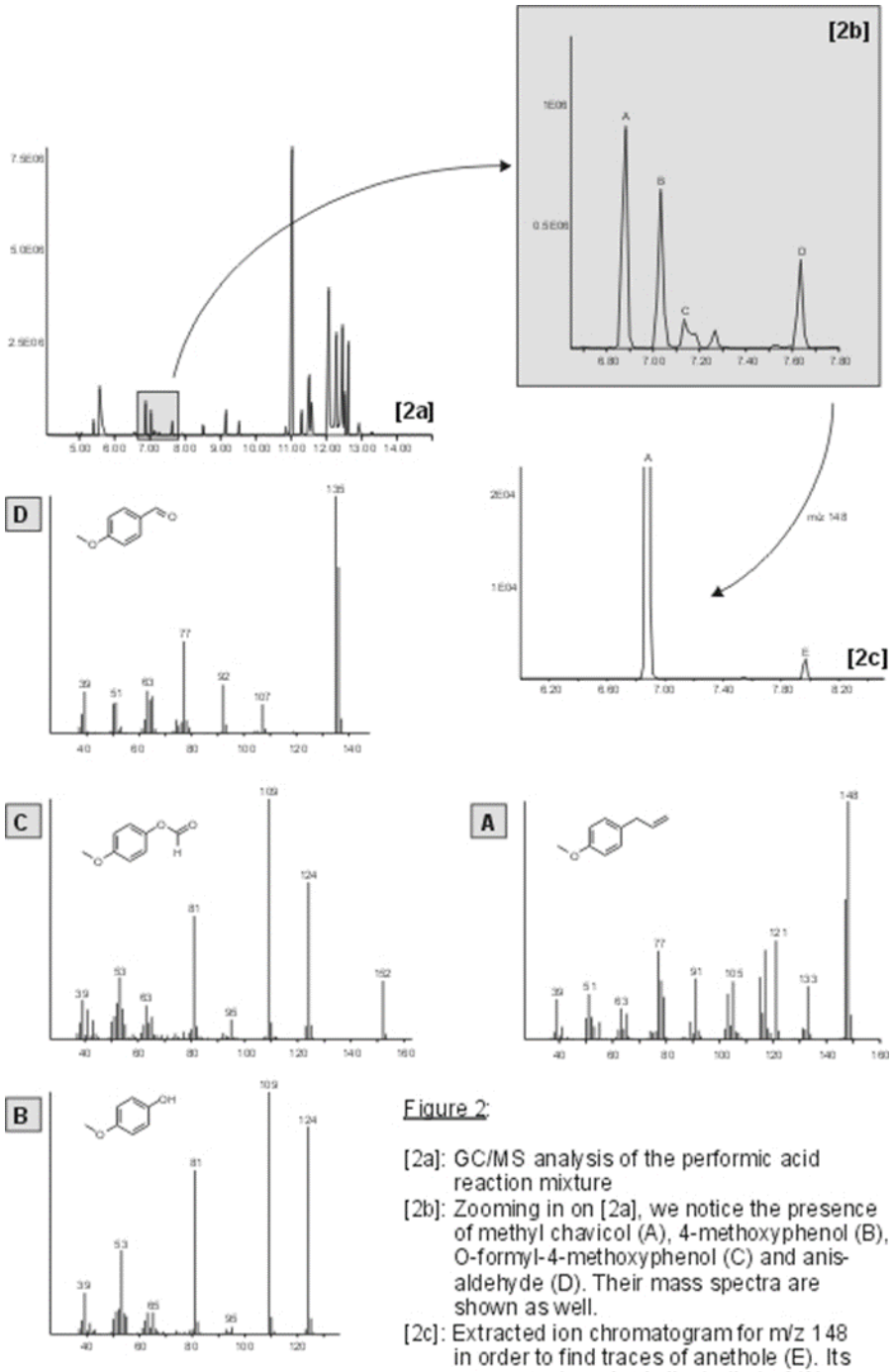


Figure 2:

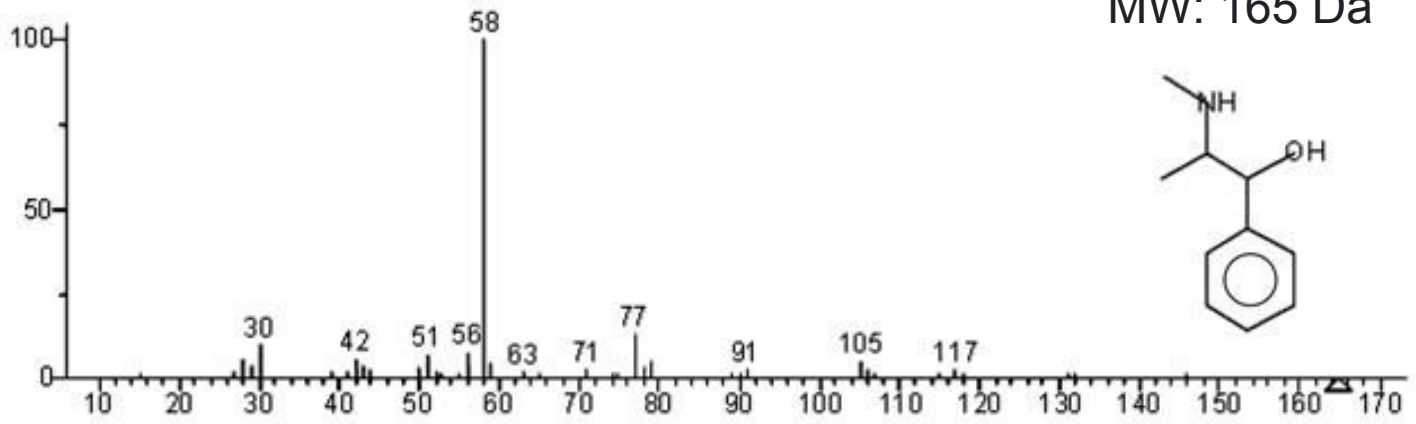
[2a]: GC/MS analysis of the performic acid reaction mixture

[2b]: Zooming in on [2a], we notice the presence of methyl chavicol (A), 4-methoxyphenol (B), O-formyl-4-methoxyphenol (C) and anis-aldehyde (D). Their mass spectra are shown as well.

[2c]: Extracted ion chromatogram for m/z 148 in order to find traces of anethole (E). Its mass spectrum is similar to that of (A).

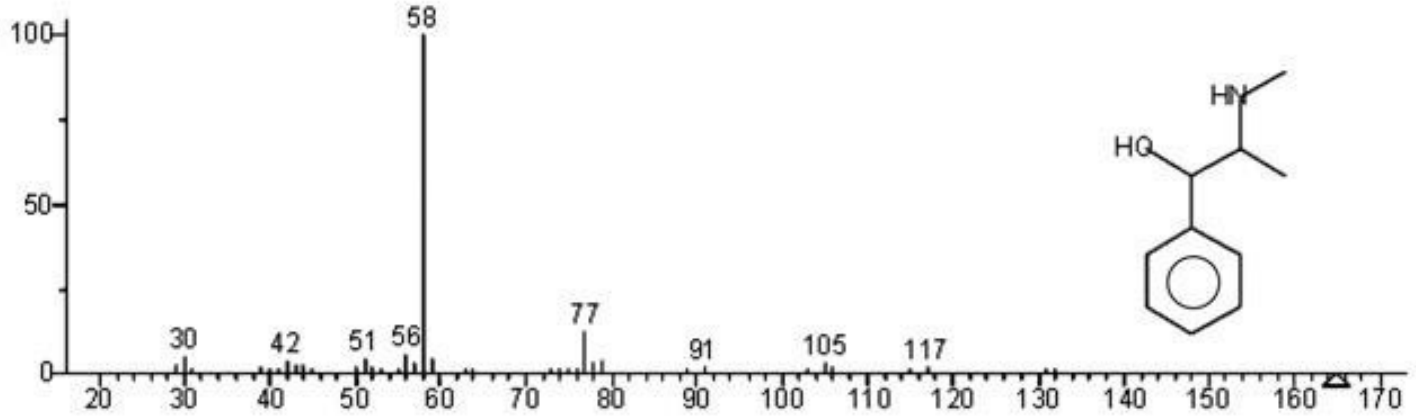
GC-MS spectra

MW: 165 Da



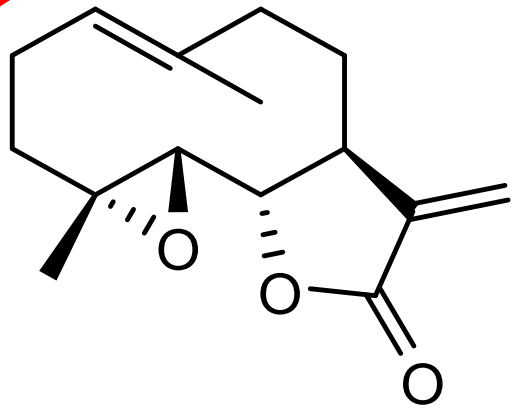
Ephedrine

MW: 165 Da



Pseudoephedrine

Collection of plant particles from feverfew ('matem' in Danish) by the use of an *high-volume air sampler*



Parthenolide

Contact allergen causing airborne contact dermatitis

MW: 248 Da

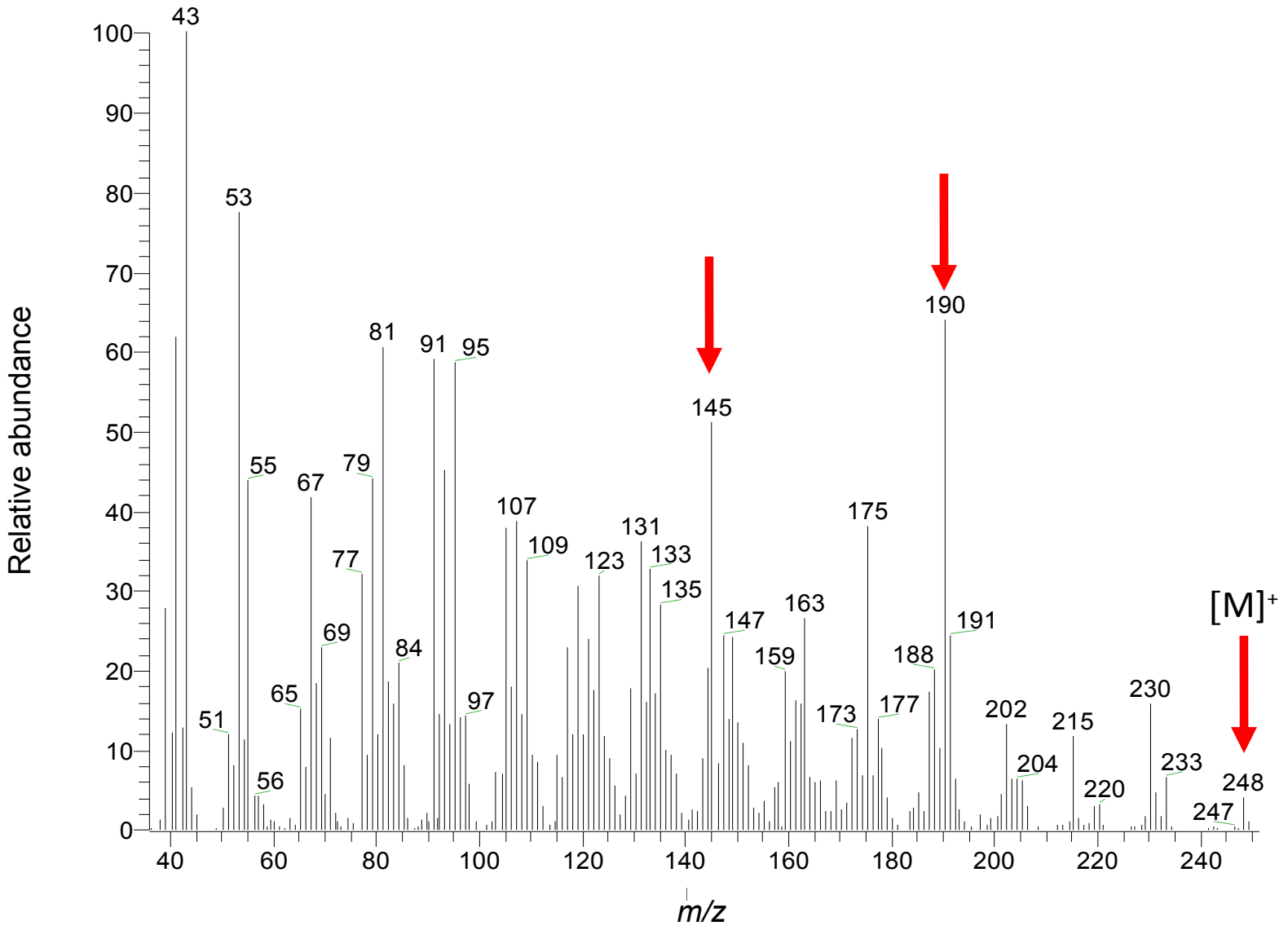
Melting point: 115-116 ºC (lit.)

Boiling point: 394.1 ± 42.0 ºC at 760 mm Hg (predicted)

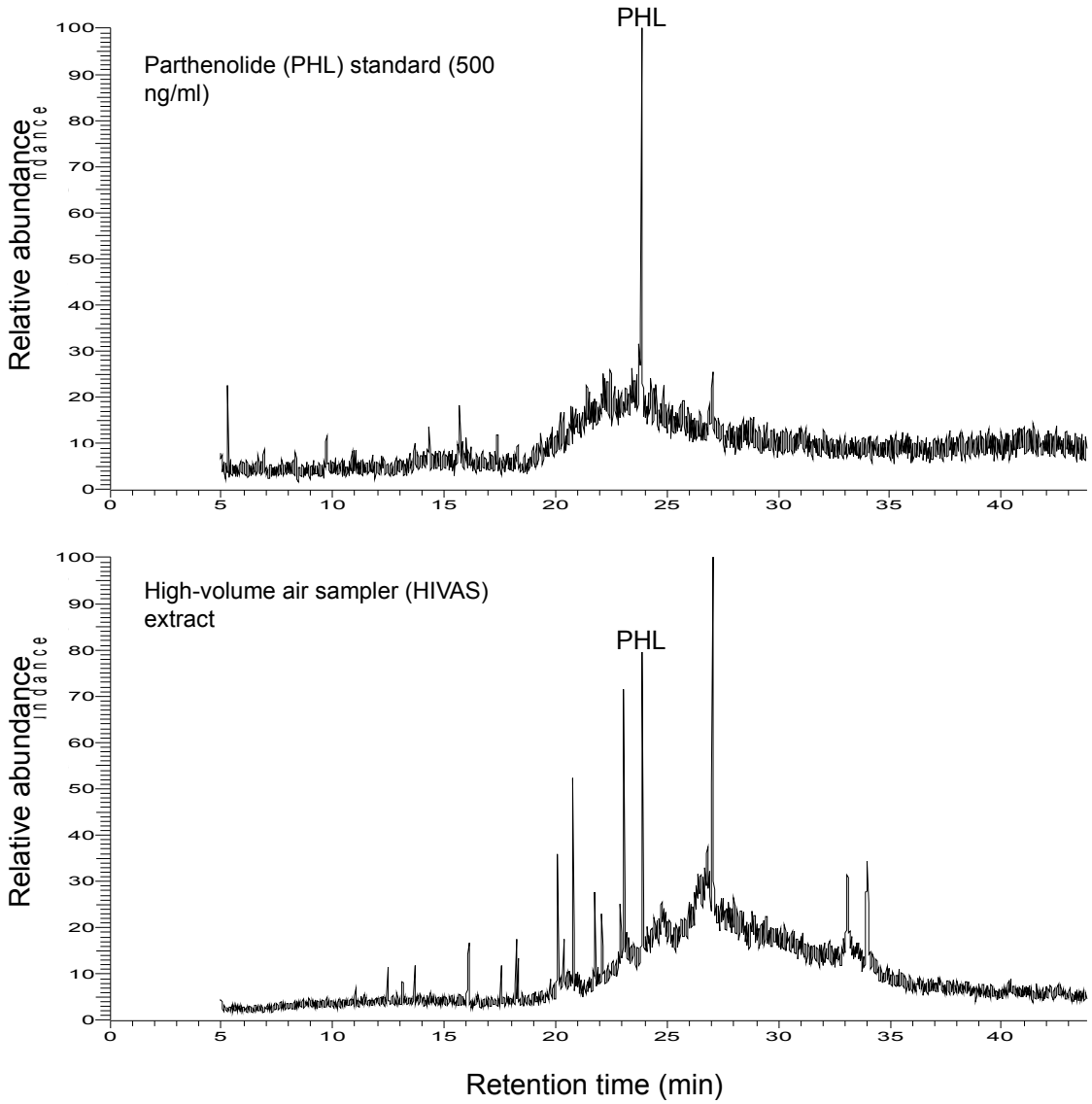

The
Science Source®
Learning Through Discovery

High-volume air sampler filter after collection of plant particles from feverfew and extracted with ethanol (HIVAS extract)

Mass spectrum (EI) of parthenolide



GC-MS extracted ion chromatogram of parthenolide (PHL) standard and a high-volume air sampler (HIVAS) filter extract



GC-MS selected ion monitoring (SIM) chromatogram of parthenolide (PHL) standard and HVIVAS extract, respectively. The selected ions in the GC-MS SIM acquisition method were *m/z* 145, 190 and 248 (molecular ion).

Experimental conditions for the analysis of parthenolide (PHL) in HIVAS extract by GC-MS

- GC-MS analysis was performed on a Trace DSQ **single quadrupole mass spec-trometer** operated at 70 eV and directly coupled to a Trace GC equipped with a split/splitless **PTV injector (split flow 10 mL/min, 200 ^{o}C)** and a **Zebron capillary column (30 m x 0.25 mm internal diameter, film thickness d_f = 0.25 µm, ZB-1, Phenomenex.).** Helium was the carrier gas at a constant column head pressure of 10 psi.

- The temperature of the column and transfer line was 200 ^{o}C. GC-MS was performed on 2 µL HIVAS extract. **Compounds in the HIVAS extract were separated by the following oven temperature gradient: 32 ^{o}C for 1 min, programmed to 260 ^{o}C at 10 ^{o}C/min followed by constant temperature for 20 min**. The mass spectrometer was operated in full scan mode over a mass range **from 39 to 650 amu and analysed for PHL**. PHL was detected in the HIVAS extracts by **selected ion monitoring (SIM) by setting the mass selective detector to repeatedly scan for the following ions *m/z* 145, 190 and 248 (molecular ion) = extracted ion chromatogram.**

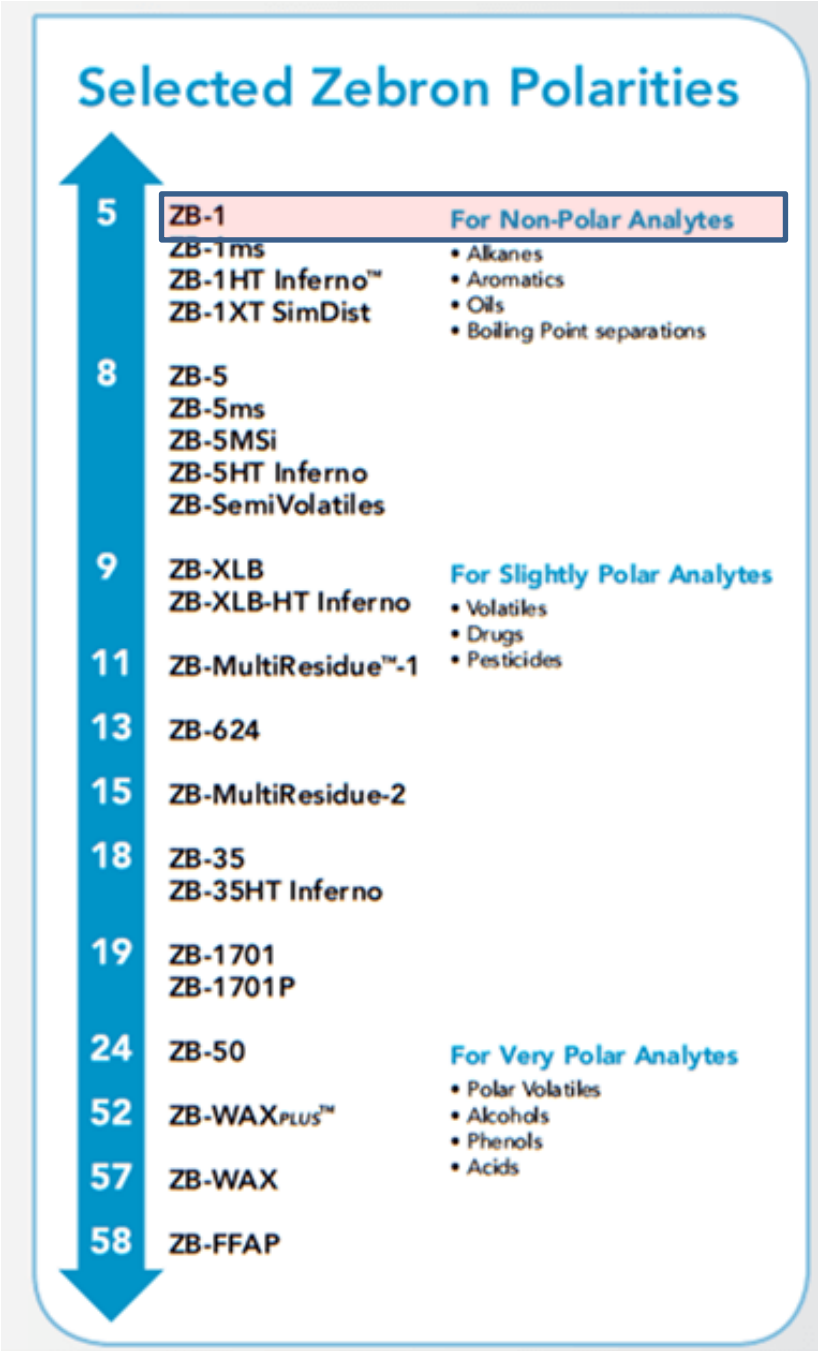
- Quantification of PHL in the HIVAS extracts was performed in SIM mode using PHL as external standard. The calibration curve was determined to be linear in the concen-tration range 50–1000 ng/mL (r^2 = 0.988). The detection limit of PHL was determined from the calibration curve to be 20 ng/mL (Signal to noise (S/N) = 10) in SIM scan mode and the quantification limit to be 50 ng/mL (S/N = 3).

Types of GC-columns from Zebron

Non-polar

Polar

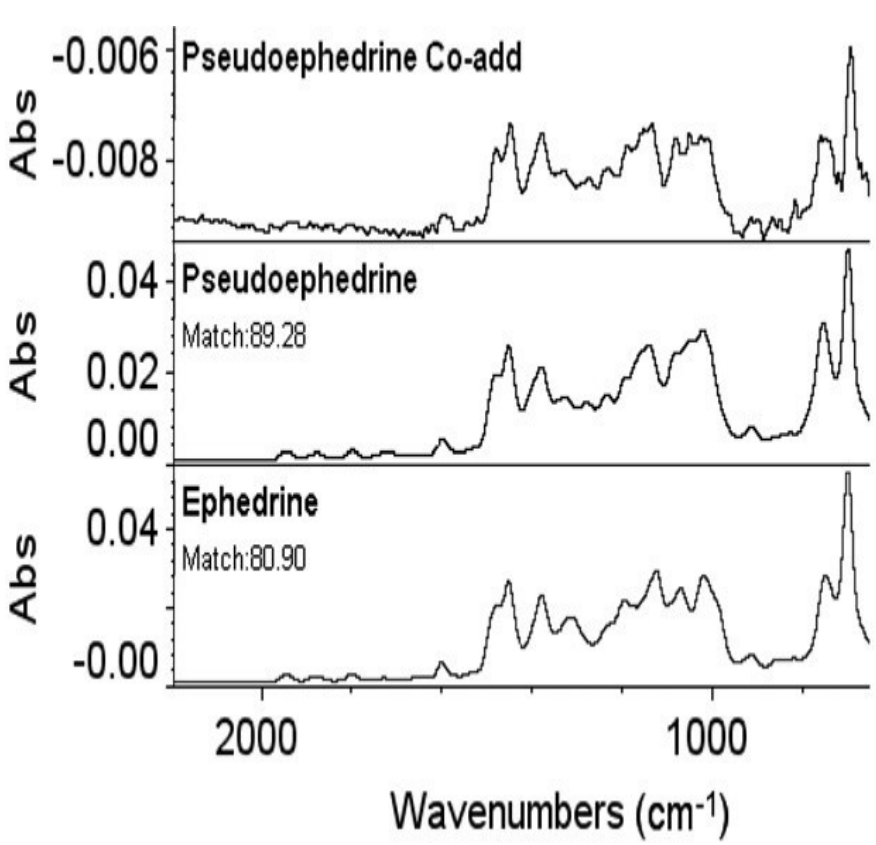
Why use a non-polar capillary column for the GC analysis of parthenolide when parthenolide is polar?

