ADVANCES IN FOOD ANALYSIS

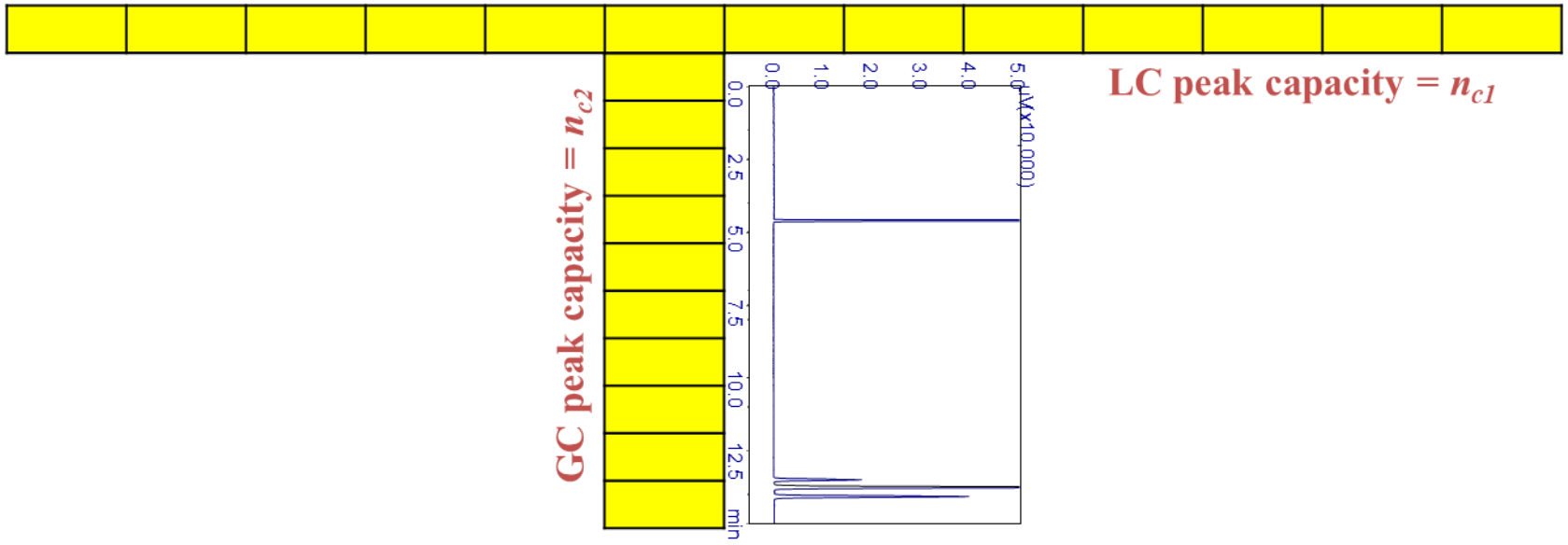
INTRODUCTION TO LC-GC

Marco Beccaria, PhD

University of Liege, Belgium
email: mbeccaria@uliege.be

Heart-cutting LC-GC: peak capacity

$$\text{LC-GC peak capacity} = n_{c1} + (x \times n_{c2})$$



LC represents a valid alternative to traditional sample preparation methods

improve separation efficiency
speed up analysis

Disadvantages of off-line LC:

- possibility of analyte loss
- possibility of sample contamination
- it is possible to inject only a part of the sample eluted from the column

Advantages of LC-GC on-line:

- possibility to transfer the whole fraction eluted from LC column
- reduced sample manipulation
- possibility to automate analysis

First on-line coupling of LC and GC
1980

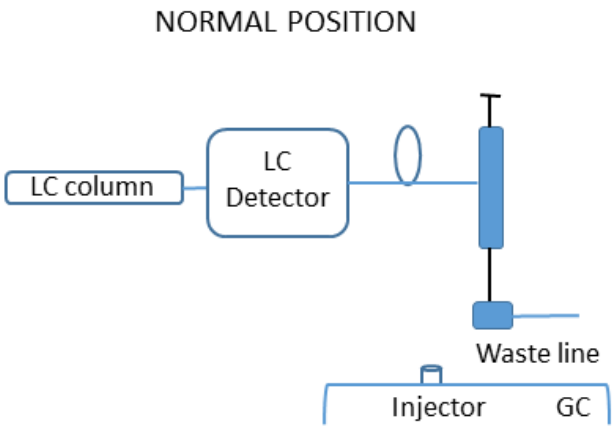
Journal of Chromatographic Science, Vol. 18, October 1980

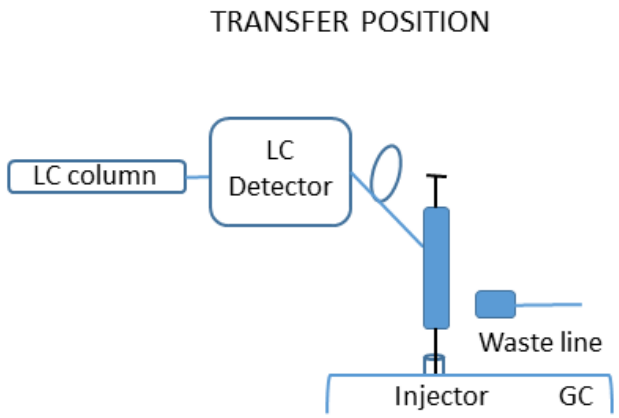
Multidimensional High Performance Liquid Chromatography*

Ronald E. Majors
Varian Instrument Group, 2700 Mitchell Drive, Walnut Creek, California 94598

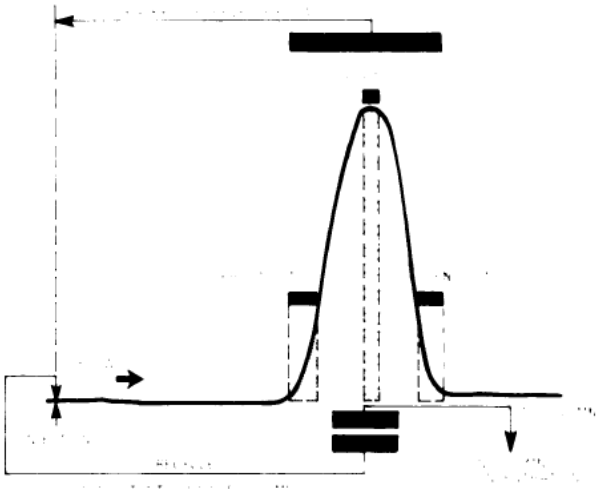
analysis of athrazine in sorghum samples

Heart-cutting LC-GC: split/splitless injector





LC: 15 cm ciano column
eluent: 2% isopropanol/hexane 2mL/min
GC: OV-101 25m glass column
Injection: **8uL in splitless**



First on-line LC-GC system: R. Majors, J. Chromatogr. Sci. 18 (**1980**) 571

Necessity of transfering the **entire fraction** was soon evident

first attempt presented by Grob in 1984 based on previous studies on on-column injection

Journal of Chromatography, 295 (1984) 55–61
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,706

COUPLING OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH CAPILLARY GAS CHROMATOGRAPHY

K. GROB, Jr.*, D. FRÖHLICH, B. SCHILLING, H. P. NEUKOM and P. NÄGELI
Kantonales Labor, P.O. Box, CH-8030 Zürich (Switzerland)

(Received March 6th, 1984)