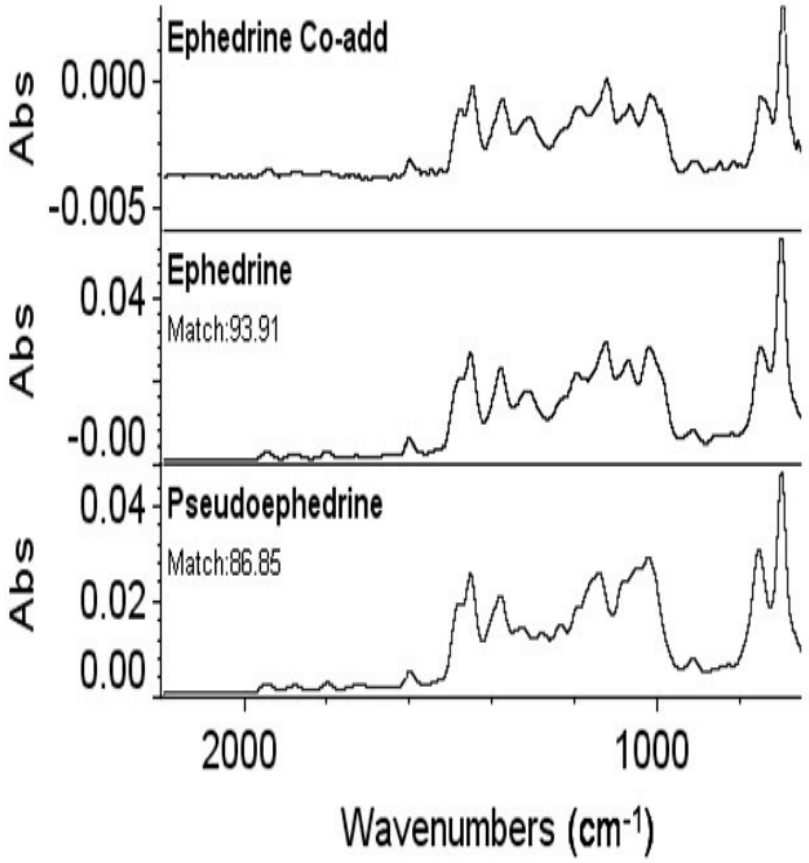


GC-Fourier-Transform Infrared spectroscopy (FTIR)

GC-FTIR allows to quick identifying functional groups in unknown substances, based on the retention behavior of the analytes and the IR absorption bands. IR complement the information afforded by MS, in achieving structural identification of volatile and semi-volatile molecules. Moreover, by measuring small energy **differences based on rotational and vibrational amplitudes between individual molecular bonds**, FTIR spectroscopy enables to overcome one limitation of MS detection, **in discriminating isomeric compounds**

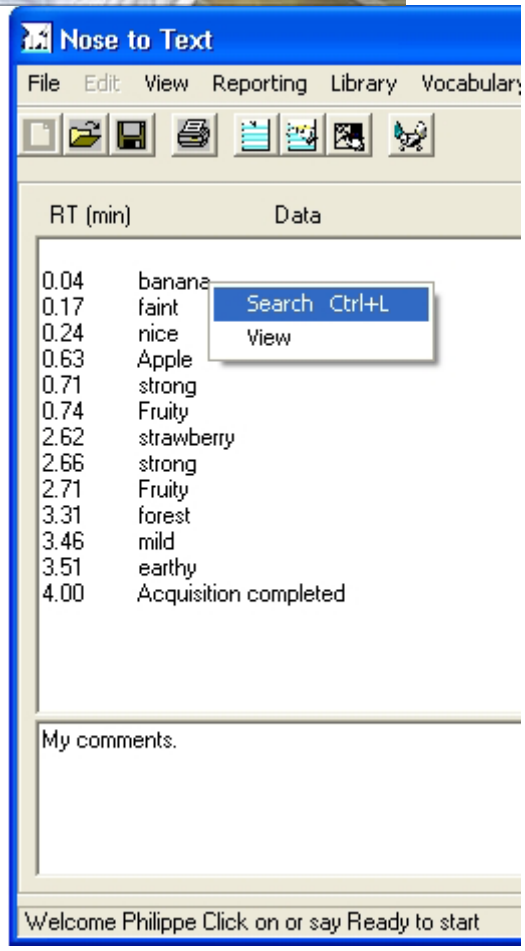
GC-FTIR


Pseudoephedrine Co-add
-0.006
-0.008
Abs
Pseudoephedrine
Match:89.28
0.04
0.02
0.00
Abs
Ephedrine
Match:80.90
0.04
-0.00
Abs
2000
1000
Wavenumbers (cm-1)


Ephedrine Co-add
0.000
-0.005
Abs
Ephedrine
Match:93.91
0.04
-0.00
Abs
Pseudoephedrine
Match:86.85
0.04
0.02
0.00
Abs
2000
1000
Wavenumbers (cm-1)

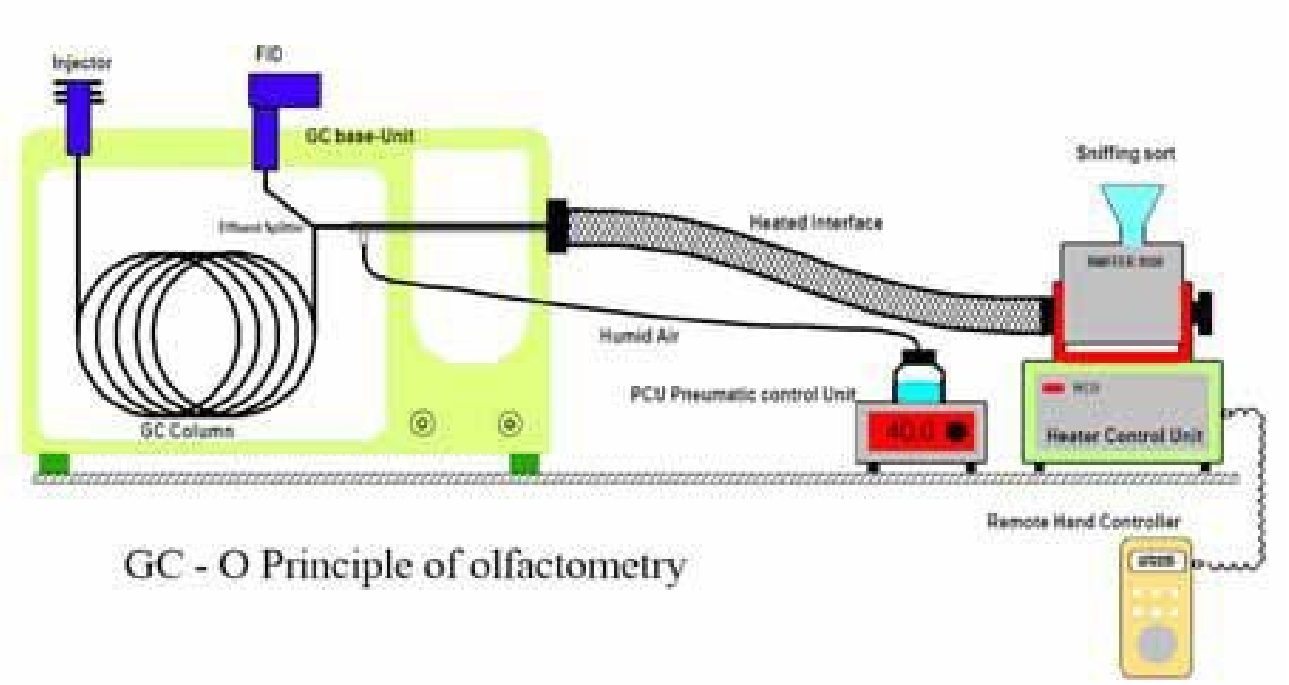
GC-O

GC-Olfactometry, GC-Sniff



GC-O

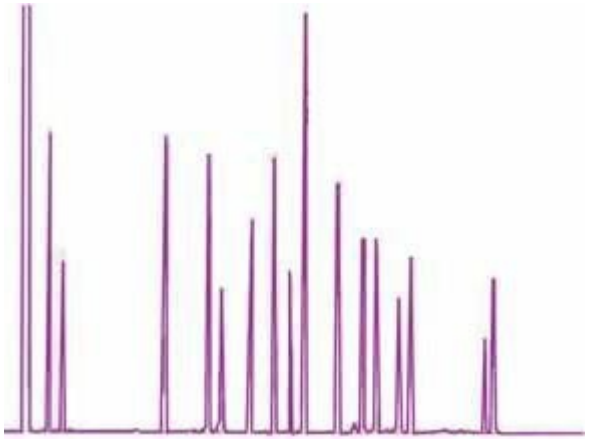
GC-Olfactometry, GC-Sniff



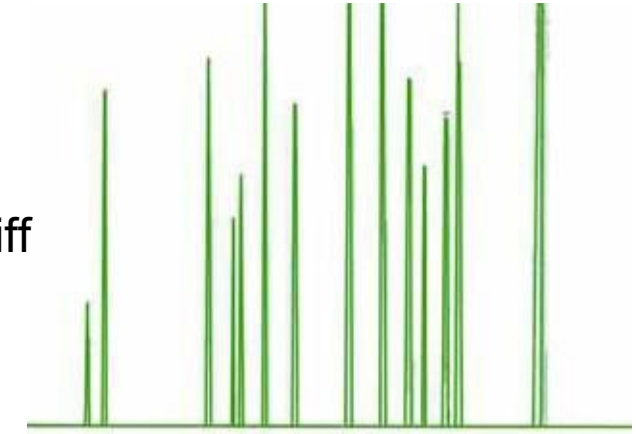
GC-O

GC-Olfactometry, GC-Sniff

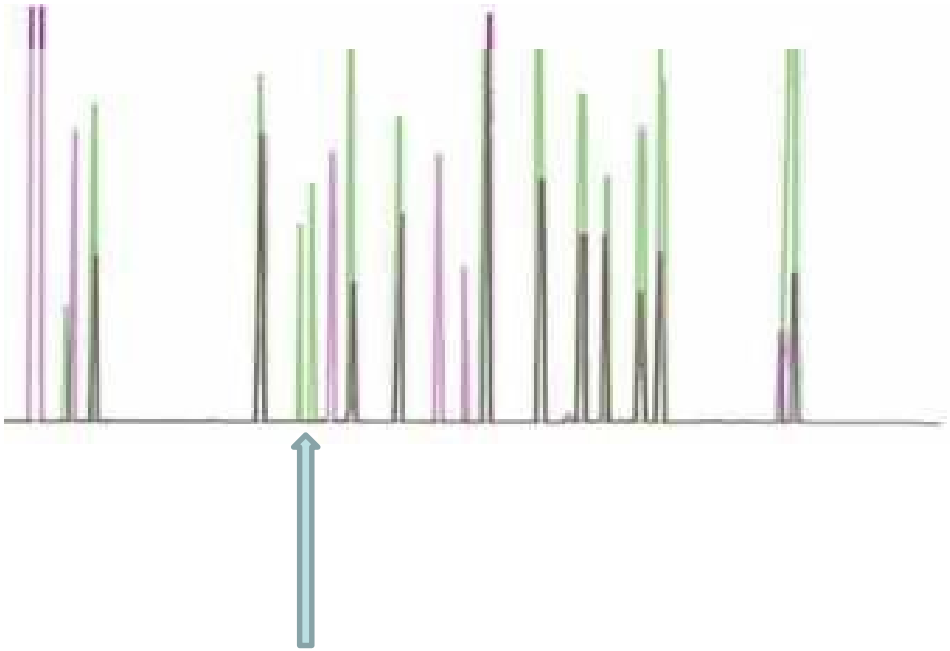
FID



Sniff



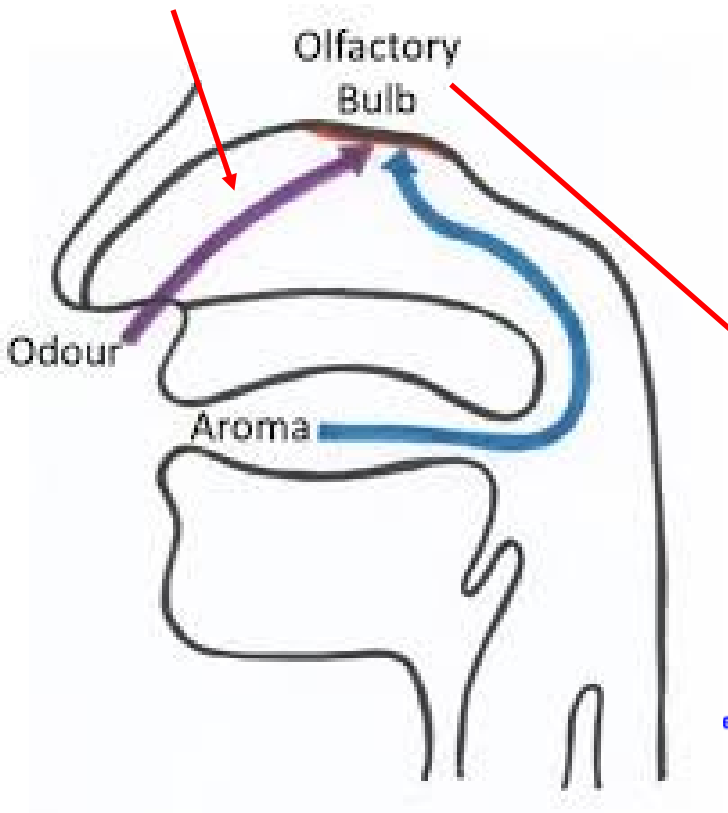
Together



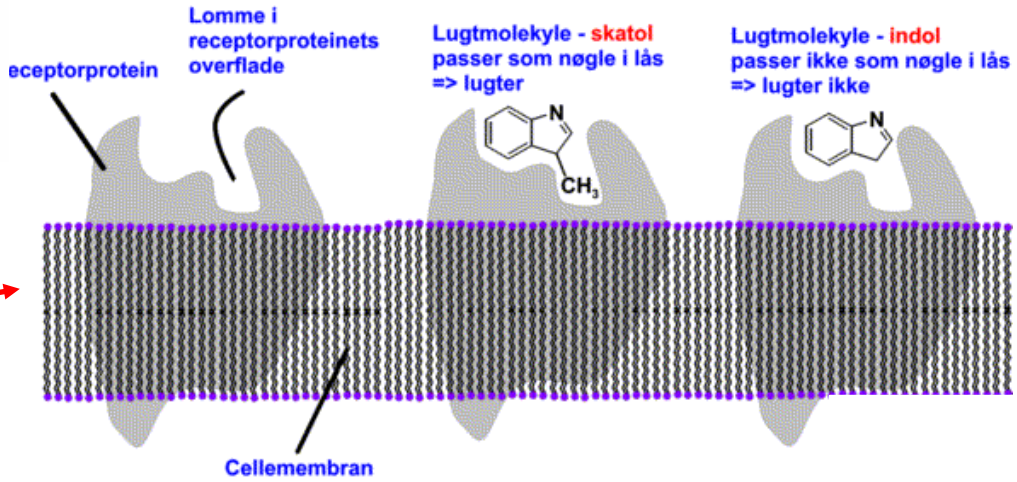
Peaks under the detection limit of GC-FID

Aroma compounds and their contribution to flavour of foods

Nasal cavity



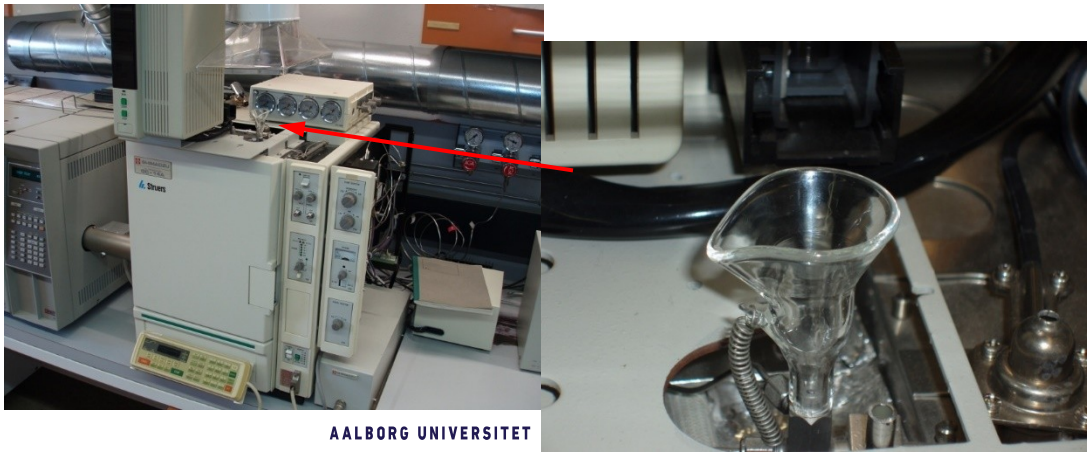
Receptor proteins



Methods for the determination of important aroma compounds in foods by ved GC-olfactometry (GC-O)

Common GC-O ('GC-sniff') methods

- **Osme** (***time-intensity***). Assessment of aroma and intensity over time of min. 4 judges at a specific concentration.
- **AEDA** (»Aroma Extraction Dilution Analysis«) [***dilution***]. Assessment of aroma by dilution of the sample. Few judges (min. 2).
- **CharmAnalysis** (***dilution***). Assessment of aroma over time by diluting the sample. Few judges (min. 2).
- **NIF** and **SNIF** (***frequency***). Assessment of aroma at one concentration (NIF) and over time (SNIF) of min. 8 judges.



Collecting aroma compounds by dynamic-headspace technique

Pretreatment

Dynamic headspace (trapping aroma compounds/volatiles on a polymer)

Thermal desorption

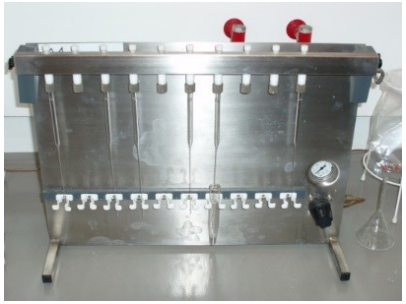
Elution of trap with a volatile organic solvent and adding an int. std.

Analysis

Identification and quantification
GC-FID, GC-MS,GC-MS/MS, LVI-GC

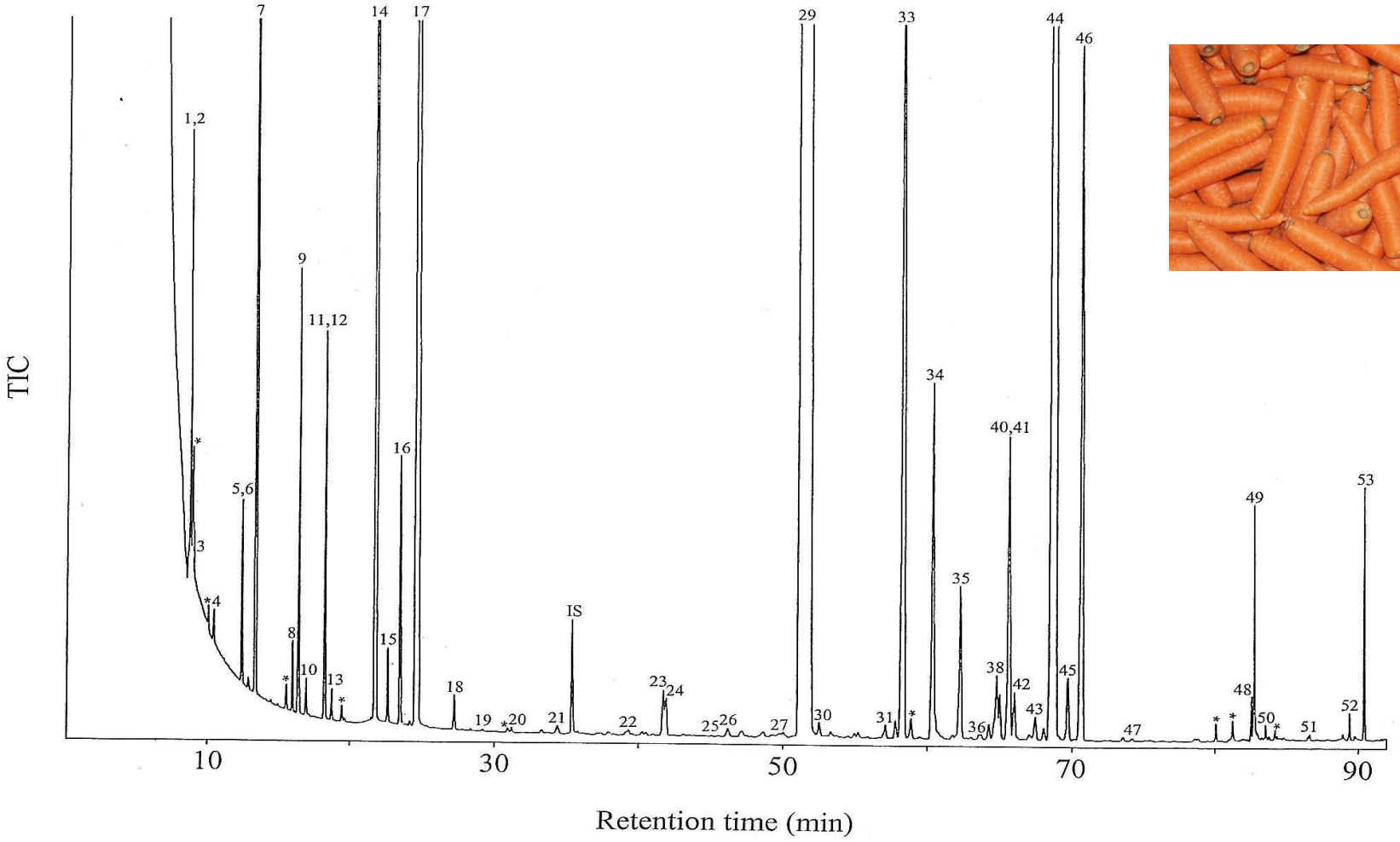
Evaporation under nitrogen

Analysis



Analysis af headspace ekstrakt from carrots by GC-MS

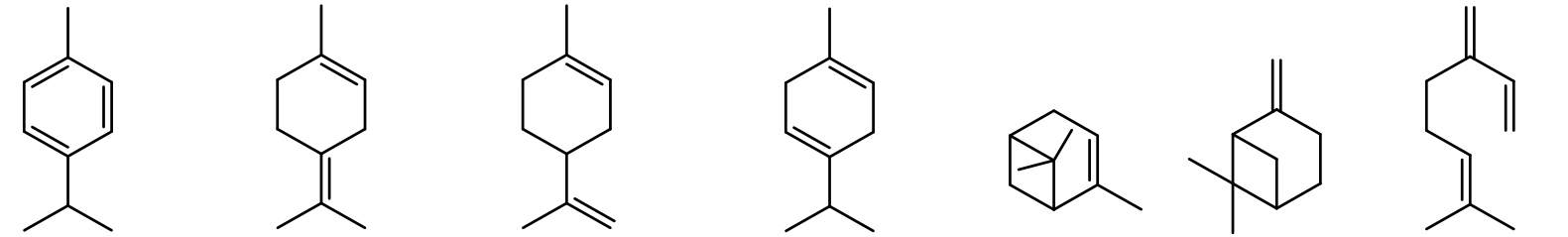
Injection af 1 µl koncentratet headspace extract sample (2 ml → 150 µl)



TIC = Total Ion Count

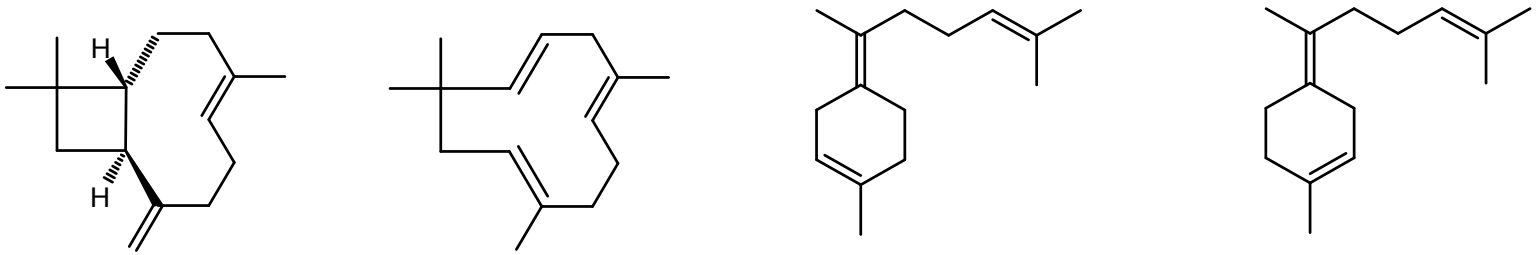
Major volatiles and central aroma compounds in carrots

Monoterpenes



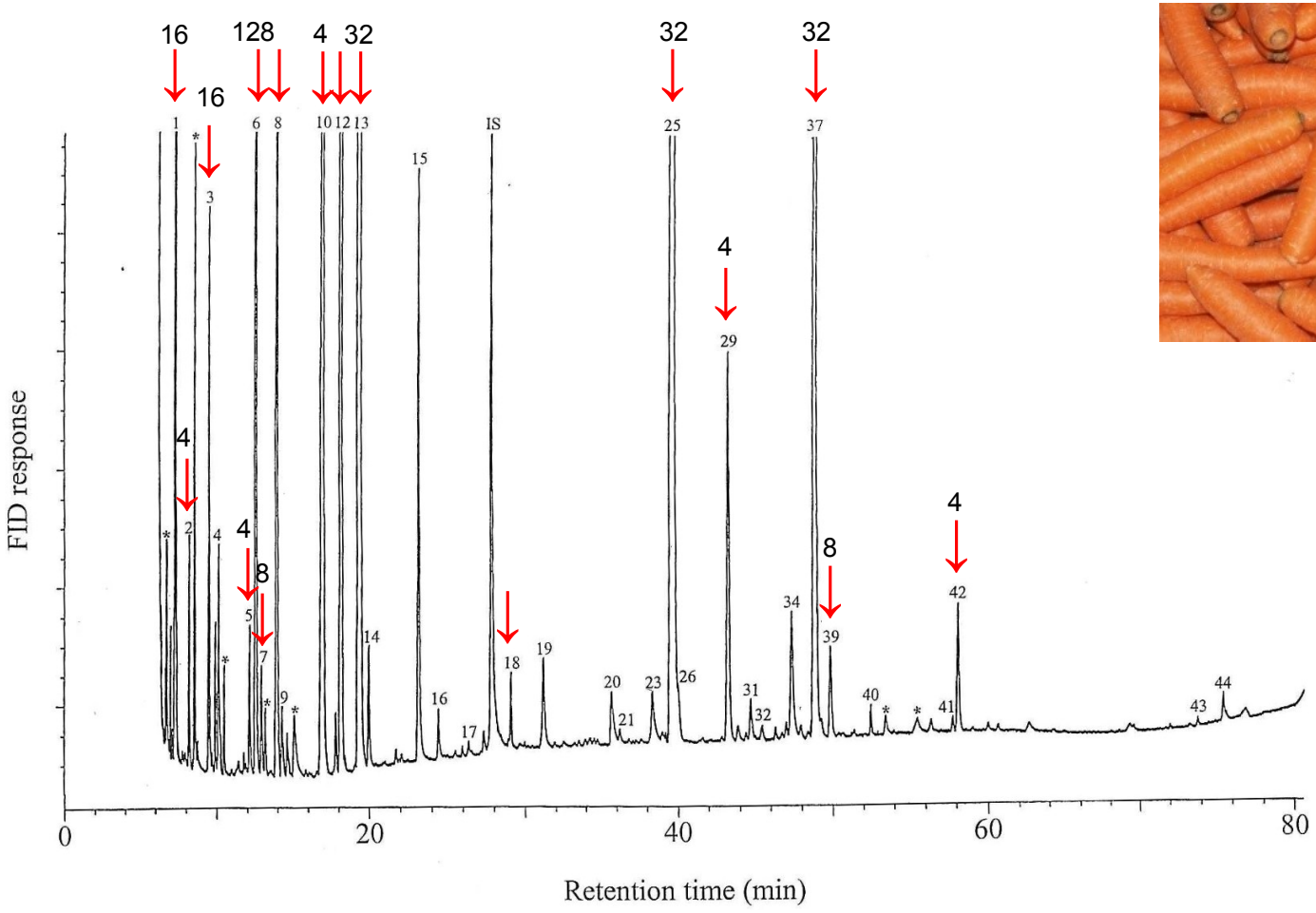
p-Cymene | Terpinolene | Limonene | □-Terpinene | *a*-Pinene | □-Pinene | *b*-Myrcene

Sesquiterpenes



□-Caryophyllene | □-Humulene | (*E*)-*g*-Bisabolene | (*Z*)-□-Bisabolene

Determination of central aroma compounds in carrots by GC-olfactometry (AEDA)



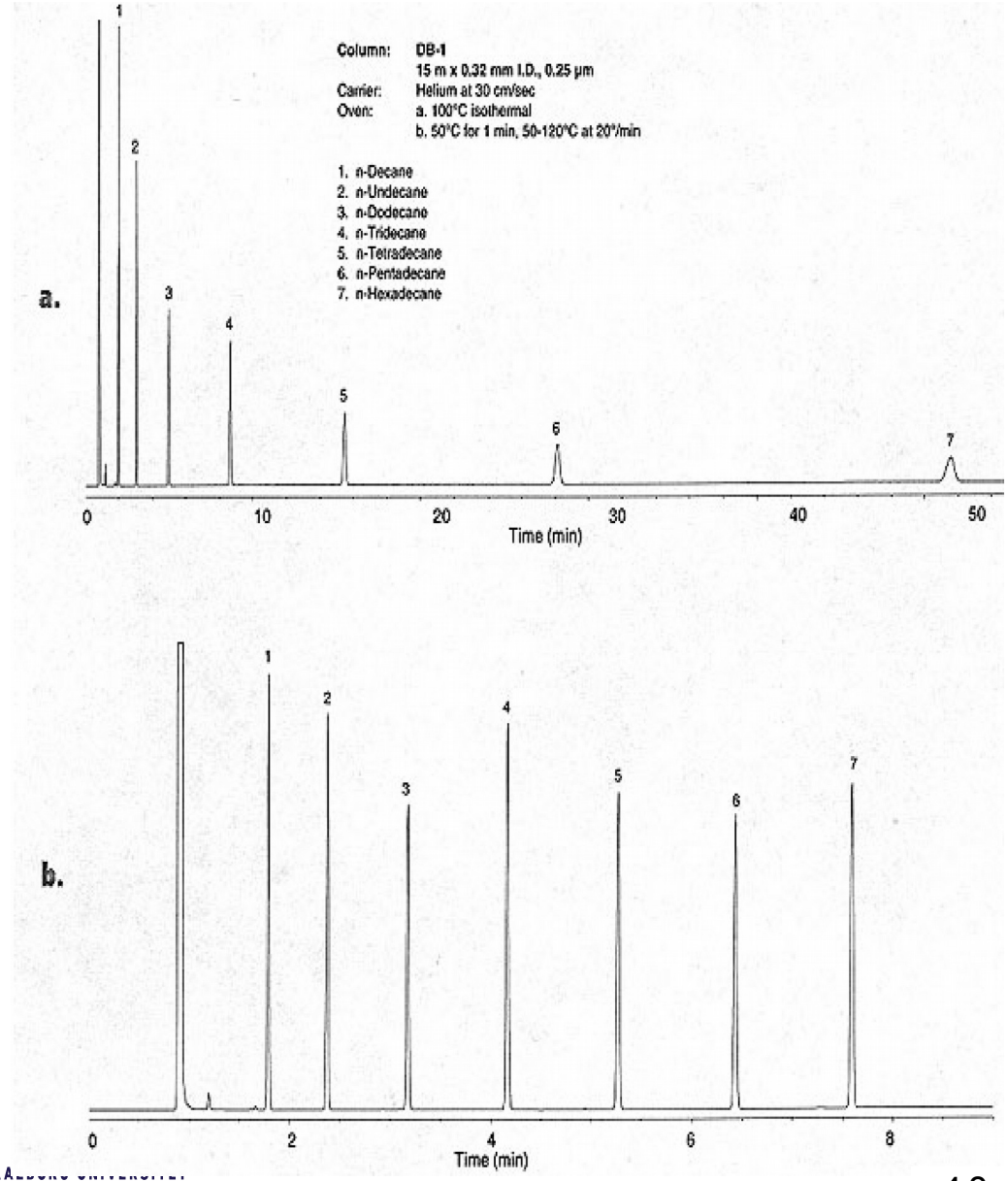
Important aroma compounds in carrots determined by GC-O (AEDA)

Identified compounds	olfactory description	flavour dilution factor	concentration ng/50g/h	
			Duke'	'Cortez'
β-Myrcene	terpene-like, sweet	128	2330b	8750a
Terpinolene	sweet, fruity, citrus	32	13500b	17700a
β-Caryophyllene	woody, spicy, terpene-like	32	11500b	40700a
(*E*)-γ-Bisabolene	soapy, spicy	32	7160b	10400a
α-Pinene	carrot top	16	7780a	4680b
Valencene	soapy, sharp,	16	756a	477b
α-Terpinene	herbaceous	8	140b	268a
(*Z*)-γ-Bisabolene	soapy	8	205b	949a
Camphene	spicy	4	194b	270a
α-Phellandrene	fresh green, carrot top	4	21b	173a
γ-Terpinene	herbaceous, citrus, fruity	4	4220b	9070a
α-Humulene	spicy, carrot top	4	740b	2540a
Caryophyllen oxide	citrus	4	230a	350a
Limonene	sweet, citrus, fruity	2	1360b	2120a
***p*-Cymene**	carrot top	2	5340a	5280a
β-Pinene	carrot top, sweet, fruity	1	1730a	1630a

Temperature programming

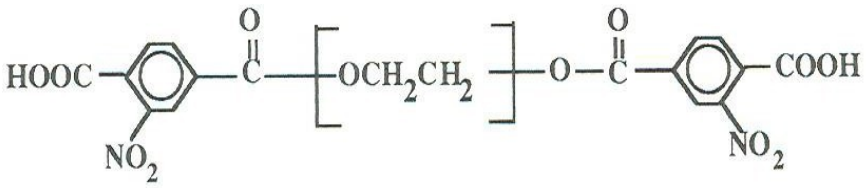
- Isothermal GC involves maintaining a constant oven temperature throughout the GC run. Isothermal temperature conditions are used for **solutes with similar retention**. Retention differences for dissimilar solutes (e.g., complex samples with analytes of different polarity and structure) can be quite severe in isothermal GC. Peak widths increase with retention for isothermal conditions (a). For these reasons, isothermal conditions are only suitable for a limited number of analyses.

- Most analyses require the use of a temperature program (temp. gradient). A temperature program involves heating the oven at a controlled rate during the run. This allows **faster analysis of solutes with dissimilar retention**, and there is very little peak broadening with an increase in retention (b).

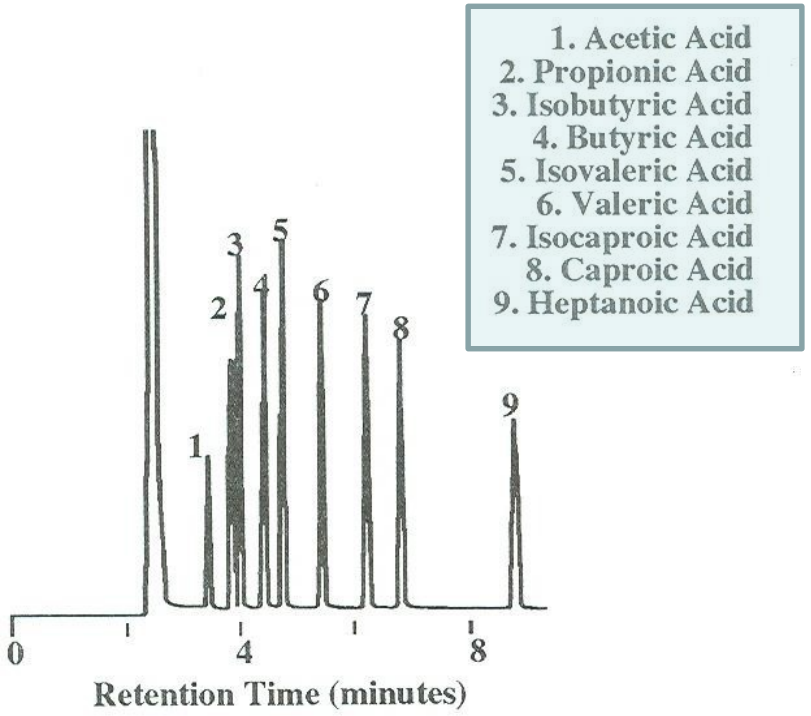


Isothermal GC analysis of related compounds

The stationary phase nitroterephthalic acid modified polyethylene glycol in a FFAP column. Highly polar column used to separate violatile fatty acids



The separation was carried out isothermally at 185°C with helium as a carrier gas having a linear velocity of 20 cm/s.

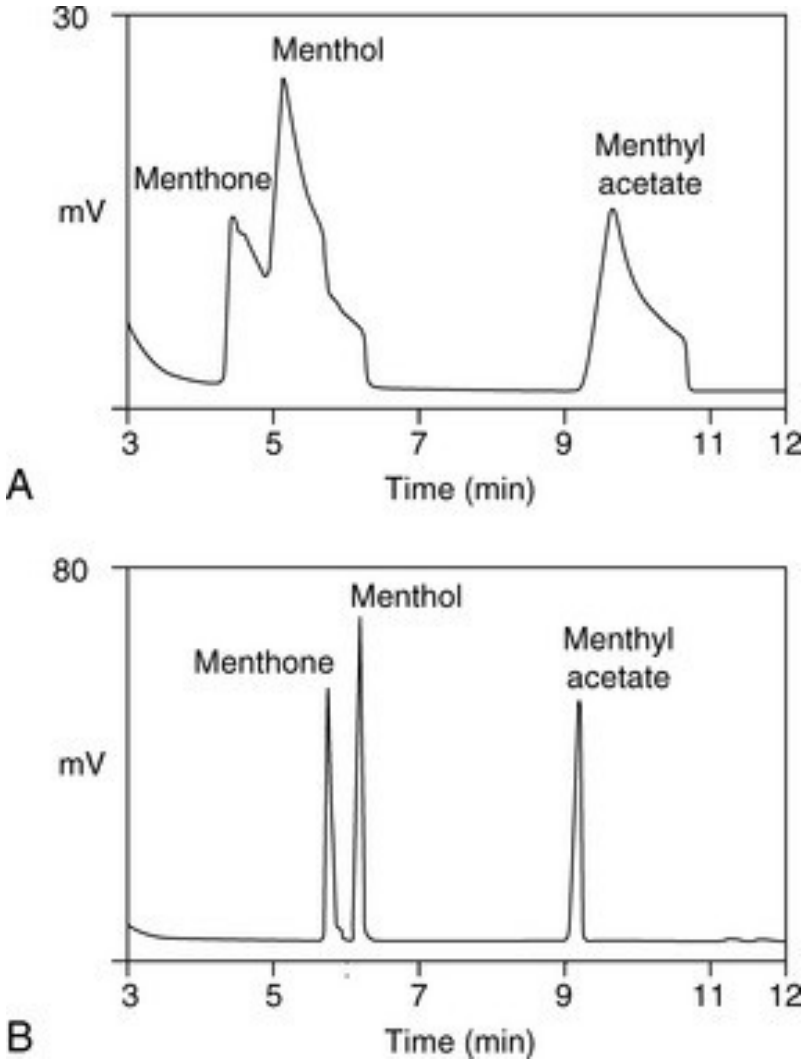


Courtesy of Supelco, Inc.

Acetic acid	C2	118 -119°C
Propionic acid	C3	141°C
Isobutyric acid	C4	155°C
Butyric acid	C4	163.5°C
Isovaleric acid	C5	175 -177°C
Valeric acid	C5	186 -187°C
Isocaproic acid	C6	199 -201°C
Caproic acid	C6	205.8°C
Heptanoic acid	C7	223°C

Effect of isotermal GC analysis in splitless mode

GC trace analysis for menthone, menthol and menthyl acetate in splitless mode under isothermal conditions (**110°C** for 12 min.) (**A**) and temperature programming (**60°C** (1 min) 30°C/min to 120°C for 9 min. (**B**).



- In the splitless mode, the sample must be efficiently trapped at the head of the column. For this to occur, it must be sufficiently involatile.
- If the sample is relatively volatile, it must be injected into the GC in a low-volatility solvent, which will condense at the head of the column, trapping the sample in the process.
- In **figure A** the effect of too high a starting temperature on the trapping of a volatile analyte resulting in misshapen peaks, which can be corrected by lowering the starting temperature and then programming the GC temperature to rise.

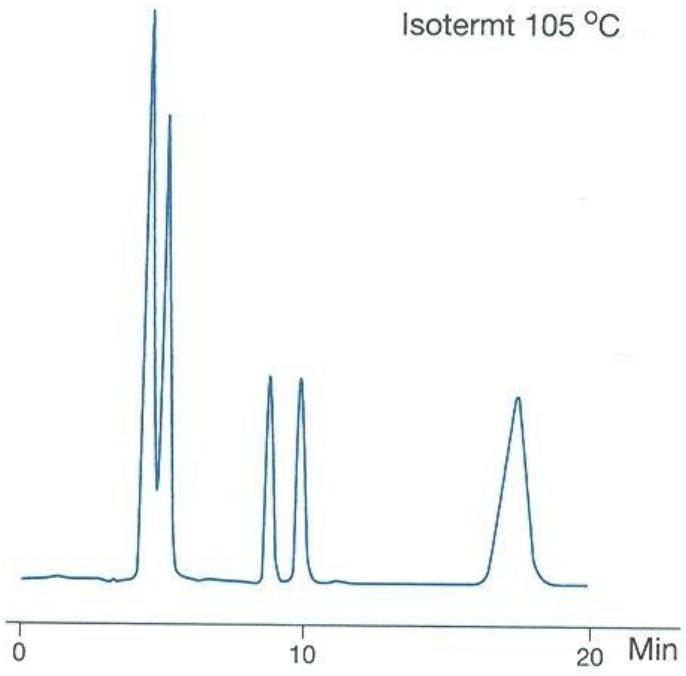
Effect of temperature increase and separation problems with isotermal GC analyses

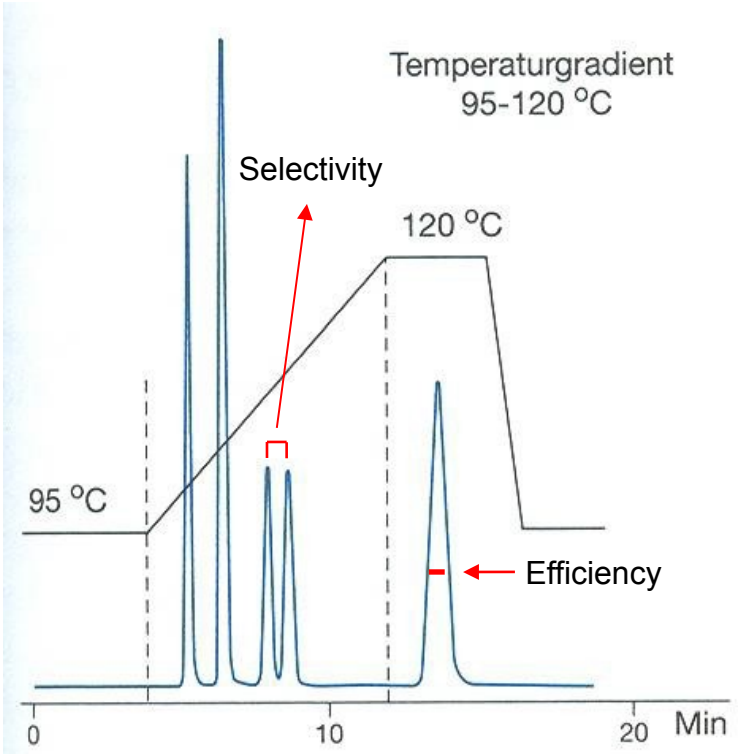
Temperature increase: °C ↗

$V_R = t_R \times F_c$ (retention volume) ↘ $t_R = t_M + t'_R$ (total retention time) ↘

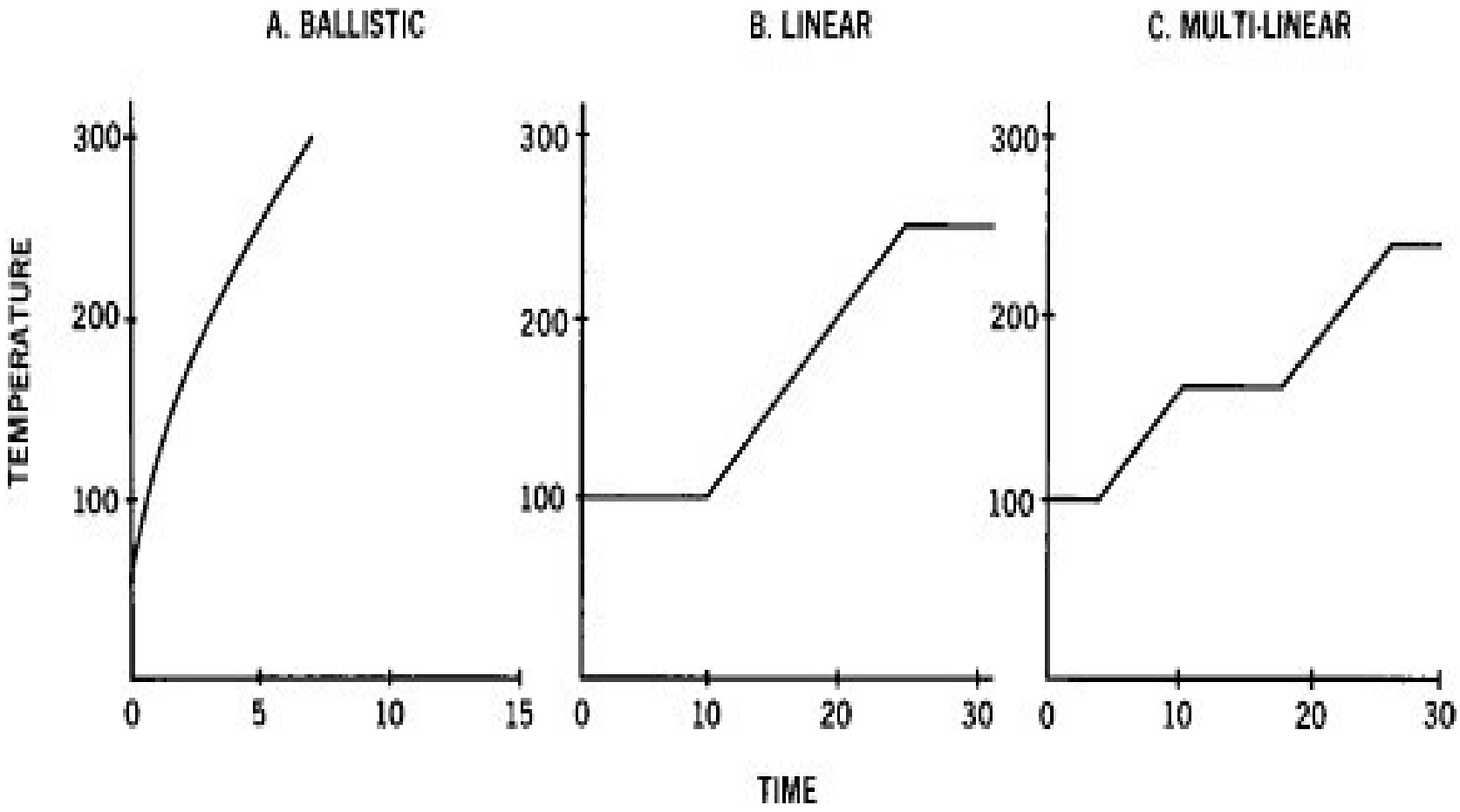
$N = (t_R/\sigma)^2 = 5.54(t_R/W_{1/2})^2$ (number of theoretical plates) = Efficiency ↗

$k = t'_R / t_M$ (retention factor) ↘ $\alpha = k_B/k_A = (t_{RB} - t_M / t_{RA} - t_M)$ (selectivity) usually ↘





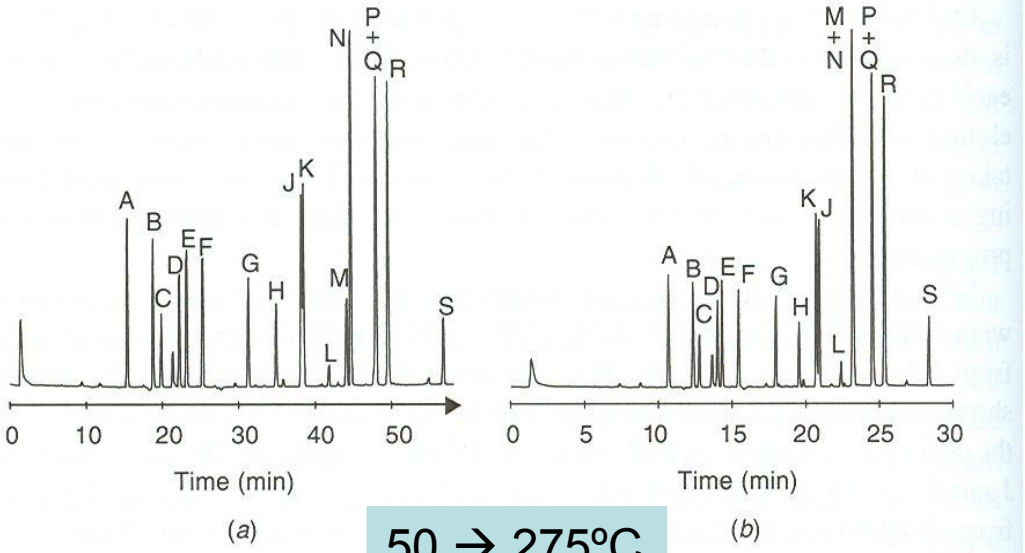
Temperature programs



Temperature gradient GC analysis

- Temperature gradient (GC) ≈ eluent strength (HPLC)

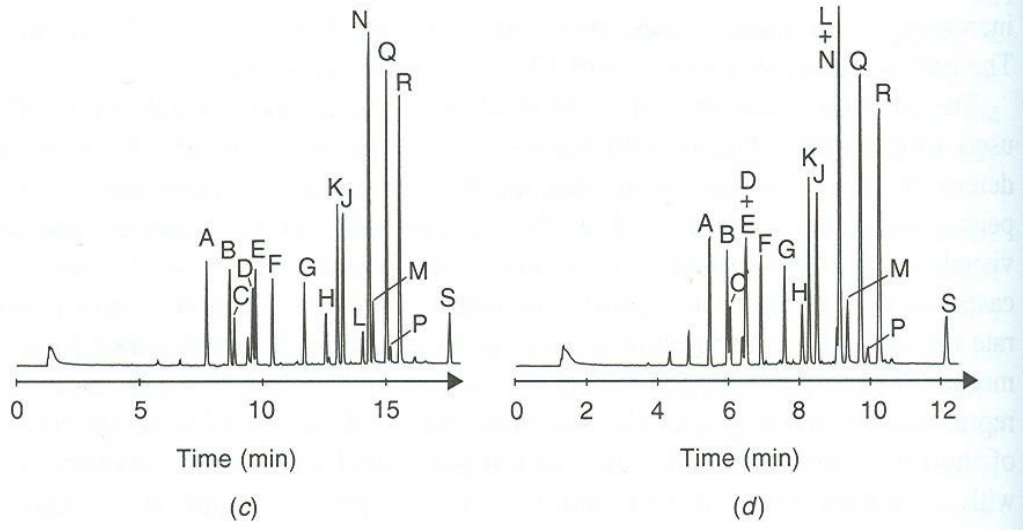
1.5 ºC/min



4 ºC/min

50 → 275ºC

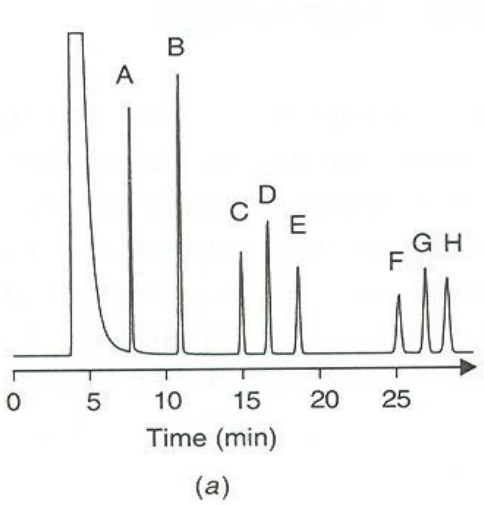
8º C/min



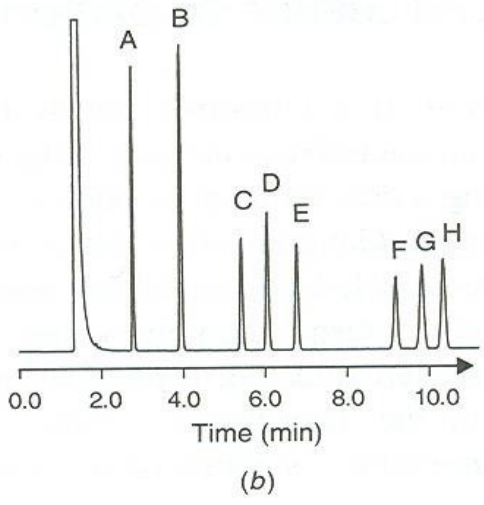
16 ºC/min

Effect of gas velocity in GC analysis

11 cm/s



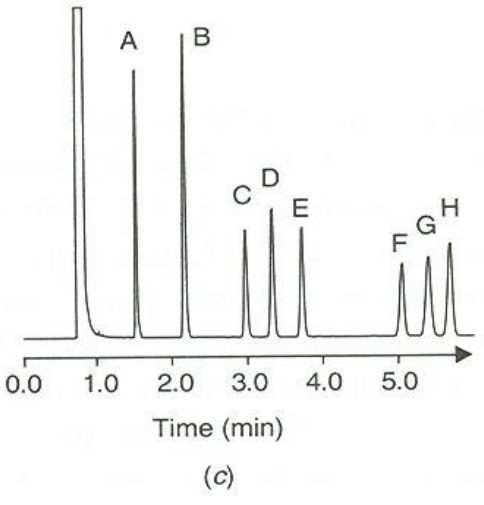
(a)



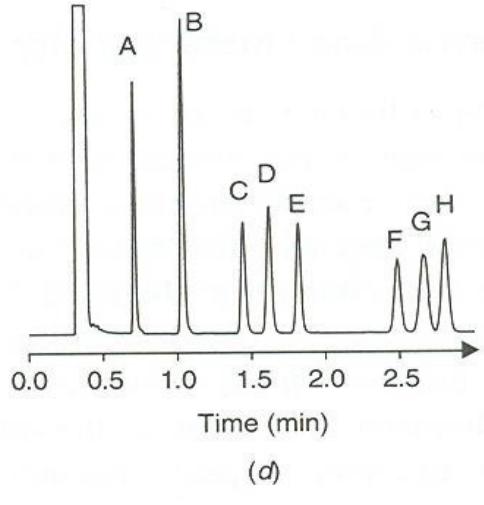
(b)

30 cm/s

53 cm/s



(c)

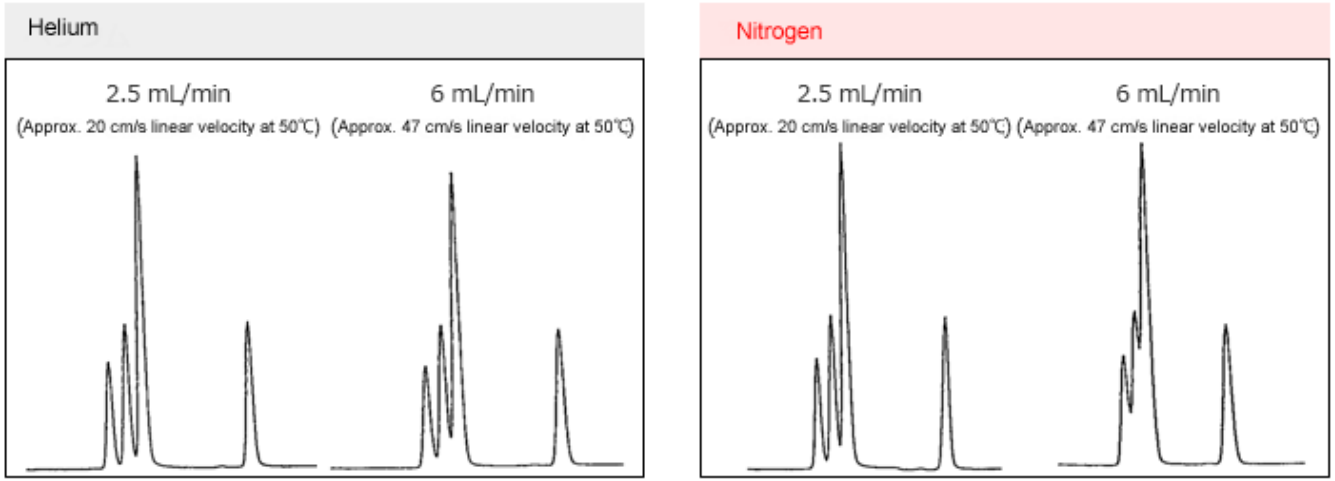


(d)

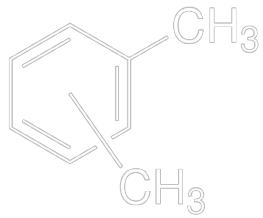
105 cm/s

Effect of gas velocity for different carrier gasses in GC analysis

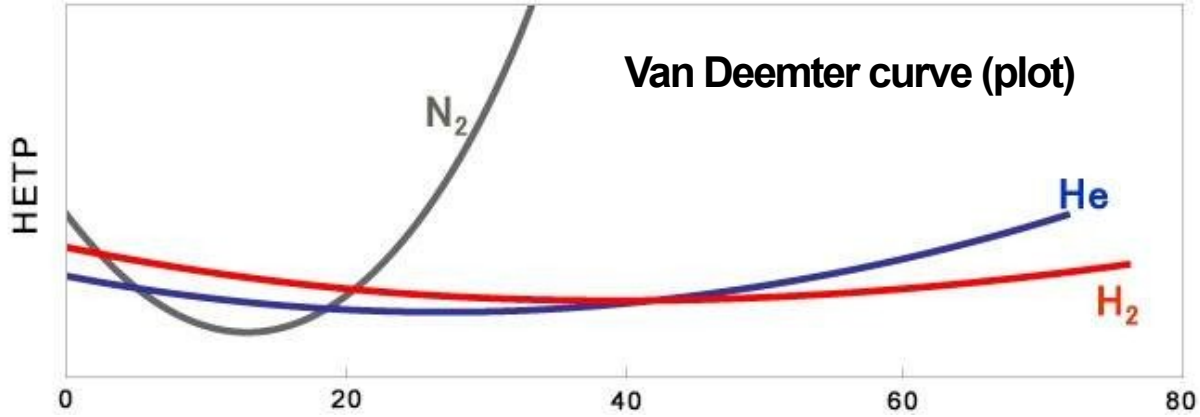
Using He gas, the separation is virtually unchanged across the range from 20 to 47 cm/s linear velocity. With N_2 gas, however, the separation deteriorates at 47 cm/s linear velocity. This occurs because the optimal linear velocity range for N_2 is narrower than for He (see below).



Sample: Xylene
Column: CBP20-W25-100 (length 25 m, inner diameter 0.53 mm, thickness 1 μm)
Column temperature: 50℃ to 150℃ at 5℃/minute



Xylene
(*ortho*, *meta* and *para*)



Qualitative analyses

Chromatographic peaks come without name !!!

Chromatographic peaks can be compared with a standard

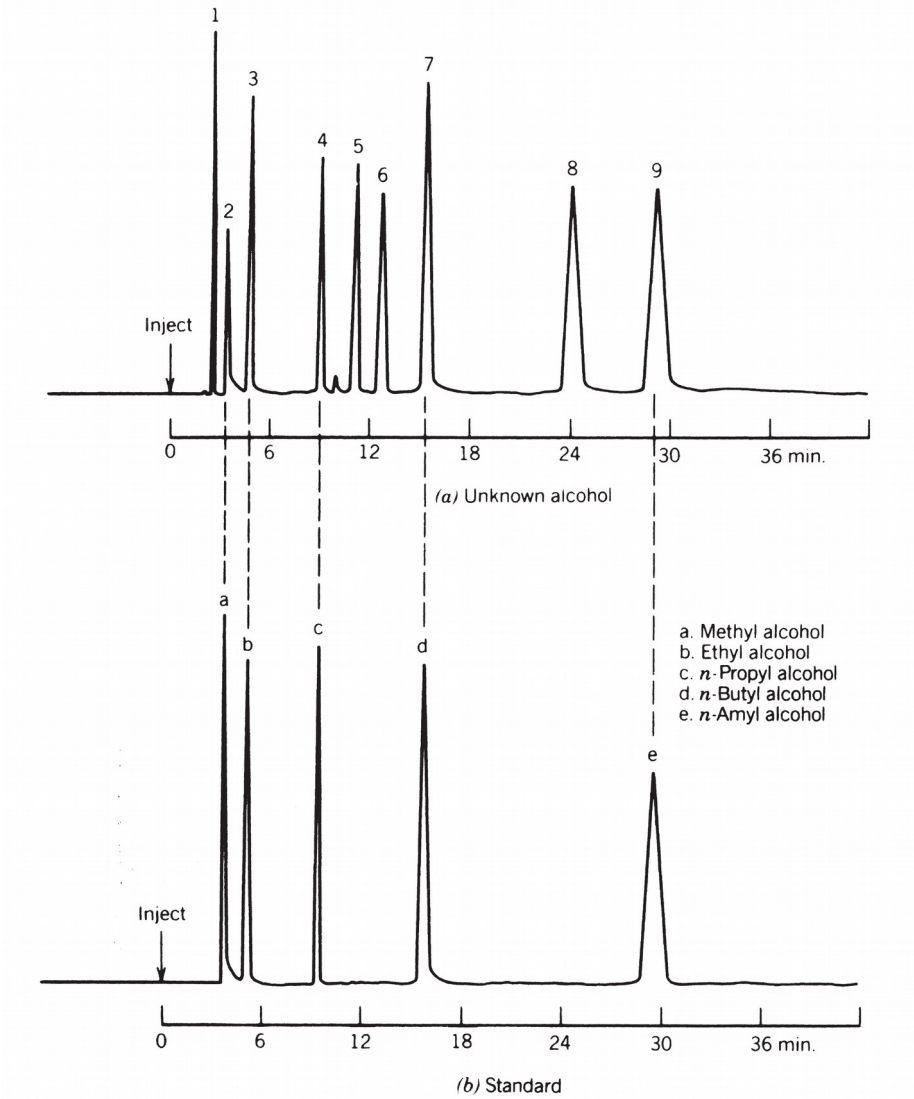
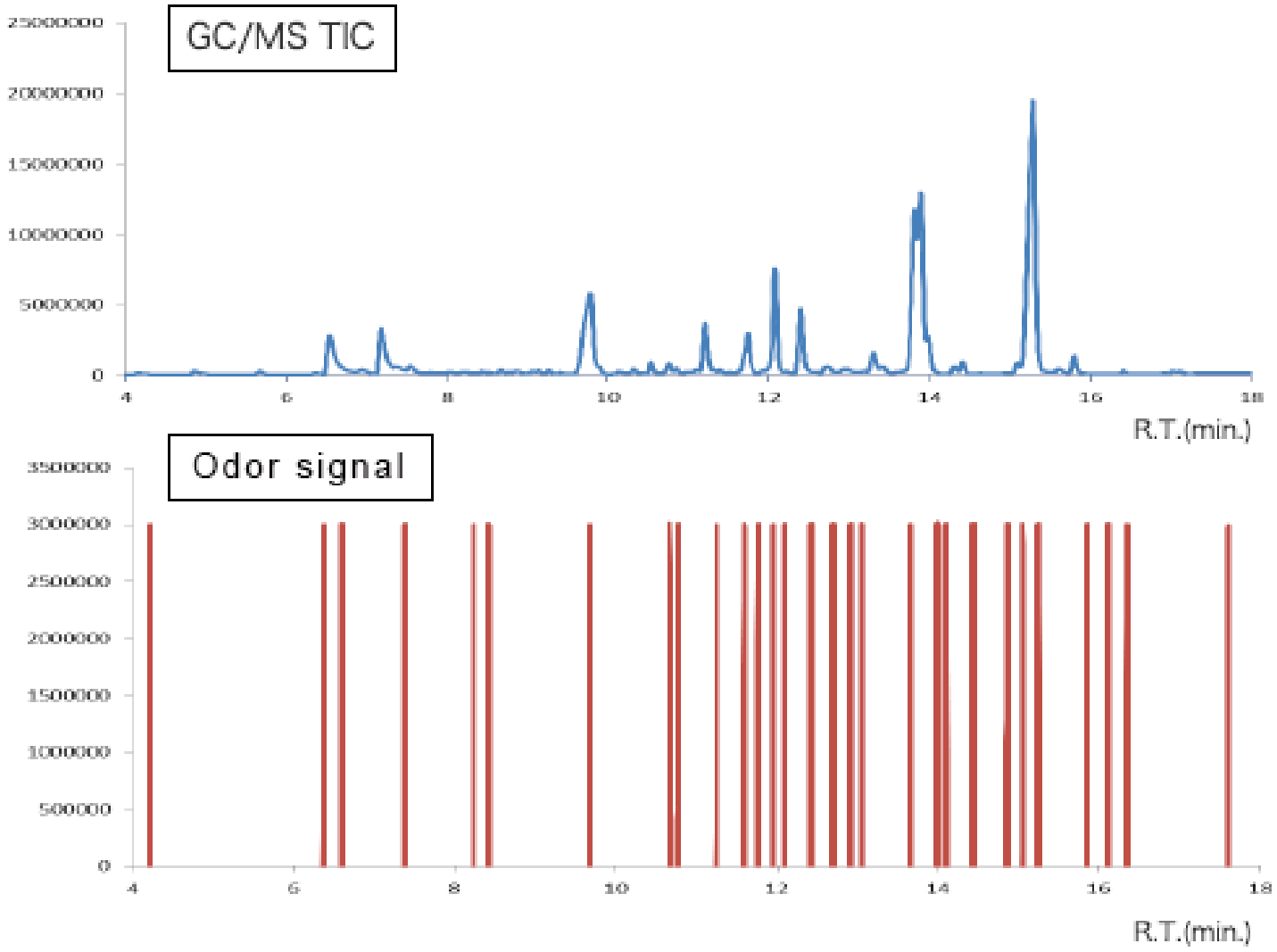


Fig. 8.1. Identification of unknown by retention times using standards. From Miller, J. M., *Chromatography: Concepts and Contrasts*, 2nd ed., John Wiley & Sons, Hoboken, NJ, 2005, p. 354. Reproduced courtesy of John Wiley & Sons, Inc.

TABLE 8.1 GC Methods for Qualitative Analysis

Method
1. Retention parameters
Retention time
Relative retention time; Retention indexes
2. Use of selective detectors
Dual channel GC
On-line
MS or MSD (mass selective detector)
FTIR
Off-line
MS, MSD
FTIR
NMR
UV
3. Other methods
Chemical derivatization
Pre-column
Post-column
Pyrolysis and chromatopyrography
Molecular weight chromatograph (gas density balance)

Dual detection



Dual detection

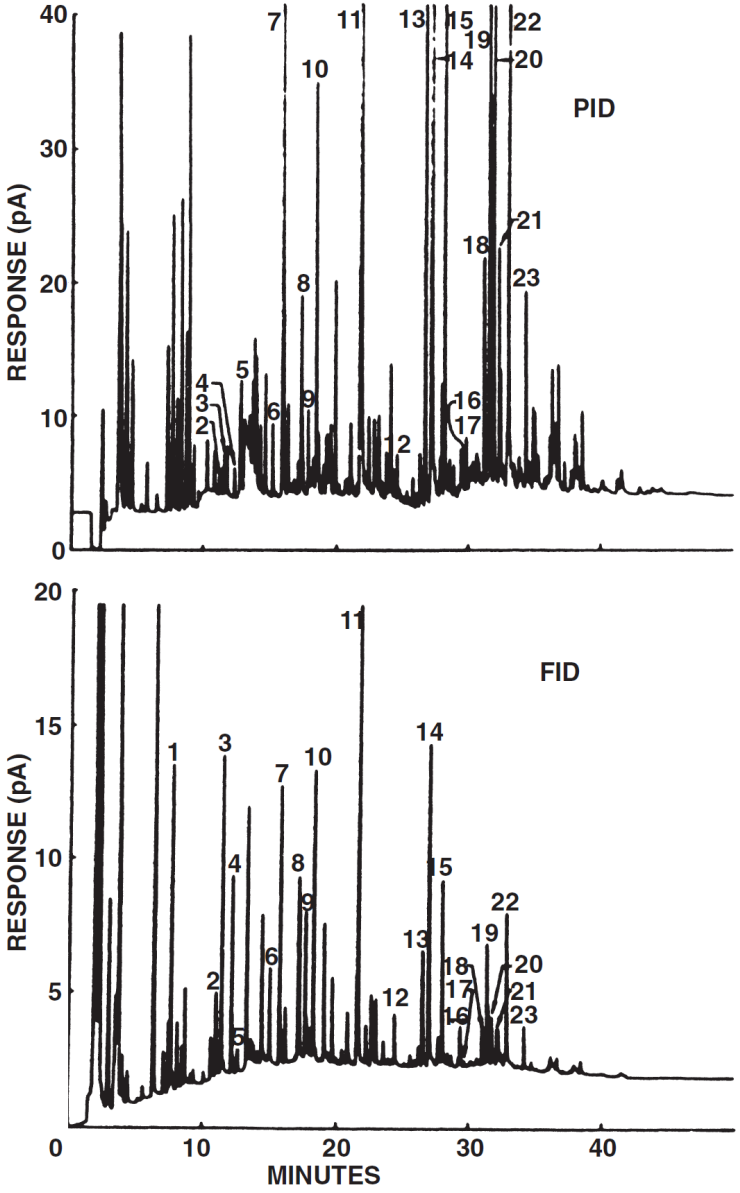


Fig. 8.3. Dual channel presentation of GC analysis of air contaminants in a parking lot. Reprinted with permission from reference 13. Copyright 1983, American Chemical Society. From Miller, J. M., *Chromatography: Concepts and Contrasts*, 2nd ed., John Wiley & Sons, Hoboken, NJ, 2005, p. 360. Reproduced courtesy of John Wiley & Sons, Inc.

Kovats Retention Index

- The Kovats retention index (Kovats index (*KI*), retention index (*I*)) constitute a reliable method for measuring the retention behavior of solutes in GC. The index is named after the Hungarian-born Swiss chemist Ervin Kováts. ***The Kovats index simply convert retention times into system-independent constants.***

- Kovats index uses a **homologous series of *n*-alkanes as standards**. For *n*-alkanes the **intermolecular forces are relatively constant**, and the separation is controlled **primarily by differences in vapor pressure (boiling points)**. The chromatogram, which is produced shows a linear logarithmic relationship between carbon numbers and adjusted retention time (see next slide).

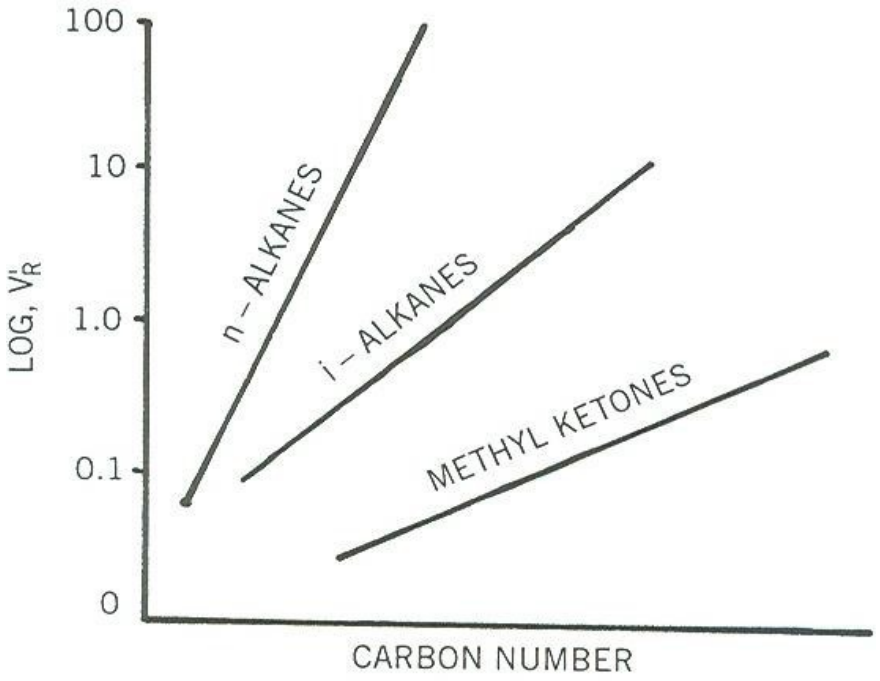
- To find the Kovats index for a given solute X on a given stationary phase, members of the *n*-alkane series are chromatographed and plotted. Then the solute X is run under the same conditions and its Index value is determined from the graph. If the **flow rate is kept constant**, then adjusted retention times or volumes can be plotted. The Kovats index for compound X in **isothermal** GC can also be calculated from the equation:

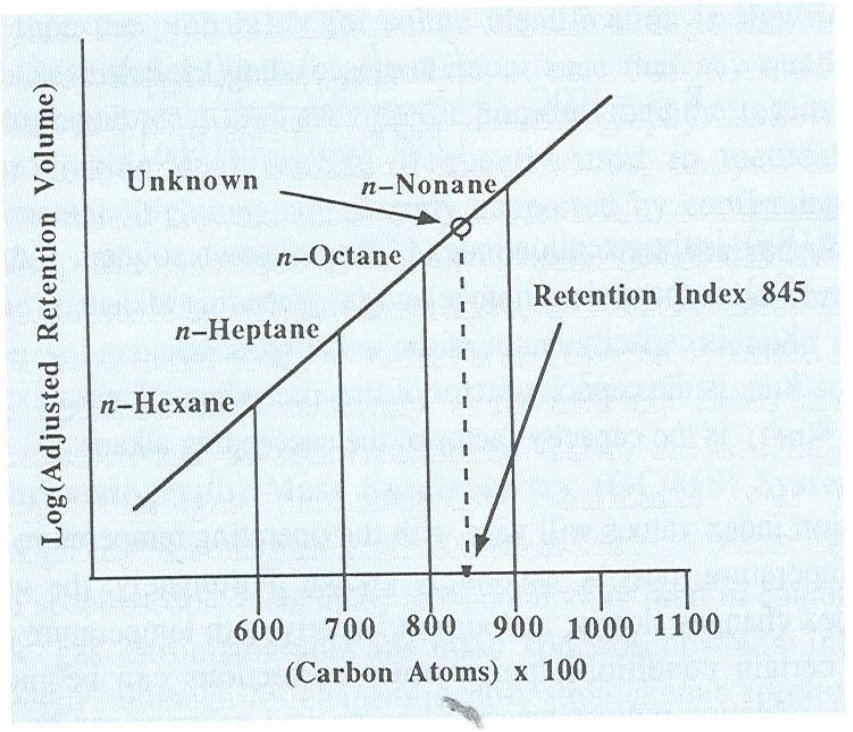
$$I_X = 100n + 100[\log(t'_{RX}) - \log(t'_{Rn})] / [\log(t'_{R(n+1)}) - \log(t'_{Rn})]$$

where t'_{Rn} and $t'_{R(n+1)}$ are adjusted retention times of the reference n-alkane hydrocarbons eluting immediately before and after chemical compound X with the adjusted retention time t'_X.

Kovats, E., *Helv. Chim. Acta*, 1958, **41**, 1915–1932.

Plot of retention time (or volume) vs. carbon number
(isothermal GC analysis)

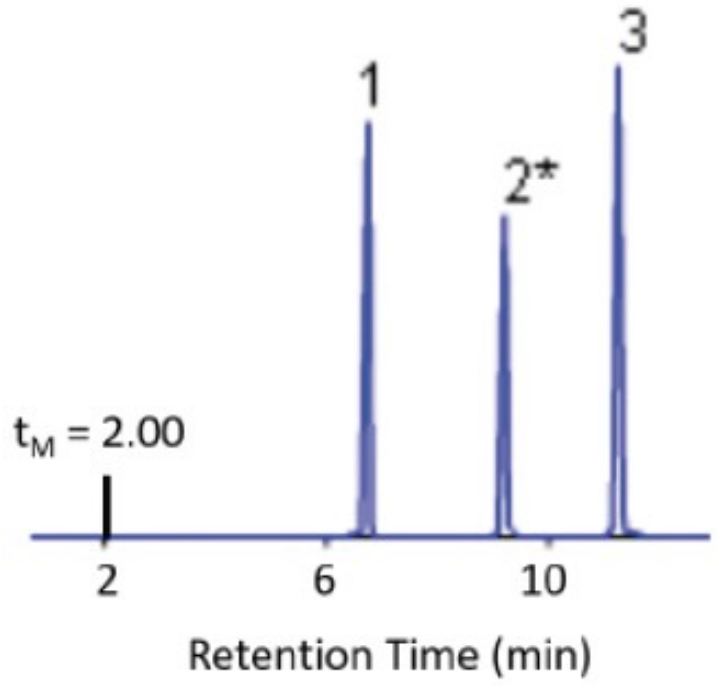




Adjusted retention volume and retention time:

$V'_R = t'_R \times F_c$, where $t'_R = t_R - t_M$ and F_c = column flow (kept constant)

Calculation of Kovats Retention Index



1. Hexane t_R = 6.50 min, t'_R = 4.50 min
2. Unknown t_R = 8.25 min, t'_R = 6.25 min
3. Heptane t_R = 11.50 min, t'_R = 9.50 min

$$I = 100(6) + 100\left[\frac{\log 6.25 - \log 4.50}{\log 9.50 - \log 4.50}\right]$$

I = 644

$$\boldsymbol{I_X = 100n + 100[\log(t'_{RX}) - \log(t'_{Rn})] / [\log(t'_{R(n+1)}) - \log(t'_{Rn})]}$$

Because $t'_R = t_R - t_M$ and t_M is the same during the isothermal GC analysis, the Kovats Retention Index equation is also often **formulated by total retention time** as follows:

$$\boldsymbol{I_X = 100n + 100[\log(t_{RX}) - \log(t_{Rn})] / [\log(t_{R(n+1)}) - \log(t_{Rn})]} = 100 \times 6 + 100 \times (\log$$

$$8.25 - 6.50 / \log 11.25 - \log 6.50) = 600 + 100 \times (0.1035/0.2382) = \mathbf{644}$$

”Kovats index” for temperature programmed gradient GC analysis (non-isothermal)

- The limitation in isothermal GC is that it is difficult to elute *n*-alkanes with a high number of carbon atoms and other compounds with high boiling points resulting in peak broadening.

- The limitations in the elution of high boiling compounds in isothermal GC can be solved by applying linear temperature programming gradient where the *n*-alkanes elute in a linear mode.

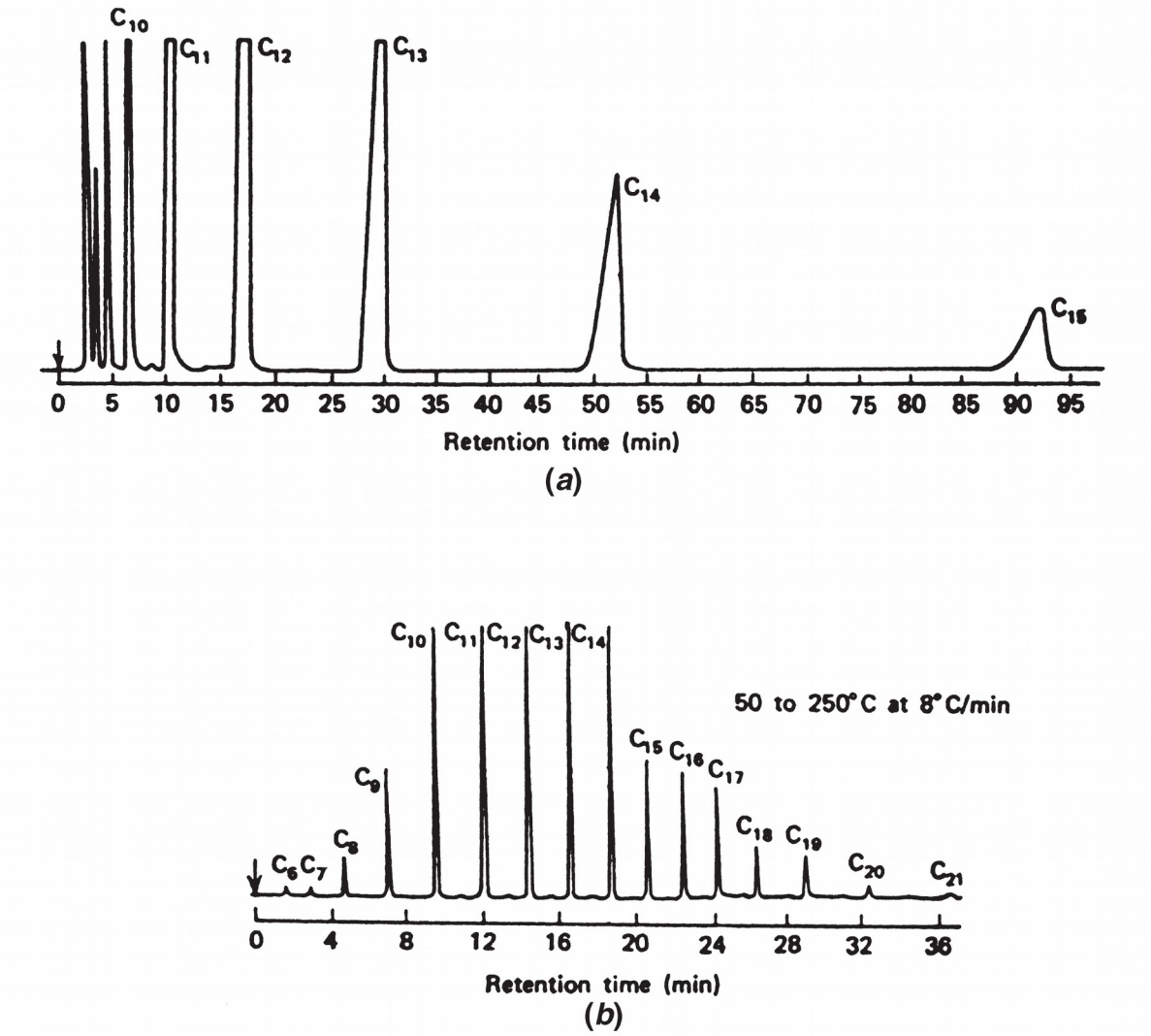


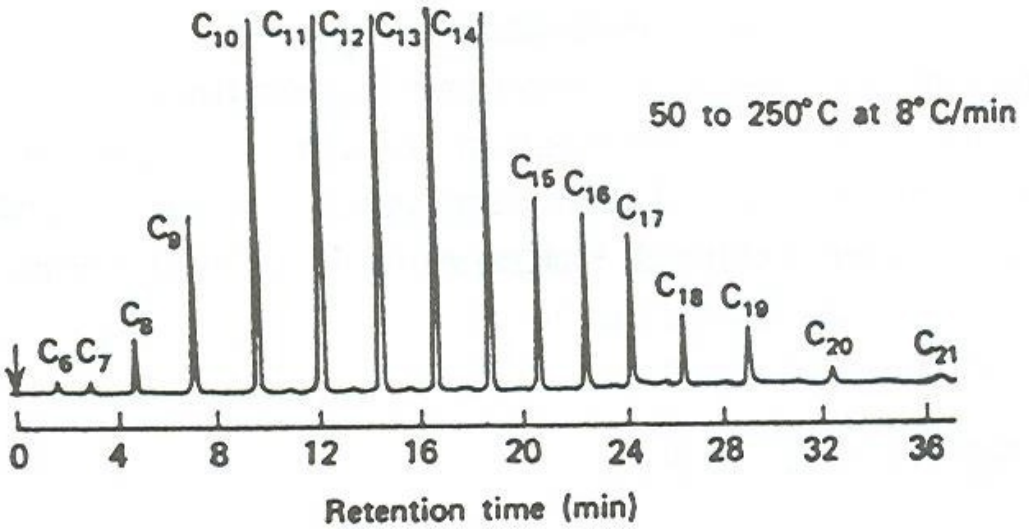
Fig. 9.2. Comparison of (*a*) isothermal and (*b*) programmed temperature separations of *n*-paraffins.

”Kovats index” for temperature programmed gradient GC analysis (non-isothermal)

- In 1963 Van den Dool and Kratz developed a relative retention index based on the work of Kovats that can be applied under linear temperature-programming conditions solving the limitations of the isothermnal Kovats Index.
- The index of Van den Dool and Kratz is often referred to as the **retention index (*RI* or *I*), linear retention index (*LRI*) or programmed-temperature retention index (*PTRI* or I^T)** and can be calculated from the following equation:

$$I_X = 100n + 100\,(t_{RX} - t_{Rn} \,/\, t_{R(n+1)} - t_{Rn})$$

where t_{Rn} and t_{n+1} are retention times of the reference *n*-alkanes eluting immediately before and after chemical compound “X”; and t_x is the retention time of compound “X”.

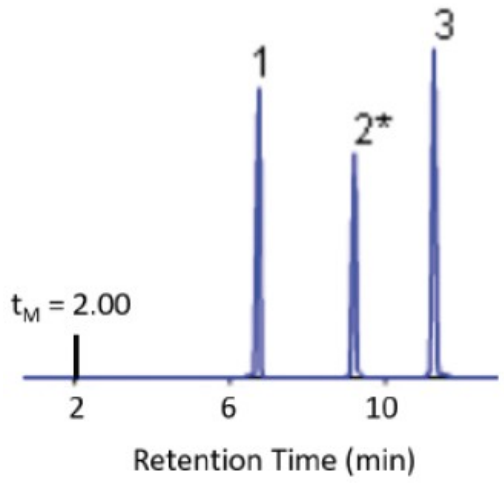


Van Den Dool, H., Kratz, P.D., *J. Chromatography*, 1963, **11**, 463-471.

Calculation of retention index depends on the GC analysis conditions

- Capillary column stationary phase, the operational variables, such as carrier gas flow-rate, stationary phase film thickness, polarity of the column and temperature programming (e.g., linear versus isothermal), can alter an analyte's retention time, thus retention index reproducibility represent an uncertainty in calculating retention indices (*I*) even though the index is relative.

- Retention index (*I*) values are, however, reliable if they are compared with *I* values obtained from capillary columns with comparable stationary phases (film thickness and polarity) and same type of temperature programming (isothernmal versus gradient), although small differences in the retention index for the same compounds are common.



1. Hexane t_R = 6.50 min, t'_R = 4.50 min
2. Unknown t_R = 8.25 min, t'_R = 6.25 min
3. Heptane t_R = 11.50 min, t'_R = 9.50 min

Retention times obtained by isothermal GC

$$I = 100(6) + 100\left[\frac{\log 6.25 - \log 4.50}{\log 9.50 - \log 4.50}\right]$$

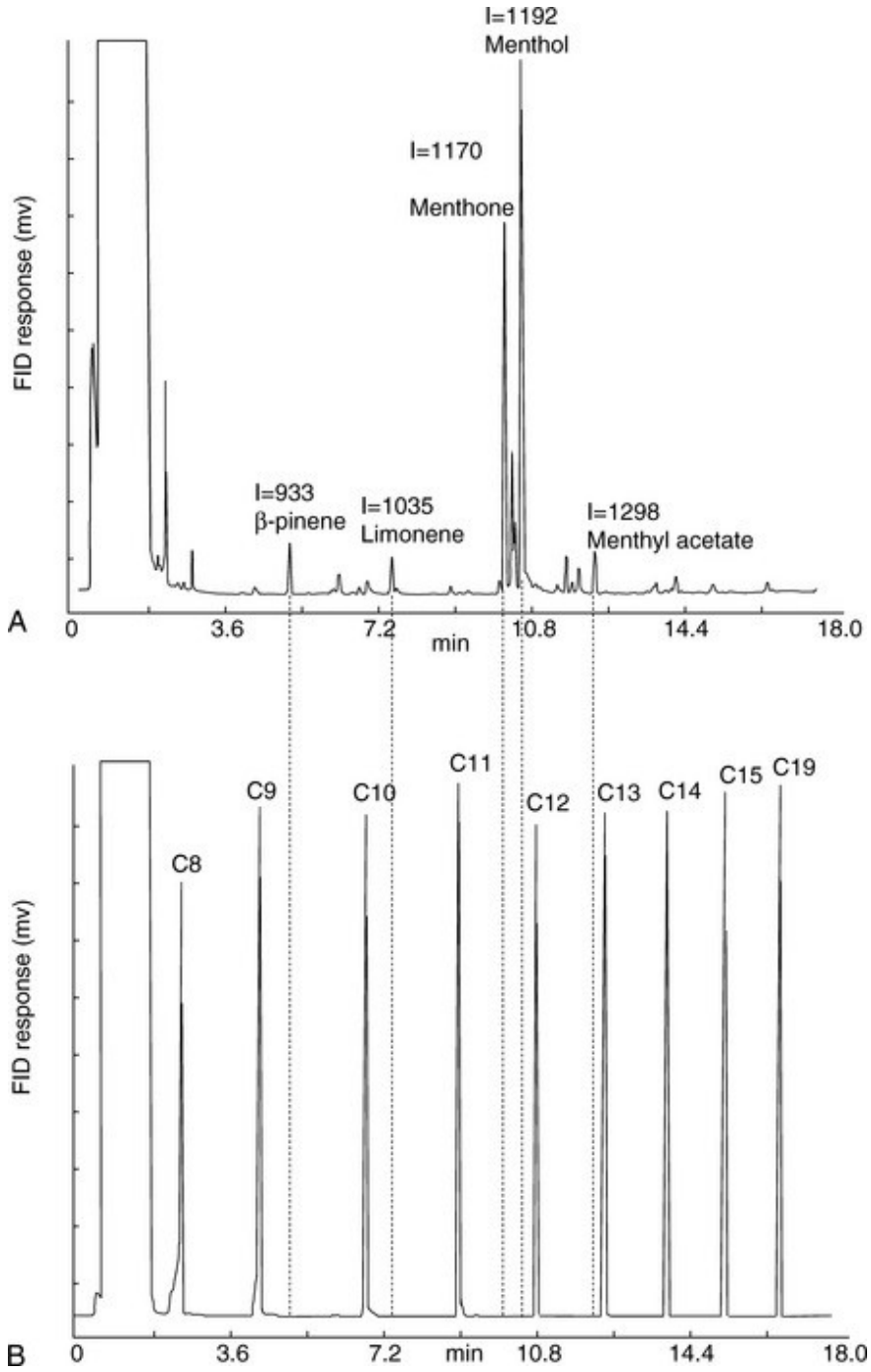
I = 644

Why are these retention index values different?

$$I_X = 100n + 100\,(t_{RX} - t_{Rn} / t_{R(n+1)} - t_{Rn}) =$$

$$= 600 + 100\,(8.25 - 6.50 / 11.50 - 6.50)) = \mathbf{635}$$

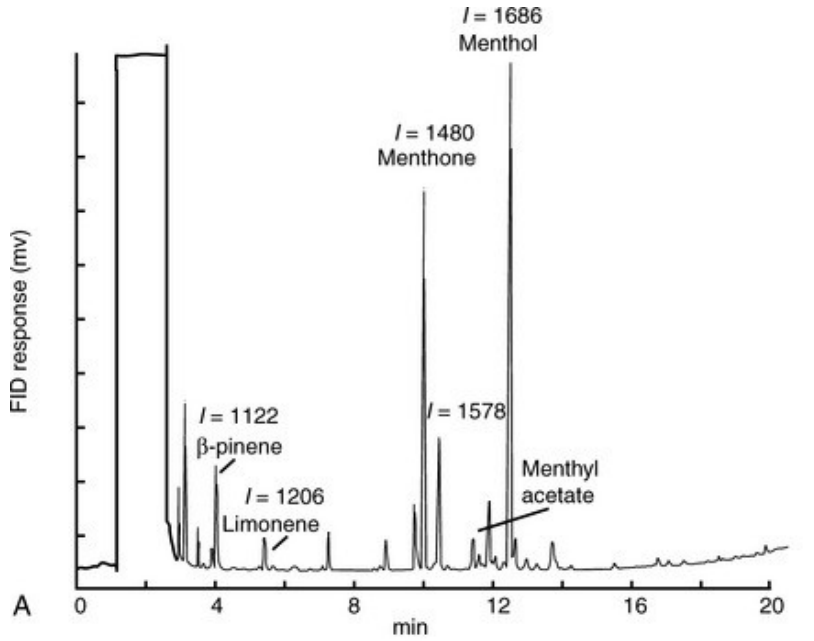
Retention index depends on the GC analysis conditions



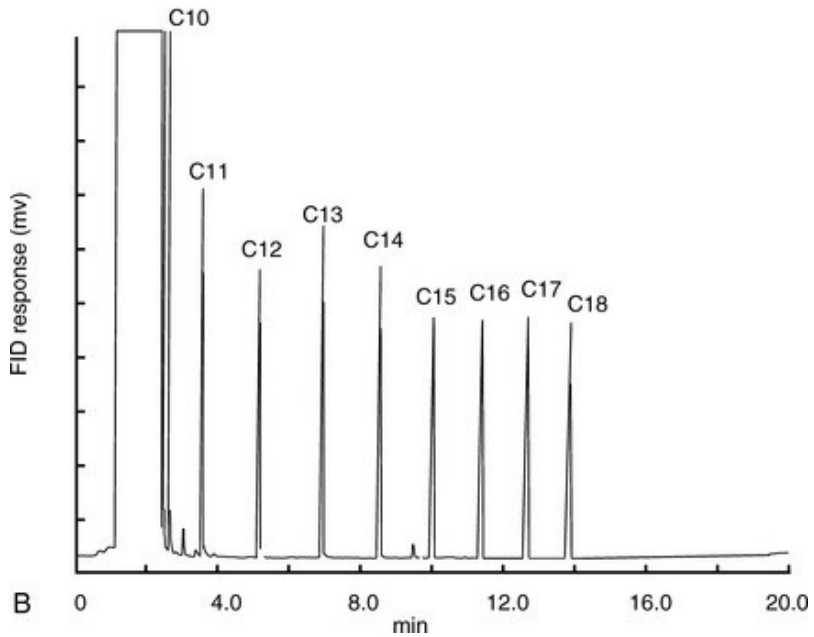
(**A**) Peppermint oil analysed on a BPX-5 column (12 m × 0.25 mm i.d. × 0.25 µm film). Programmed 50°C (1 min), then 5°C/min to 70°C, then 10°C/min to 200°C.

(**B**) *n*-alkanes C8-C16 chromatographed under the same conditions as in (**A**).

Retention index depends on the GC analysis conditions



(**A**) Peppermint oil analysed on a carbowax column (15 m × 0.25 mm i.d. × 0.5 µm film). Programmed 50°C (1 min), then 5°C/min to 70°C, then 10°C/min to 200°C. (B) *n*-alkanes C10-C18 chromatographed under the same conditions.



(**B**) *n*-alkanes C10-C18 chromatographed under the same conditions as in (**A**).

Data from Kjeldsen, Christensen & Edelenbos. *J. Agric. Food Chem.* 2003, **51**, 5400−5407

Determination of Retention Indices. The GC RI of the isolated components were determined externally with a series of *n*-alkanes (C_{10}−C_{25}). GC retention indices were determined on the CP-Wax 52CB column and the chiral column, respectively, and calculated according to the formula given by van den Doll and Kratz (*27*). The GC conditions were the same as described above except that the oven temperature was linearly programmed from 32 °C (1 min isothermal) to 220 °C at 1 °C/min.

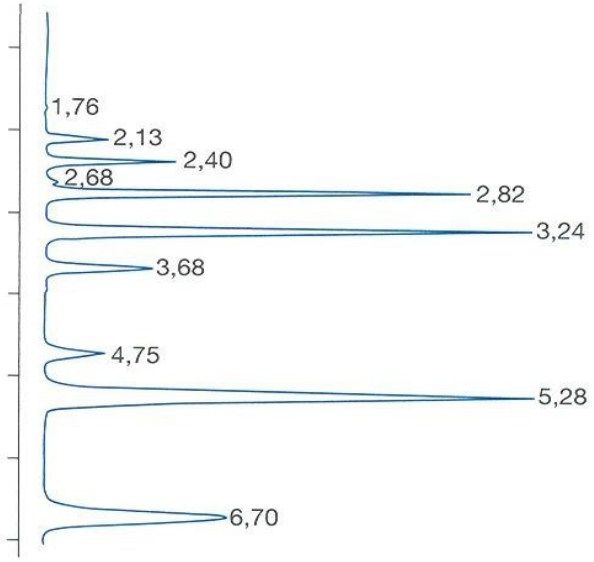
Important in the calculation retention index:

1. The series of standards (typically *n*-alkanes) that have been used to calculate the retention index.
2. Indicate the type of column used.
3. Temperature programming.

Table 1. Odor Description and Identification of Volatiles Isolated from Carrots by Dynamic Headspace Sampling

peak	compound[a]	chemical group[c]	retention index CP-Wax 52CB	retention index β-cyclodextrin	odor description[d]	content[e] (ng/g) refrigerated[f] min	refrigerated[f] max	frozen[f] min	frozen[f] max	CV[g] (%)
1	(−)-α-pinene	MT	1008	1010	sharp, pine, carrot top	34	140	31	46	10.3
2	(+)-α-pinene	MT	1008	1019	pine, carrot top	11	47	11	16	10.3
3	(−)-α-thujene	MT	1010	1002		nq	nq	nq	nq	
4	(−)-camphene	MT	1044	1033		3.7	21	3.3	6.0	17.3
5	(−)-β-pinene	MT	1086	1063	carrot top, fresh green	16	45	15	22	13.9
6	(+)-β-pinene	MT	1086	1067	pine, fresh green	10	29	9.4	14	13.9
7	(+)-sabinene	MT	1105	1044	carrot-like, fresh green	202	421	148	248	11.5
8	(−)-α-phellandrene	MT	1147	1048	herbaceous, green, carrot top	6.6	19	6.1	8.7	12.9
9	β-myrcene	MT	1153	1025	green, terpene-like	89	219	80	113	11.7
10	α-terpinene	MT	1162	1059		13	37	12	23	13.6
11	(−)-limonene	MT	1183	1081	sweet, citrus, fruity	28	73	27	38	10.3
12	(+)-limonene	MT	1183	1085	citrus, fruity	23	60	23	31	10.3
13	(−)-β-phellandrene	MT	1191	1030		19	45	18	32	13.9
14	γ-terpinene	MT	1230	1107	herbaceous, citrus, fruity	145	444	136	205	9.3
15	(*E*)-β-ocimene	MT	1241	1074		11	41	10	12	13.0
16	*p*-cymene	MT	1252	1085	carrot top	28	64	25	60	10.2
17	terpinolene	MT	1266	1125	sweet, fruity, citrus	387	1350	371	570	12.4
18	octanal	FAD	1274	1204		nq	3.6	nq	0.3	66.6
19	6-methyl-5-hepten-2-one	IT	1346	1096	herbaceous, green, spicy	0.2	2.2	2.1	5.2	26.7
20	unknown (*m/z* 135, 150, 91, 79, 107, 65)		1376	1211		1.1	3.6	3.5	4.4	16.6
21	unknown (*m/z* 135, 91, 150, 79, 107, 65)		1389	1390	fresh green	nq	3.7	nq	0.1	26.9
22	unknown monoterpene (*m/z* 79, 110, 95, 77, 67, 119, 152)	MT	1422	1241		1.5	13	2.8	6.8	20.7
23	(−)-α-copaene	ST	1457	1384		3.4	26	2.0	3.9	15.3
24	unknown sesquiterpene (*m/z* 161, 121, 105, 134, 91, 93, 79, 204)	ST	1459	1398	dry hay	2.2	13	1.4	1.9	25.0
25	(−)-camphor	MT	1507	1331		nq	3.1	nq	2.0	46.4
26	unknown sesquiterpene (*m/z* 161, 105, 91, 119, 204, 133, 147)	ST	1518	1417		1.4	6.1	0.7	1.2	38.4
27	(*E*)-α-bergamotene[b]	ST	1565	–[h]		nq	nq	nq	nq	
28	(+)-bornyl acetate	MT	1570	1367	green	nq	nq	nq	nq	
29	β-caryophyllene	ST	1576	1472	terpene-like, spicy, woody	1110	6060	998	1190	10.2
30	thymol methyl ether	MT	1587	1286		2.9	32	2.7	4.8	15.5
31	(+)-aromadendrene	ST	1622	1494		1.5	15	1.4	2.8	21.0
32	(*Z*)-β-farnesene	ST	1632	1433		0.8	5.5	nq	0.8	27.2
33	α-humulene	ST	1640	1510	woody	67	294	56	64	10.5
34	(*E*)-β-farnesene	ST	1650	1488		9.2	71	4.4	9.1	14.6
35	(+)-valencene	ST	1671	1529		4.6	34	1.9	4.5	19.7
36	(+)-α-terpinyl acetate	MT	1679	1429		0.5	5.8	nq	1.0	38.2
37	santalene[b]	ST	1688	–[h]		nq	nq	nq	nq	
38	(+)-borneol	MT	1698	1411		3.7	15	1.6	3.1	17.1
39	unknown sesquiterpene (*m/z* 119, 93, 91, 77, 79, 105, 107, 161, 204)	ST	1700	1465		0.9	2.2	1.1	1.8	28.5
40	(*E,E*)-α-farnesene	ST	1708	1535	green grass	7.1	37	3.7	6.7	14.6
41	(−)-β-bisabolene	ST	1708	1542	sweet	3.3	18	1.9	3.4	14.6
42	unknown sesquiterpene (*m/z* 93, 91, 67, 204, 79, 119, 161, 133, 147)	ST	1713	1567		1.0	2.8	nq	0.8	27.5
43	unknown sesquiterpene (*m/z* 67, 93, 79, 107, 147, 161, 133, 189, 204)	ST	1722	1422	green	1.5	4.7	0.3	1.5	20.3
44	(*E*)-γ-bisabolene	ST	1737	1557	soapy, spicy	117	532	53	112	10.8
45	α-zingiberene[b]	ST	1745	1583		1.9	7.9	0.9	1.6	16.5
46	(*Z*)-γ-bisabolene	ST	1756	1578	fatty, woody	21	126	11	21	13.0
47	(+)-cuparene	ST	1776	1599		nq	0.6	nq	nq	44.0
48	thymohydroquinone dimethyl ether[b]	PP	1873	–[h]		0.2	1.6	0.3	0.7	28.0
49	β-ionone	IT	1909	1622	sweet	0.4	1.6	nq	0.8	37.6
50	caryophyllene oxide isomer[b]	ST	1951	1709		0.4	1.3	nq	0.5	28.3
51	(−)-caryophyllene oxide	ST	1969	1717	citrus	0.9	5.0	0.6	1.4	23.5
52	3-oxo-β-ionone[b]	IT	2025	–[h]		nq	1.1	0.5	0.7	37.7
53	eugenol methyl ether	PP	2036	1532		nq	1.6	nq	nq	42.9
54	eugenol	PP	2151	1525		nq	1.1	nq	0.3	36.0
55	elemicin	PP	2202	1669		1.3	2.8	0.9	1.1	38.4
56	myristicin	PP	2225	1631	spicy	1.2	5.7	1.2	3.0	22.8

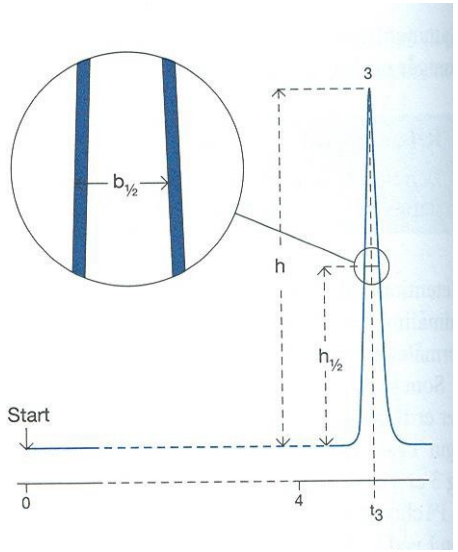
Quantitative analyses



RT	Type	Area	Height	Area%	Name
1,76	BV	9452	3509	0,008	
2,13	BB	2754621	114408	2,438	
2,40	BB	4362766	232952	3,861	
2,68	BV	59431	34296	0,053	
2,82	VB	20194752	721106	17,872	
3,24	BB	25527930	810689	22,591	
3,68	BB	5844274	199226	5,172	
4,75	BB	4328590	108366	3,831	
5,28	BB	31867345	865730	28,201	
6,70	BB	18050371	376286	15,974	

Figur 17.13. Eksempel på integratorudskrift.

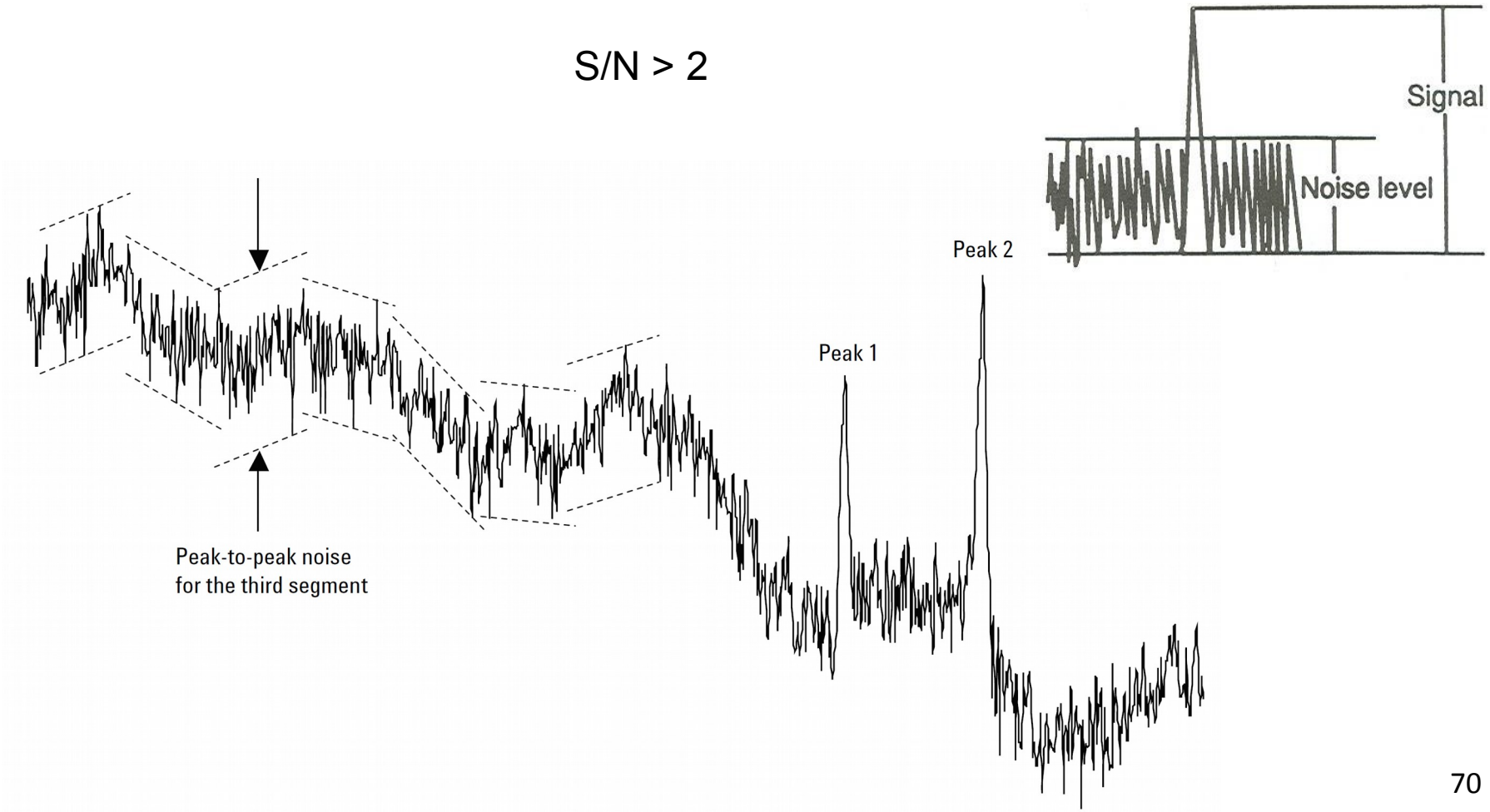
$$\text{Area} = b_{1/2} \times h$$



Noise (‘Støj’)

- Noise is signal from the detector without sample.
- The minimum signal, that can be assumed to be generated by a compound in the sample must be 2 times the noise N:

S/N > 2



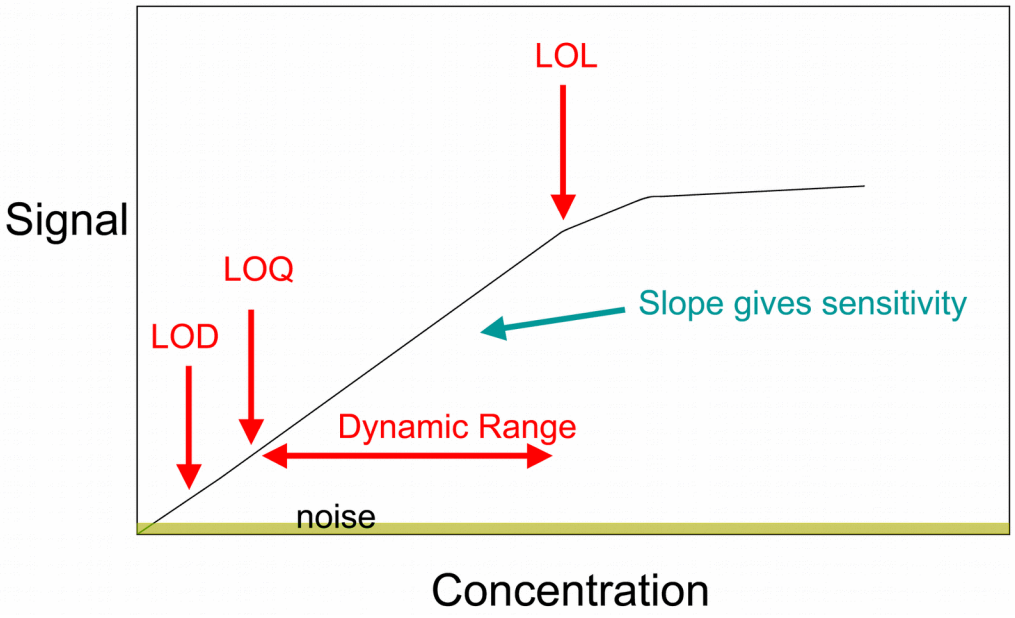
Dynamic range and minimum detectable level

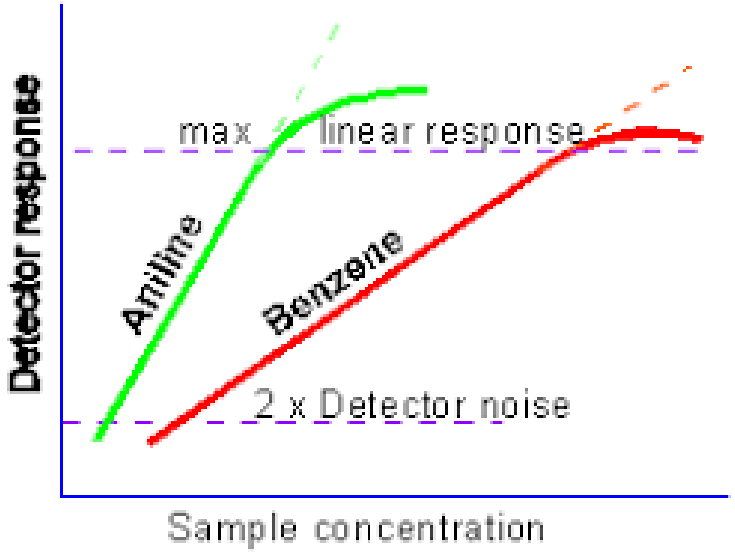
Minimum detectable level (MDL) or LOD of a sample component in the mobile phase that gives a detector signal is equal to twice the noise level and can be calculated from the measured **sensitivity** (S) and **noise** (N):

$$D = 2 \times N/S$$

where D is the **minimum detectability**, expressed either as concentration or mass-flow of the substance of interest in the mobile phase at the detector. Both sensitivity and minimum detectability must be determined for the same substance.

The **Limit of Quantification (LOQ)** is the lowest analyte concentration that can be quantitatively detected with a stated accuracy and precision (will be discussed in the lecture 'Method validation').





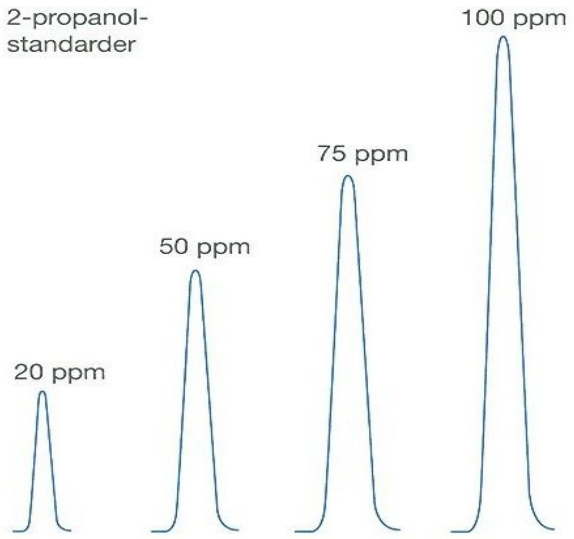
External standard method

- Compound X is to be quantified in a sample
  - A_x (area) is known
  - C_x (concentration) is unknown
- Compound X is quantified by an external standard
  - Both Area (A_{std}) and concentration are known (C_{std})

$$C_x = \frac{A_x}{A_{std}} \times C_{std}$$

Calibration curve

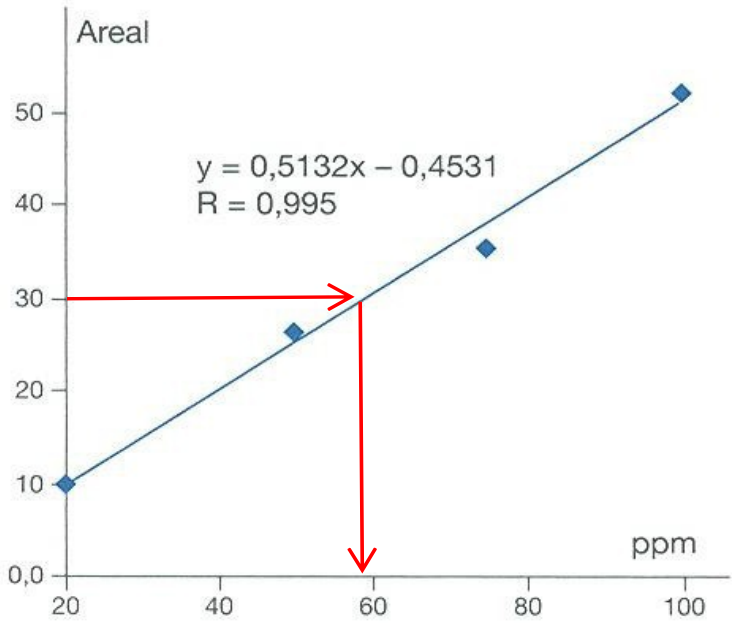
- Make a range of standards in the desired concentration area (check linearity)



Figur 17.16. Koncentrationsbestemmelse med kalibre-ringskurve.

Toppenes arealer aflæses fra integrator:

c (ppm)	20	50	75	100
A ($x10^{-6}$)	9,92	26,3	35,5	52,2



Figur 17.17. Kalibreringskurve.

Internal standard method

- The response factor accounts for differences in detector response between the analyte(s) and the internal standard (IS). Calculation of relative response factor (F) between an analyte X and the internal standard (IS) is calculated as follows:

$A_X = k_X \times n_X = k_X \times C_X V_X$ (analyte X) and $A_{IS} = k_{IS} \times n_{IS} = k_{IS} \times C_{IS} V_{IS}$

where k_X and k_{IS} are the the sensitivities for analyte X and internal standard (IS) towards the detector, respectively, and n = number of moles, C = concentration and V = volume.Taking the ratio of the two signals where $V_X = V_{IS}$ (same sample) gives:

$(A_X / A_{IS}) = (k_X / k_{IS}) \times (C_X / C_{IS})$

The above equation is defined in terms of a ratio of the analyte(s) sensitivity and the internal standard sensitivity, and this ratio is called the relative response factor F_{rel}. Thus, F_{rel} between an analyte X and IS is given by the equation:

$$F_{rel} = (A_X C_{IS} / A_{IS} C_X)$$

F_{rel} can be calculated when A_X, A_{IS}, C_X, and C_{IS} are known.

Internal standard method

- The sample is analyzed with the IS added
  - IS area (A_{is}) and concentration (C_{is}) are known and the analyte area in the sample (A_x) and the response factor between the analyte and IS is known, then the concentration of the analyte (C_x) can be calculated.

$$C_X = (A_X / F_{rel}\, A_{IS}) \times C_{IS}$$

- Often for complex samples the relative response factor (F_{rel}) is assumed to be 1 for all analytes. This is especially the case for complex volatile samples. *This equation is similar to the external standard equation*.

$$C_X = (A_X / A_{IS}) \times C_{IS}$$

Derivatisation

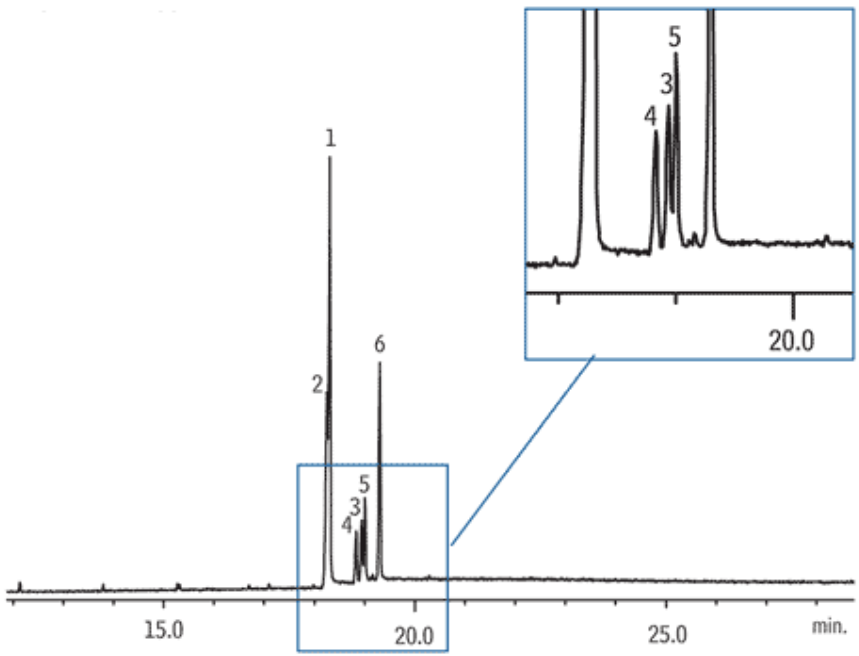
- Compounds that have poor volatility, poor thermal stability, or that can be adsorbed in the injector will exhibit non reproducible peak areas, heights and shapes.

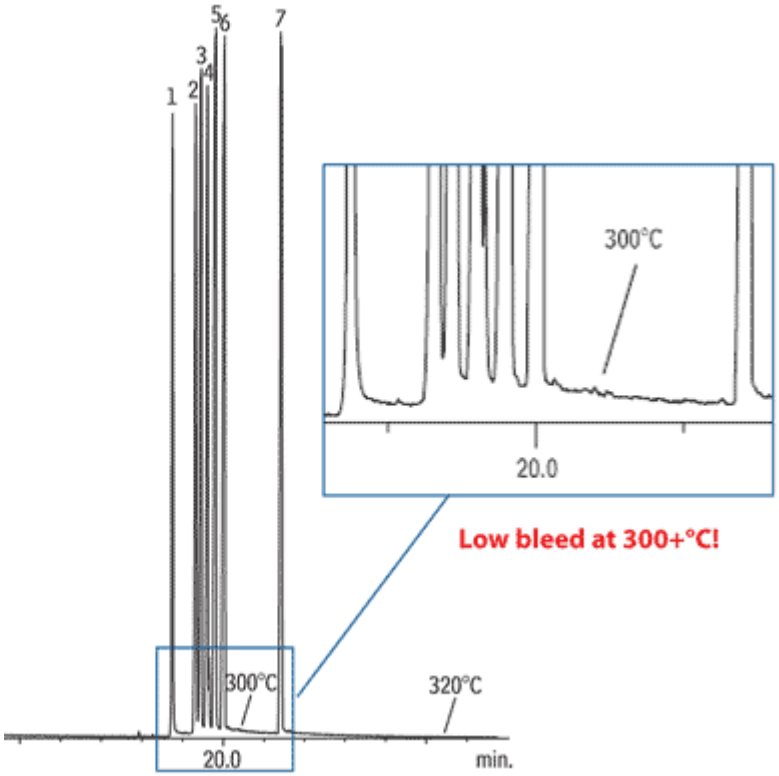
- Usually derivatisation is used to improve the volatility and polarity and thereby to reduce the analysis time and temperature programming at high temperatures.

- A good derivatizing reagent and procedure should produce the desired chemical modification of the compound(s) of interest, and to be reproducible, efficient, and nonhazardous.

Derivatisation and GC analysis of steroids

1. androsterone
2. dehydroepiandrosterone (DHEA)
3. 17-α-estradiol
4. estrone
5. 17-β-estradiol
6. testosterone
7. derivatization by-product

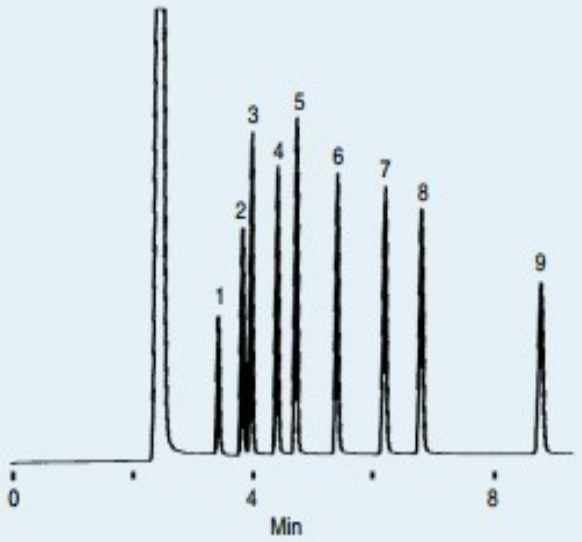




Fatty acids (FA)

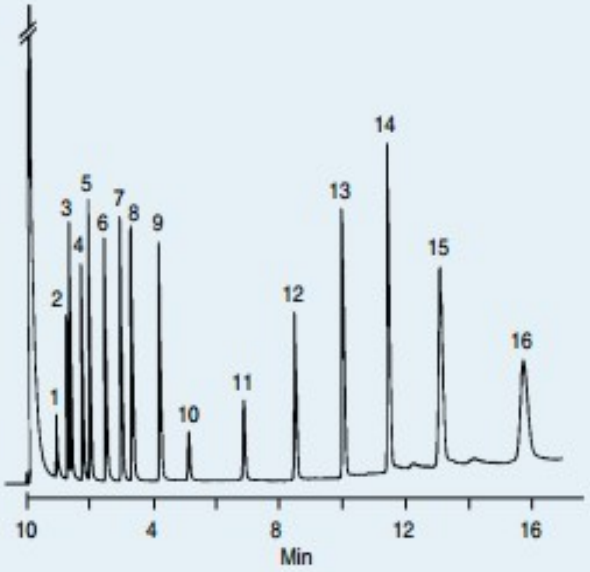
column: Nukol, 30 m x 0.25 mm I.D., 0.25 µm (24107)
oven: 185 °C
det.: FID
carrier gas: helium, 20 cm/sec
injection: 1 µL, 100:1 split
sample: Volatile Free Acid Mix (46975-U), each analyte at 10 mM in deionized water

1. Acetic acid
2. Propionic acid
3. Isobutyric acid
4. Butyric acid
5. Isovaleric acid
6. Valeric acid
7. Isocaproic acid
8. Caproic acid
9. Heptanoic acid



column: Nukol, 15 m x 0.53 mm I.D., 0.50 µm (25326)
oven: 100 °C, 10 °C/min. to 220 °C
det.: FID
carrier gas: helium, 30 mL/min.
injection: 0.5 µL, direct injection
sample: 16 analytes, at various concentrations from 50 to 800 µg/mL

1. Acetic acid
2. Propionic acid
3. Isobutyric acid
4. Butyric acid
5. Isovaleric acid
6. Valeric acid
7. Isocaproic acid
8. Caproic acid
9. Heptanoic acid
10. Octanoic acid
11. Decanoic acid
12. Dodecanoic acid
13. Tetradecanoic acid
14. Hexadecanoic acid
15. Octadecanoic acid
16. Eicosanoic acid



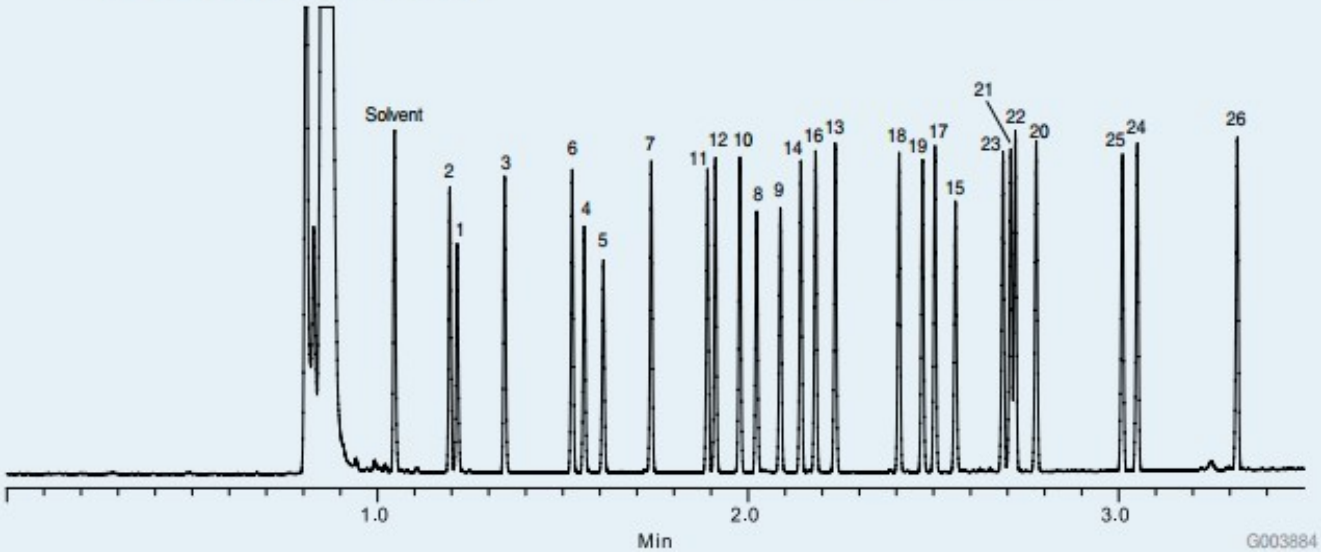
Fatty Acid Methyl Esters (FAME)

Figure 4. Esterification Reaction

$$R\text{-}COOH + CH_3OH \xrightarrow{BCl_3} R\text{-}COO\text{-}CH_3 + H_2O$$

column: Equity-1, 15 m x 0.10 mm I.D., 0.10 µm (28039-U)
oven: 175 °C, 30 °C/min. to 275 °C (1 min.)
inj.: 280 °C
det.: FID, 280 °C
carrier gas: hydrogen, 45 cm/sec constant
injection: 0.5 µL, 200:1 split
liner: 4 mm I.D., split, cup design
sample: Bacterial Acid Methyl Ester (BAME) Mix (47080-U), methyl ester derivatives total concentration of 10 mg/mL in methyl caproate

1. Methyl 2-hydroxydecanoate (2-OH-C10:0)
2. Methyl undecanoate (C11:0)
3. Methyl dodecanoate (C12:0)
4. Methyl 2-hydroxydodecanoate (2-OH-C12:0)
5. Methyl 3-hydroxydodecanoate (3-OH-C12:0)
6. Methyl tridecanoate (C13:0)
7. Methyl tetradecanoate (C14:0)
8. Methyl 2-hydroxytetradecanoate (2-OH-C14:0)
9. Methyl 3-hydroxytetradecanoate (3-OH-C14:0)
10. Methyl pentadecanoate (C15:0)
11. Methyl 13-methyltetradecanoate (i-C15:0)
12. Methyl 12-methyltetradecanoate (α-C15:0)
13. Methyl hexadecanoate (C16:0)
14. Methyl 14-methylpentadecanoate (i-C16:0)
15. Methyl-2-hydroxyhexadeanoate (2-OH-C16:0)
16. Methyl cis-9-hexadecenoate ($C16:1^{9}$)
17. Methyl heptadecanoate (C17:0)
18. Methyl 15-methylhexadecanoate (i-C17:0)
19. Methyl cis-9,10-methylenehexadecanoate ($C17:0^{\Delta}$)
20. Methyl octadecanoate (C18:0)
21. Methyl cis-9-octadecenoate ($C18:1^{9}$)
22. Methyl trans-9-octadecenoate ($C18:1^{9}$) and Methyl cis-11-octadecenoate ($C18:1^{11}$)
23. Methyl cis-9,12-octadecadienoate ($C18:2^{\Delta 9,12}$)
24. Methyl nonadecanoate (C19:0)
25. Methyl cis-9,10-methyleneoctadecanoate ($C19:0^{\Delta}$)
26. Methyl eicosanoate (C20:0)



Analysis of FAMEs

Figure 23. Detailed Analysis of cis/trans C18 FAME Isomers on the 75 m SP-2560

column: SP-2560, 75 m x 0.18 mm I.D., 0.14 µm (23348-U)
oven: 180 °C
inj.: 220 °C
det.: FID, 220 °C
carrier gas: hydrogen, 25 cm/sec @ 180 °C
injection: 0.5 µL, 100:1 split
liner: 4 mm I.D., split, cup design
sample: Mixture of C18:1, C18:2, and C18:3 FAMEs in methylene chloride

1. C18:1 Δ 6t
2. C18:1 Δ 6c
3. C18:1 Δ 7t
4. C18:1 Δ 7c
5. C18:1 Δ 9t
6. C18:1 Δ 9c
7. C18:1 Δ 11t
8. C18:1 Δ 11c
9. C18:1 Δ 12t
10. C18:1 Δ 12c
11. C18:1 Δ 13t
12. C18:1 Δ 13c
13. C18:2 Δ 9t,12t
14. C18:2 Δ 9c,12t
15. C18:2 Δ 9t,12c
16. C18:2 Δ 9c,12c
17. C18:3 Δ 9t,12t,15t
18. C18:3 Δ 9c,12t,15c
19. C18:3 Δ 9t,12c,15t
20. C18:3 Δ 9c,12c,15t
21. C18:3 Δ 9c,12t,15t
22. C18:3 Δ 9c,12t,15c
23. C18:3 Δ 9t,12c,15c
24. C18:3 Δ 9c,12c,15c

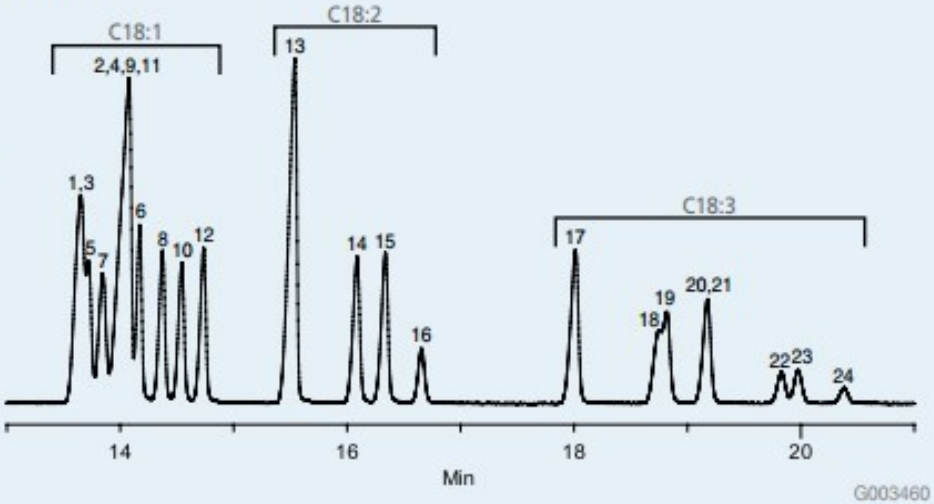
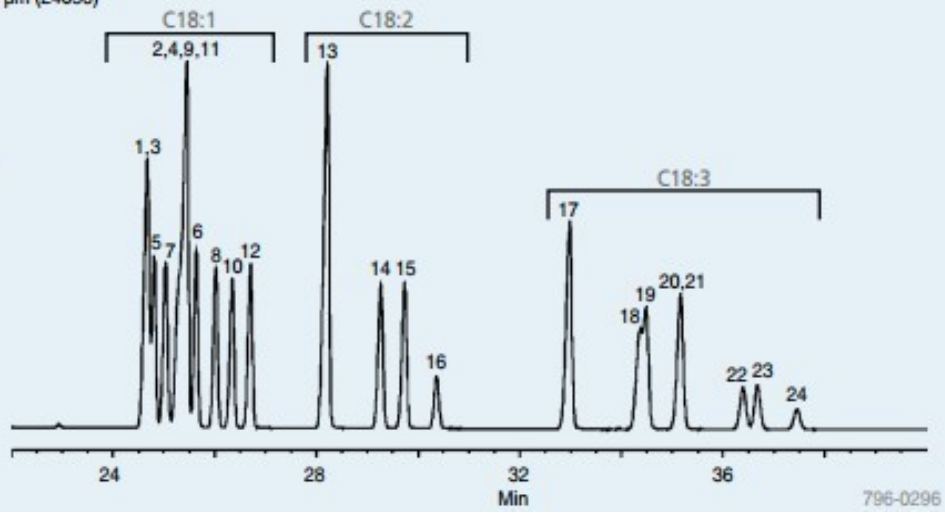


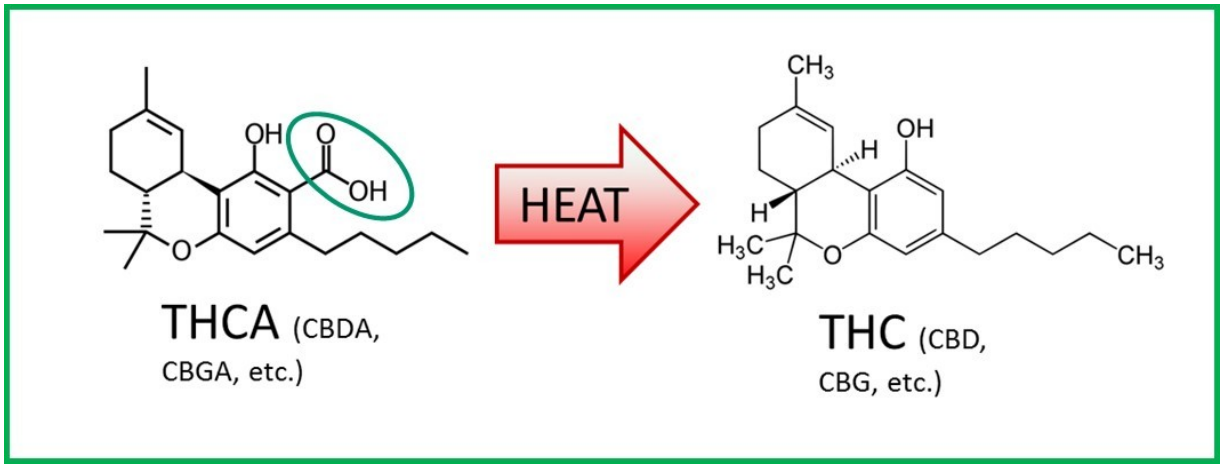
Figure 24. Detailed Analysis of cis/trans C18 FAME Isomers on the 100 m SP-2560

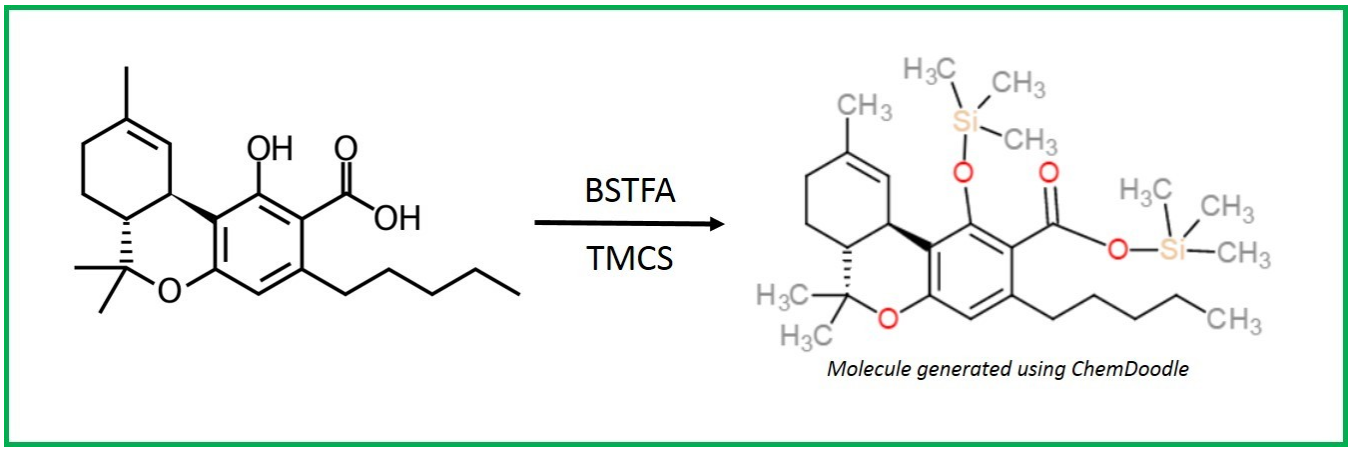
column: SP-2560, 100 m x 0.25 mm I.D., 0.20 µm (24056)
oven: 175 °C
inj.: 210 °C
det.: FID, 250 °C
carrier gas: helium, 20 cm/sec @ 175 °C
injection: 1.0 µL, 100:1 split
sample: Mixture of C18:1, C18:2, and C18:3 FAMEs in methylene chloride

Same Peak IDs as Figure 23



Analysis of cannabinoids by GC





The derivatization technique that can be used for the analysis of unstable cannabinoids involves N,O-Bis(trimethylsilyl)trifluoracetamide + 1% trimethylchlorosilane (BSTFA + 1% TMCS). This derivatization reagent targets –OH groups and replaces the hydrogens i with a trimethylsilyl group maling the compounds more volatile and thermostable.

GC-FID analysis of derivatized cannabinoids

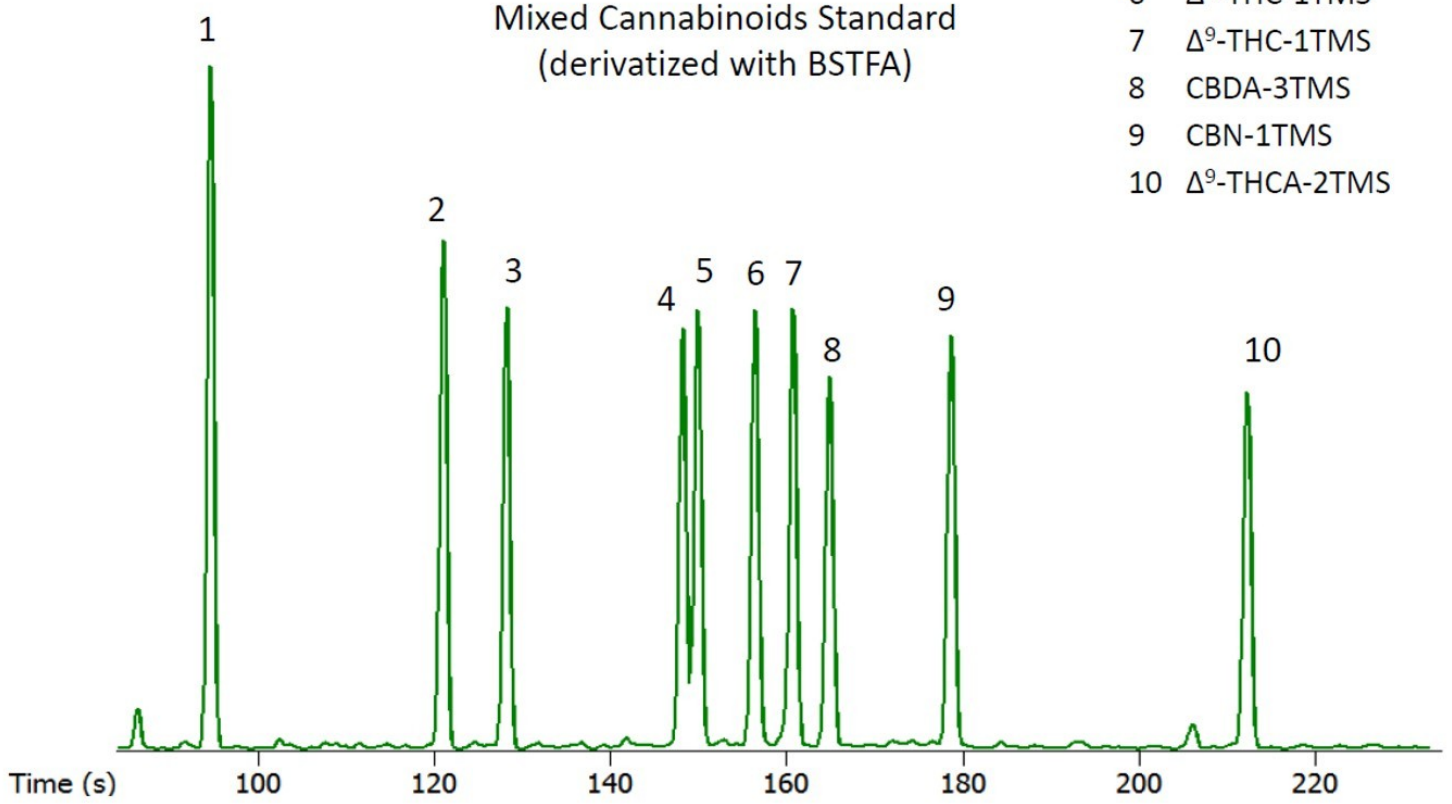
15m x 0.25mm x 0.25µm Rxi-35Sil MS

H_2 at 2.5 mL/min constant flow

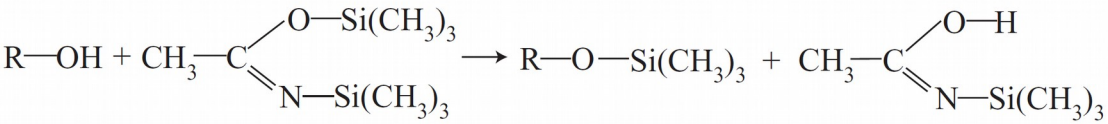
Oven: 180°C (0.1 min), 25°C/min to 320°C

Run time 5.7 min

1 CBDV-2TMS
2 CBD-2TMS
3 THCV-1TMS
4 CBC-1TMS
5 CBG-2TMS
6 Δ^8-THC-1TMS
7 Δ^9-THC-1TMS
8 CBDA-3TMS
9 CBN-1TMS
10 Δ^9-THCA-2TMS



Derivatisation of various functional groups for GC analysis



The reagent is bis(trimethylsilyl)-trifluoroacetamide (BSTFA) + silylation reagent (e.g., trimethylchlorosilane (TMCS))

In general, the ease of reaction follows the order:

alcohols > phenols > carboxylic acids > amines > amides

TABLE 13.3 Guide to Derivatization

Functional Group	Method	Derivatives
Acids	Silylation	$RCOOSi(CH_3)_3$
	Alkylation	RCOOR′
Alcohols and phenols—unhindered and moderately hindered	Silylation	$R—O—Si(CH_3)_3$
	Acylation	R—O—C(=O)—PFA
	Alkylation	R—O—R′
Alcohols and phenols—highly hindered	Silylation	$R—O—Si(CH_3)_3$
	Acylation	R—O—C(=O)—PFA
	Alkylation	R—O—R′
Amines (1°& 2°)	Silylation	$R—N—Si(CH_3)_3$
	Acylation	R—N—C(=O)—PFA
	Alkylation	R—N—R′
Amines (3°)	Alkylation	PFB carbamate
Amides	SiIylation (a)	(a) RC(=O)—NHSi(CH3)3(unstable)
	Acylation (b)	(b) RC(=O)—NH—C(=O) PFA
	Alkylation (c)	(c) RC(=O)—NHCH3
Amino acids	Esterification/acylation	
	Silylation (a)	(a) RCHCOOSi(CH3)3 / N-Si(CH3)3
	Acylation + silylation (b)	(b) RCHOOSi(CH3)3 / N—TFA
	Alkylation (c)	(c) RCHCOOR′ / NHR′
Catecholamines	Acylation + silylation (a)	(a) R—N—HFB; O Si(CH3)3; O Si(CH3)3
	Alcylation (b)	(b) R—N—HFB; OHFB; OHFB
Carbohydrates and sugars	Silylation (a)	(a) O Si(CH3)3 / —(CH2)x—
	Acylation (b)	(b) OTFA / —(CH2)x—
	Alkylation (c)	(c) OR / —(CH2)x—
Carbonyls	Silylation	TMS—O—N=C
	Alkylation	CH3—O—N=C

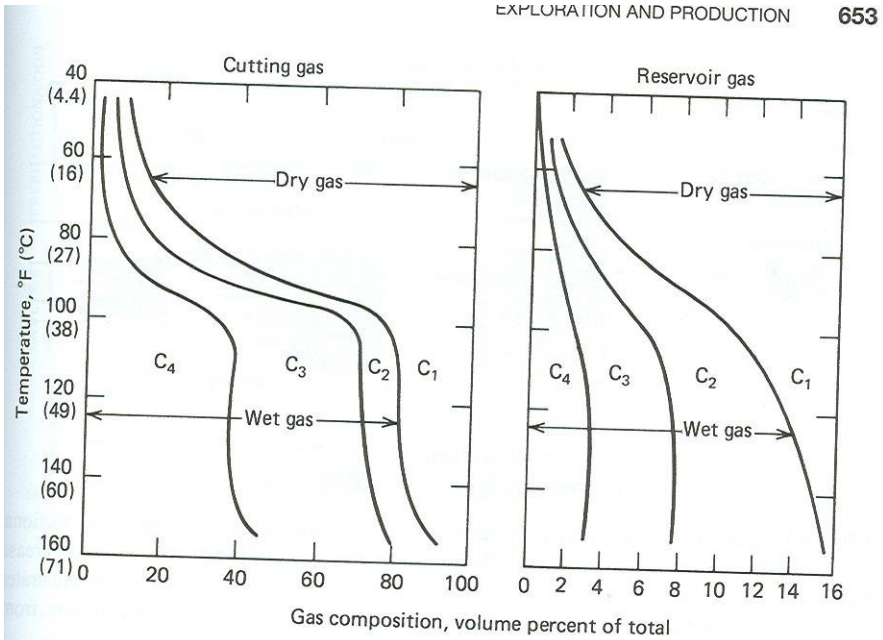
Abbreviations: TMS, trimethyl silyl; PFA, perfluoroacyl; TFA, trifluoracetyl; HFB, heptafluorobutyryl.

Source: Courtesy of Regis Chemical.

Examples of applications

- Oil/petrol industry / geology
- Pharmaceutical companies
- Narcotics
- Environment
- Biology (taxonomy)
- Food and aroma analysis

Geology for oil prospecting



IGURE 13.3 The composition of Cretaceous gas in source and reservoir rocks versus ıbsurface temperature. (Reprinted with permission from Reference 24.)

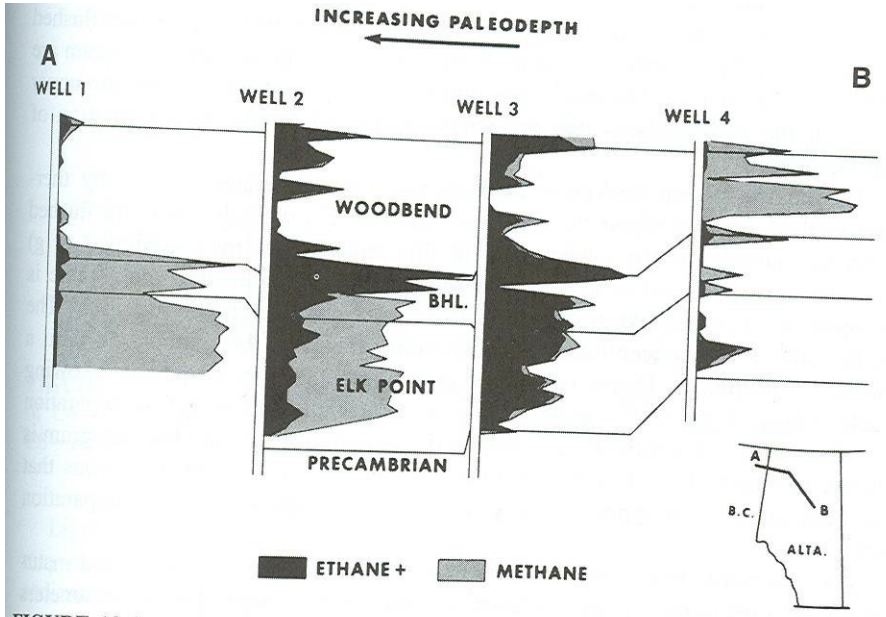


FIGURE 13.4 Cuttings-gas (C1–C4) composition (in log cross-sectional form) of Upper and Middle Devonian strata. The maximum paleodepth increases from east to west. The initial decrease and subsequent decrease in wet gas in this direction illustrate the transition from immaturity to maturity to metamorphism with increasing temperature. (Reprinted with permission from Reference 25.)

Oil industry

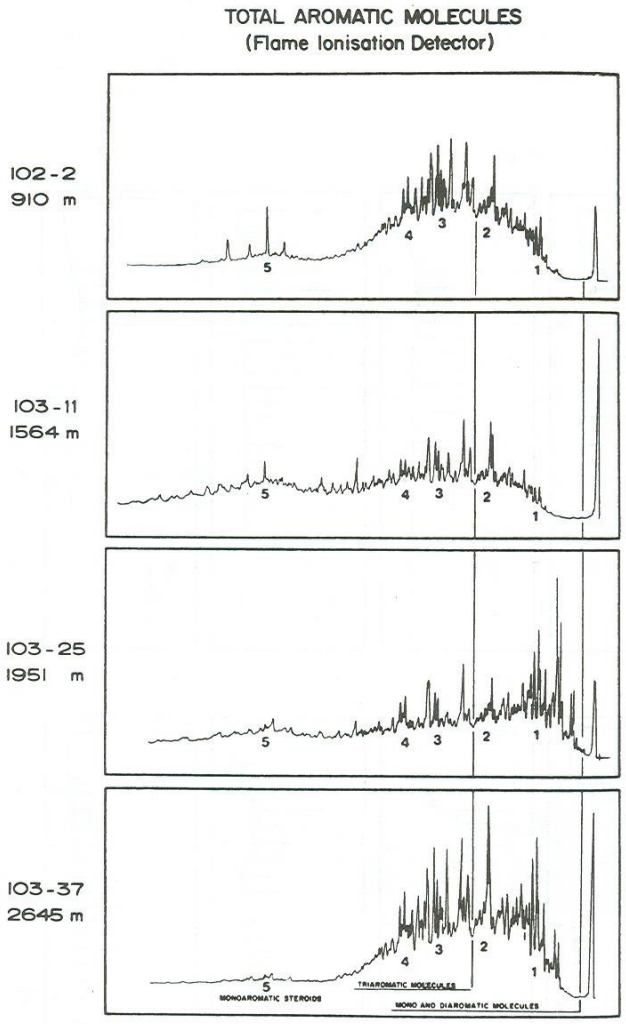


FIGURE 13.12 Analyses of the aromatic fraction of some of the same oil shown in Figure 13.11 showing the change in composition and distribution as a function of maturity. (Reprinted with permission from Reference 50, Copyright 1976, Pergamon Press, Ltd.)

Oil industry

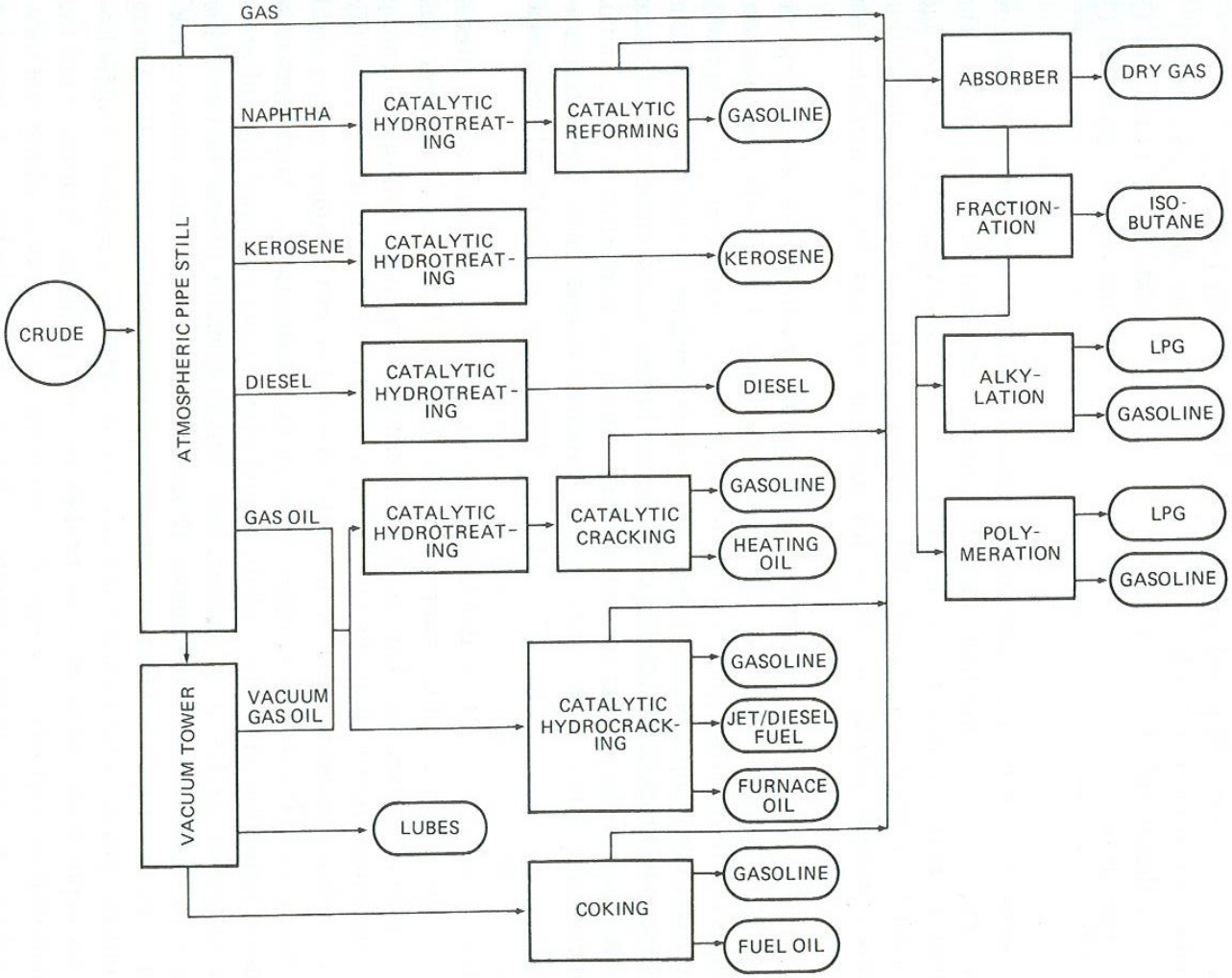
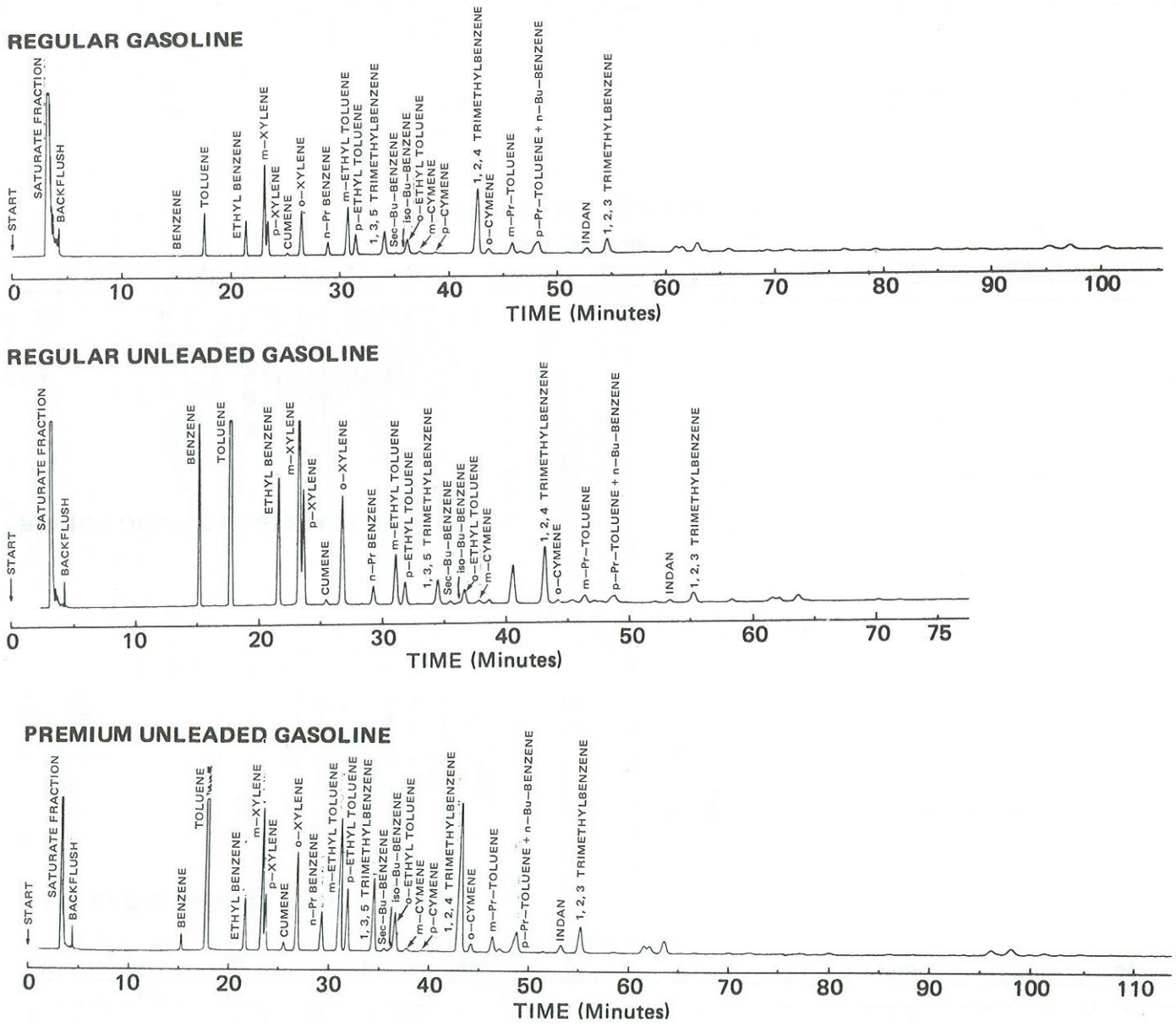


FIGURE 13.17 Simplified flow diagram of a petroleum refining process.

Oil industry



Oil industry

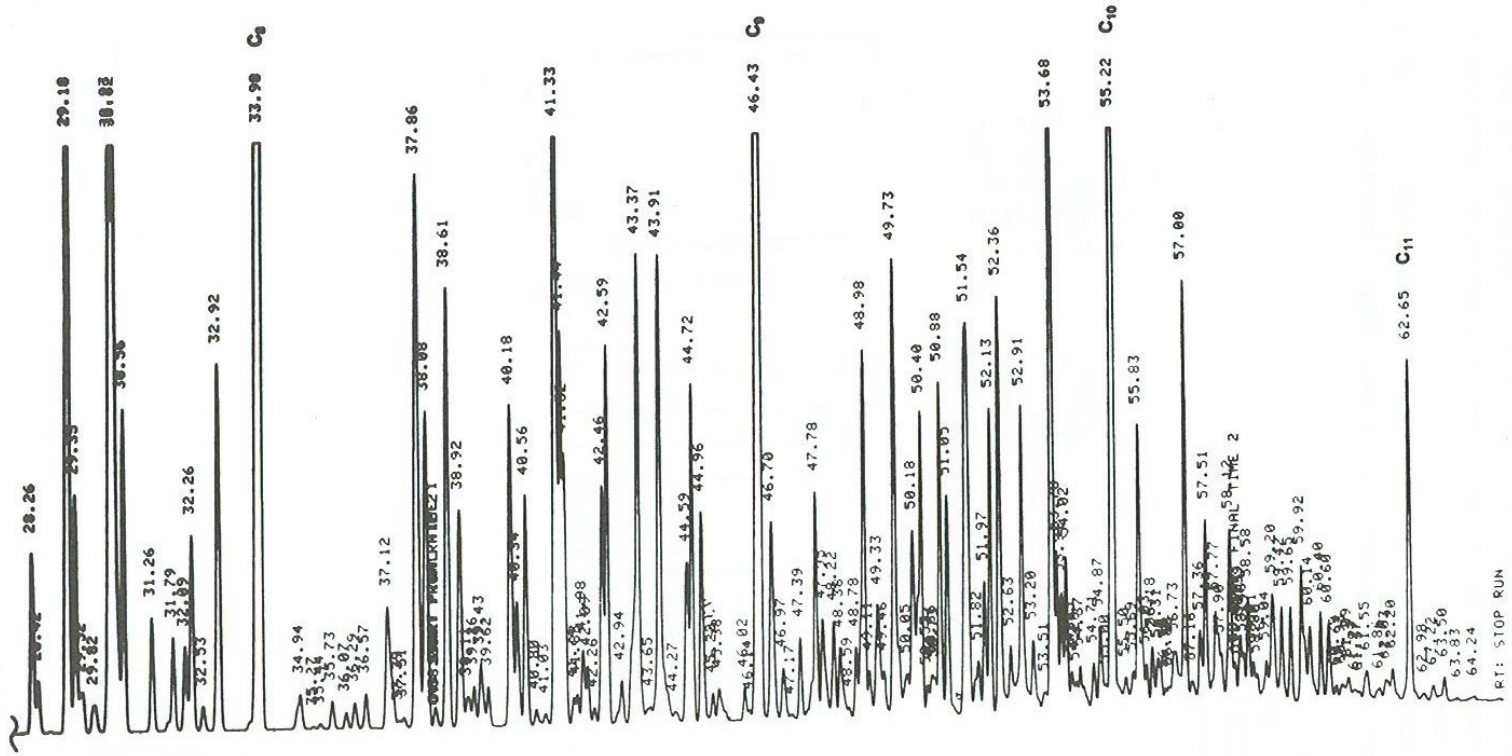


FIGURE 13.33 Typical chromatogram for PONA analysis of a naphtha sample. (Reprinted with permission from Reference 96, Hewlett–Packard Co.)

Pharmaceuticals

TABLE 14.7 Recoveries for Antiepileptic Drugs

Substance	Recovery (%)
Valproic acid	85.1
Caprilic acid	78.0
Ethosuximide	31.5
Phenobarbital	96.0
Primidone	75.0
Carbamazepina	88.0
Phenytoin	100.0

TABLE 14.8 Retention Times for Antiepileptic Drugs

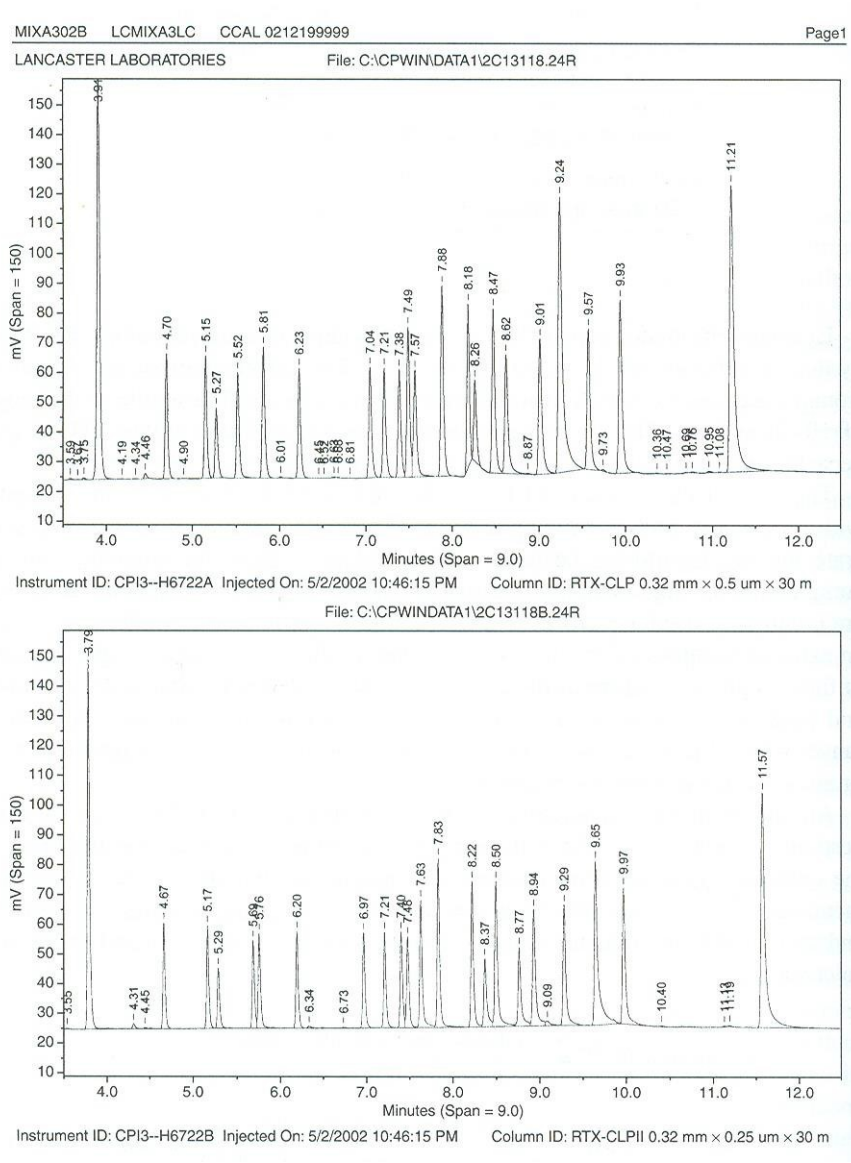
Substance	Retention Time (min)
Valproic acid	6.1
Caprilic acid	6.4
Ethosuximide	7.1
Hexobarbital	13.1
Phenobarbital	14.2
Primidone	20.2
Carbamazepina	23.1
Phenytoin	23.5
MMPH	27.6

Narcotics

TABLE 14.10 Retention Times of Drugs of Abuse

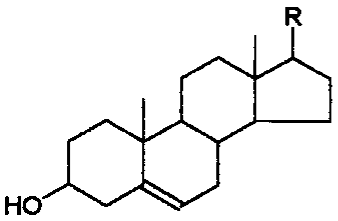
Drug	Retention Time (s)
Amphetamine	19
Methamphetamine	21
Butabarbital	41
Amobarbital	43
Meperidine	45
Pentobarbital	46
Secobarbital	47
Glutethimide	48
Phencyclidine	51
Phenobarbital	53
Methadone	65
Methaqualone	66
Amitrptyline	67
Cocaine	68
Imipramine	70
Desipramine	72
Pentazocine	73
Codeine	80
Heroin	90

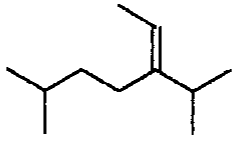
Environmental analyses

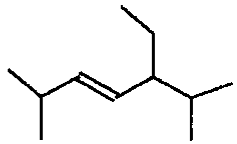


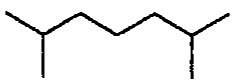
RT CLP1 (min)	RT CLP2 (min)	Compound Name	Conc. ng/mL
3.91	3.79	TCX	40
4.70	5.17	α-BHC	10
5.15	5.17	γ-BHC	10
5.27	5.29	β-BHC	10
5.52	5.70	δ-BHC	10
5.82	5.76	heptachlor	10
6.23	6.20	aldrin	10
7.05	6.97	hept. epoxide	10
7.21	7.21	γ-chlordane	10
7.38	7.40	α-chlordane	10
7.57	7.48	endosulfan I	10
7.49	7.63	4,4'-DDE	20
7.88	7.83	dieldrin	20
8.18	8.23	endrin	20
8.26	8.37	4,4'-DDD	20
8.47	8.50	endosulfan II	20
8.62	8.77	4,4'DDT	20
9.01	8.94	endrin aldehyde	20
9.24	9.65	methoxychlor	100
9.57	9.29	endosulfan sulf.	20
9.93	9.97	endrin ketone	20
11.21	11.57	DCB	40

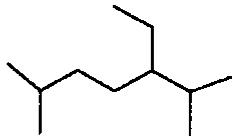
Taxonomy

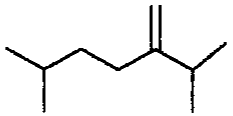


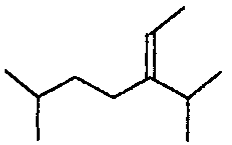


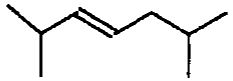


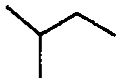


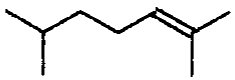


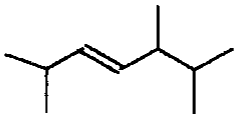


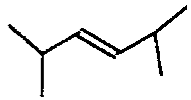












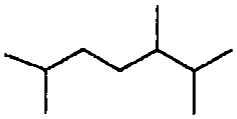
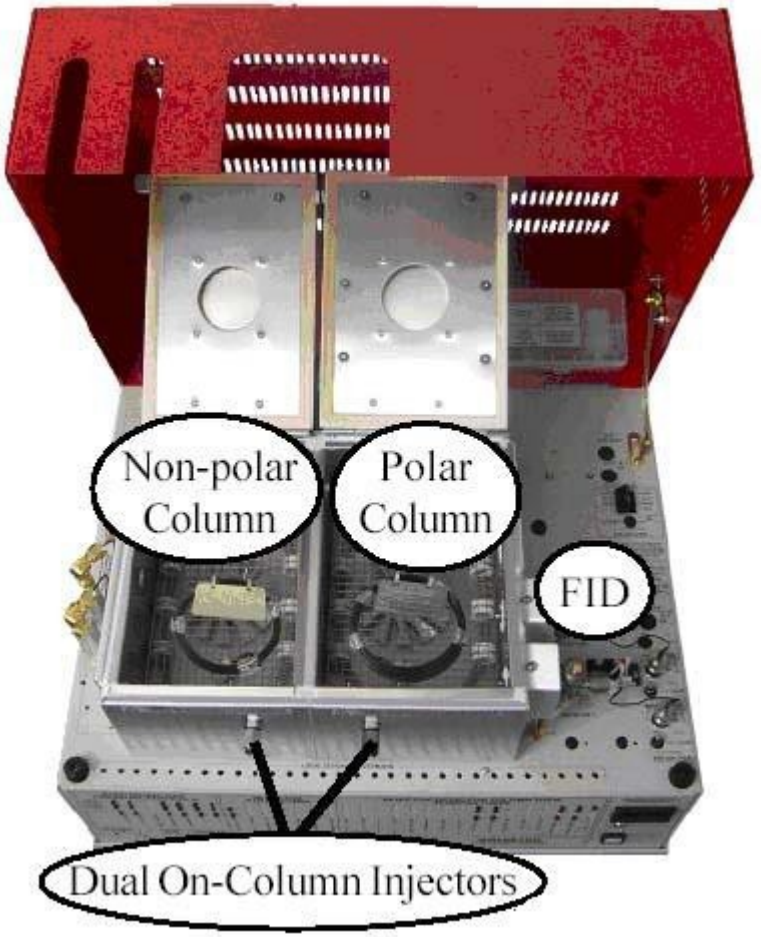


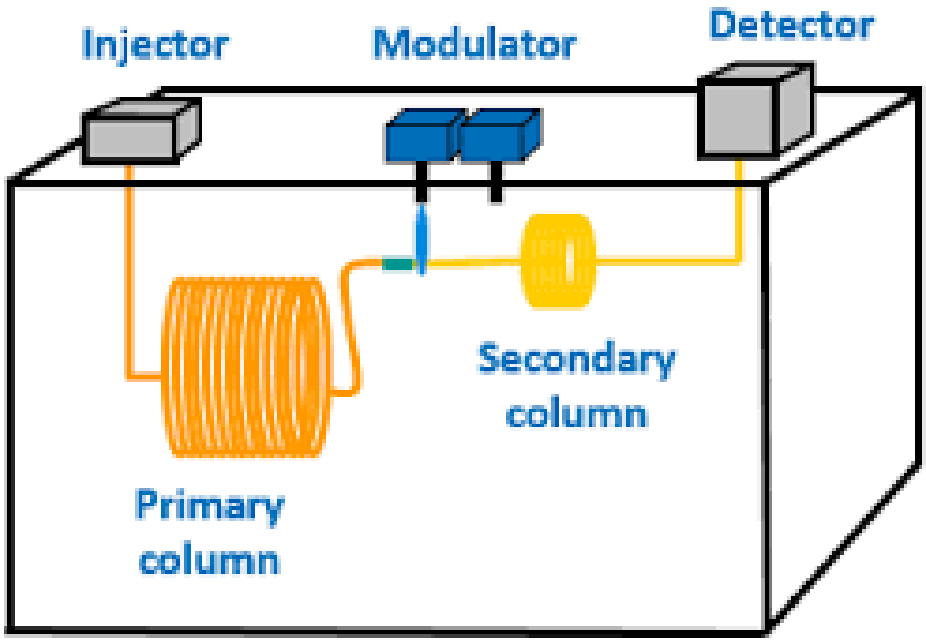
Table I. Sterol composition of *Zanardinia prototypus* *Striaria attenuata* (% of the total sterol mixture*)

Sterol (Fig. 1)	*Z. proto-typus*	*S. nu*
Fucosterol (1)	85	16
Cholesterol (2)	6	22
24-Methylenecholesterol (3)	5	18
22E-Dehydrocholesterol (4)	tr.**	–
Desmosterol (5)	tr.	tr.
Brassicasterol (6)	tr.	2
Campesterol (7)	tr.	tr.
Stigmasterol (8)	2	11
Sitosterol (9)	–	16
Isofucosterol (10)	1	6
11	tr.	–
13	–	tr.

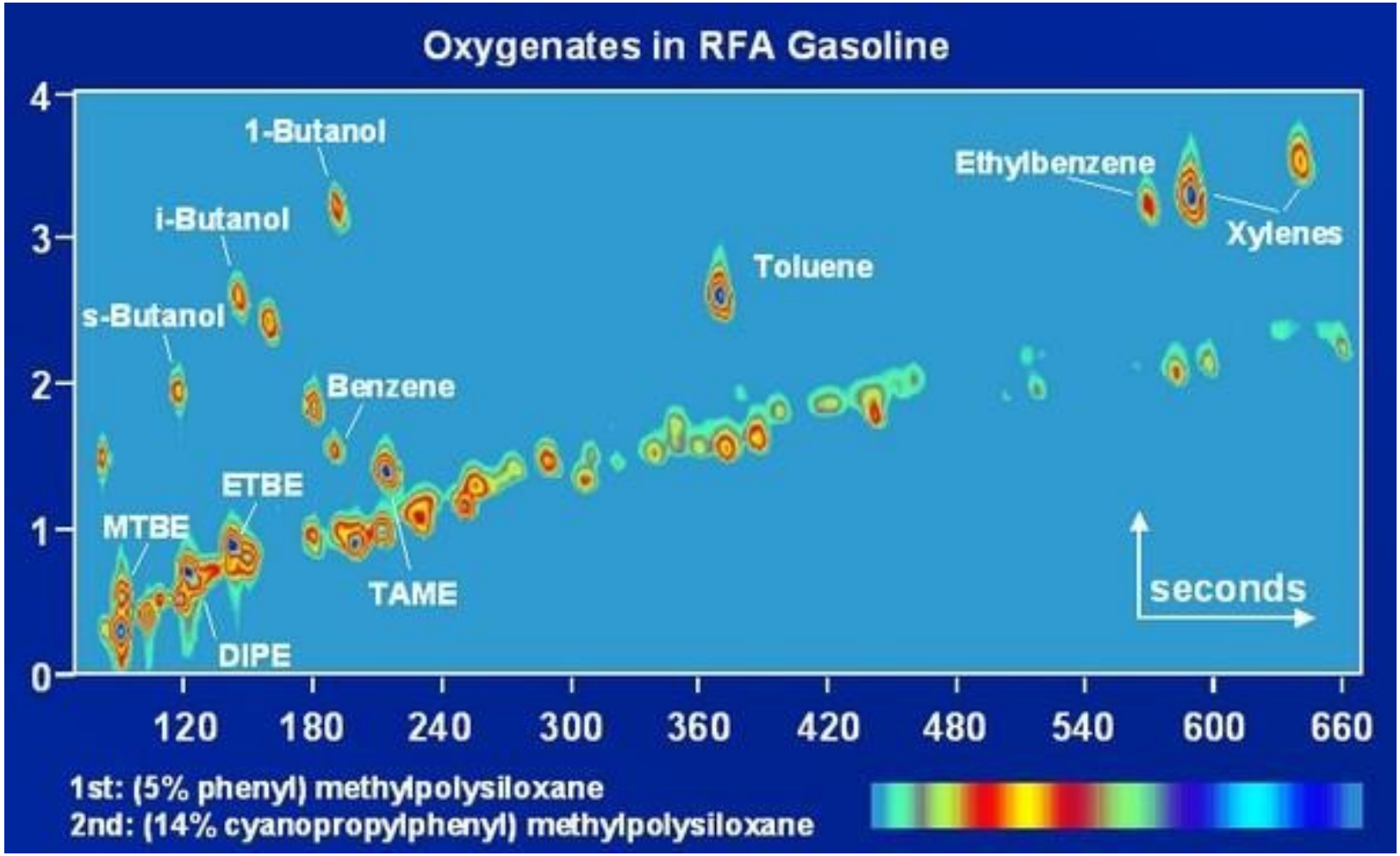
* Values, obtaines from three parallel measurements
** tr.< 0.5%

2D-GC

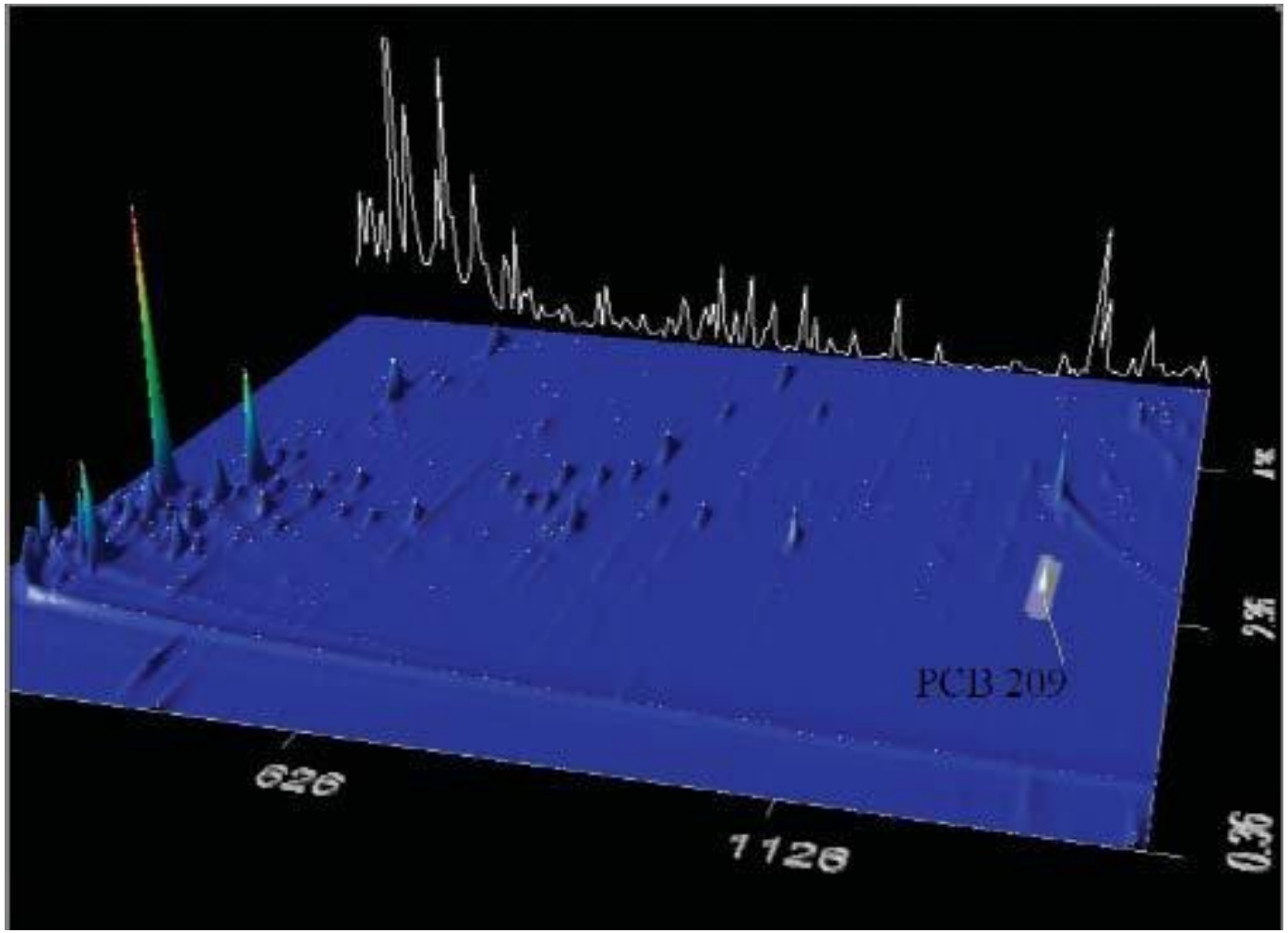

Non-polar Column
Polar Column
FID
Dual On-Column Injectors


Injector
Modulator
Detector
Secondary column
Primary column

2D-GC


Oxygenates in RFA Gasoline
1-Butanol
i-Butanol
s-Butanol
Benzene
ETBE
MTBE
TAME
DIPE
Toluene
Ethylbenzene
Xylenes
seconds
4
3
2
1
0
120
180
240
300
360
420
480
540
600
660
1st: (5% phenyl) methylpolysiloxane
2nd: (14% cyanopropylphenyl) methylpolysiloxane

2D-GC



Organochlorine pesticides in tomatoes

Latest developments

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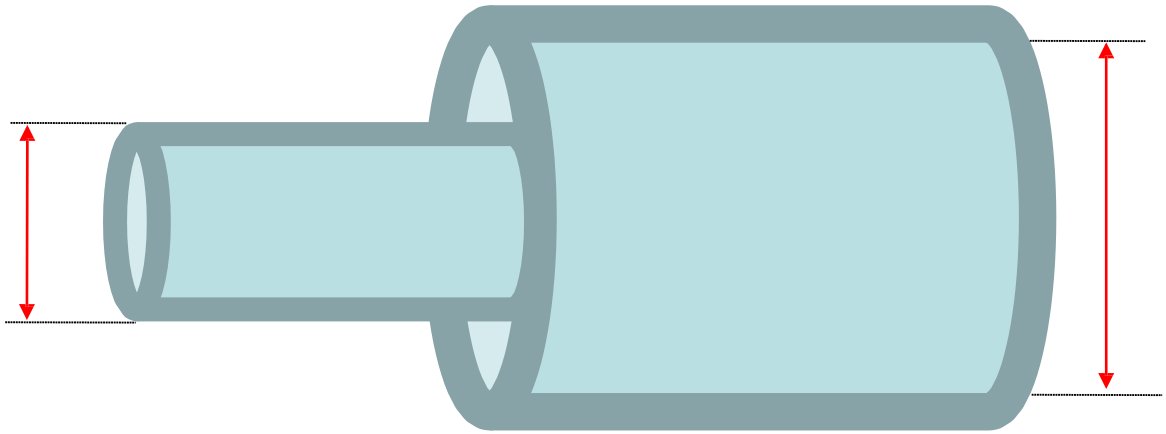
Single fiber-in-capillary annular column for gas chromatographic separation

Pengzhi Li [a], Zemin Xu [a], Xiupei Yang [a], Weiwen Bi [a], Dan Xiao [a,b,*], Martin M.F. Choi [c,**]

[a] College of Chemistry, Sichuan University, Chengdu 610064, China
[b] College of Chemical Engineering, Sichuan University, Chengdu 610065, China
[c] Department of Chemistry, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China



Latest developments

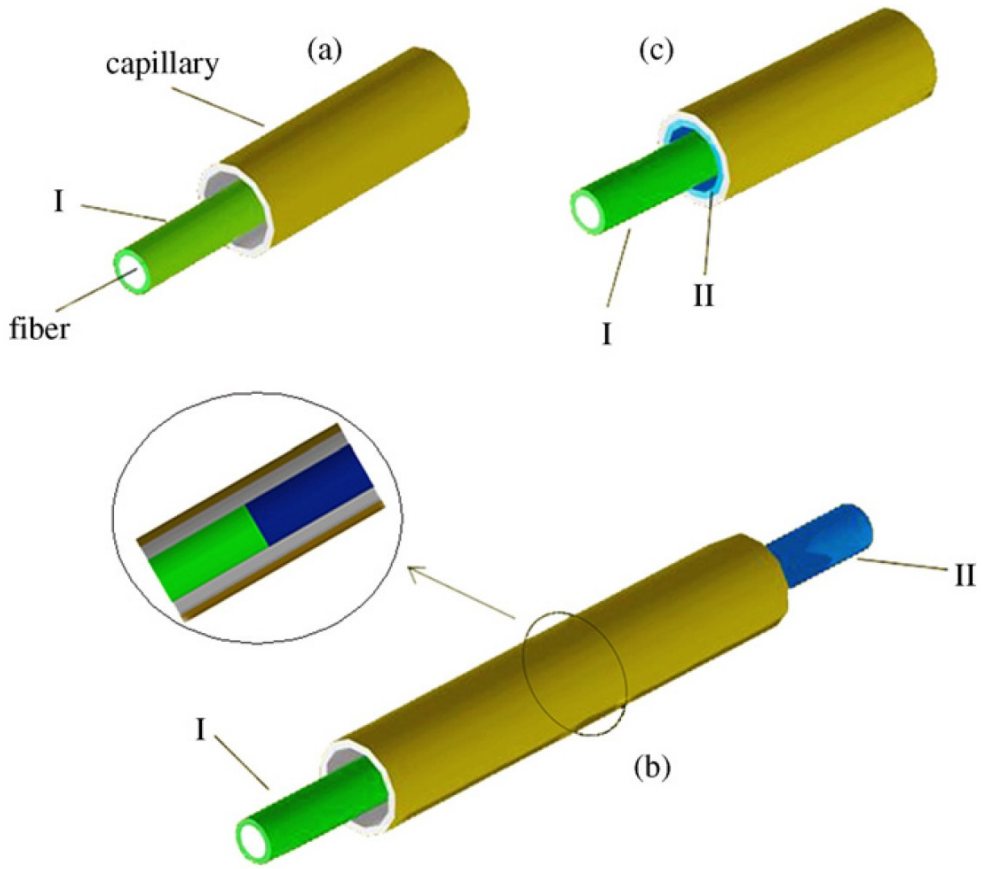


Fig. 1. Schematic diagrams of various types of single fiber-in-capillary annular columns prepared in this work. (a) Stationary phase-coated fiber-in-capillary, (b) dual stationary phase-coated fiber-in-capillary and (c) stationary phase-coated fiber-in-stationary phase-coated capillary annular columns. Stationary phases I and II can be of the same or different types.

The inserted stationary phase-coated fiber can decrease void volume, facilitate faster mass transfer, and thus improve column efficiency. Selectivity can be adjusted by using various stationary phase-coated fiber-in-stationary phase-(un)coated capillary annular columns.

Latest developments

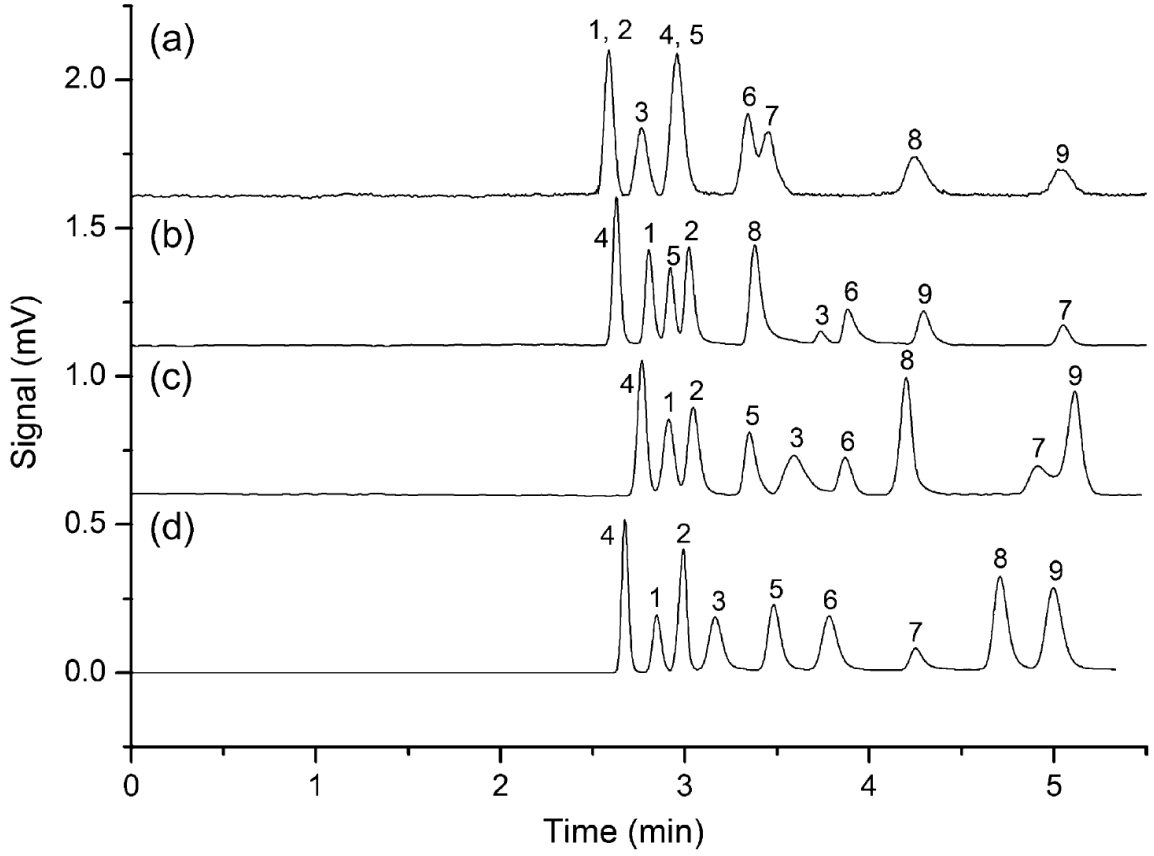


Fig. 5. Separation of a complex mixture of organic compounds on (a) SE-30-coated OTC, (b) PEG-20M-coated OTC, (c) SE/PEG-CF/C annular column (12 m × 0.20 mm, 1.0 μm), and (d) SE-30-coated OTC (6 m × 0.20 mm, 1.0 μm) series-coupled with PEG-20M-coated OTC (6 m × 0.20 mm, 1.0 μm) with a pressure valve at the junction. Solutes 1: *n*-propanol, 2: isobutanol, 3: isoamylol, 4: isooctane, 5: butyl acetate, 6: amyl acetate, 7: 1-hexanol, 8: *n*-decane, and 9: *n*-undecane. *Conditions*: linear carrier gas velocity 25 cm/s; temperature program from 65 to 110 °C at 7 °C/min; and splitless injection. For the series-coupled column, linear carrier gas velocity for SE-30-coated OTC and PEG-20M-coated OTC are 18 and 32 cm/s, respectively.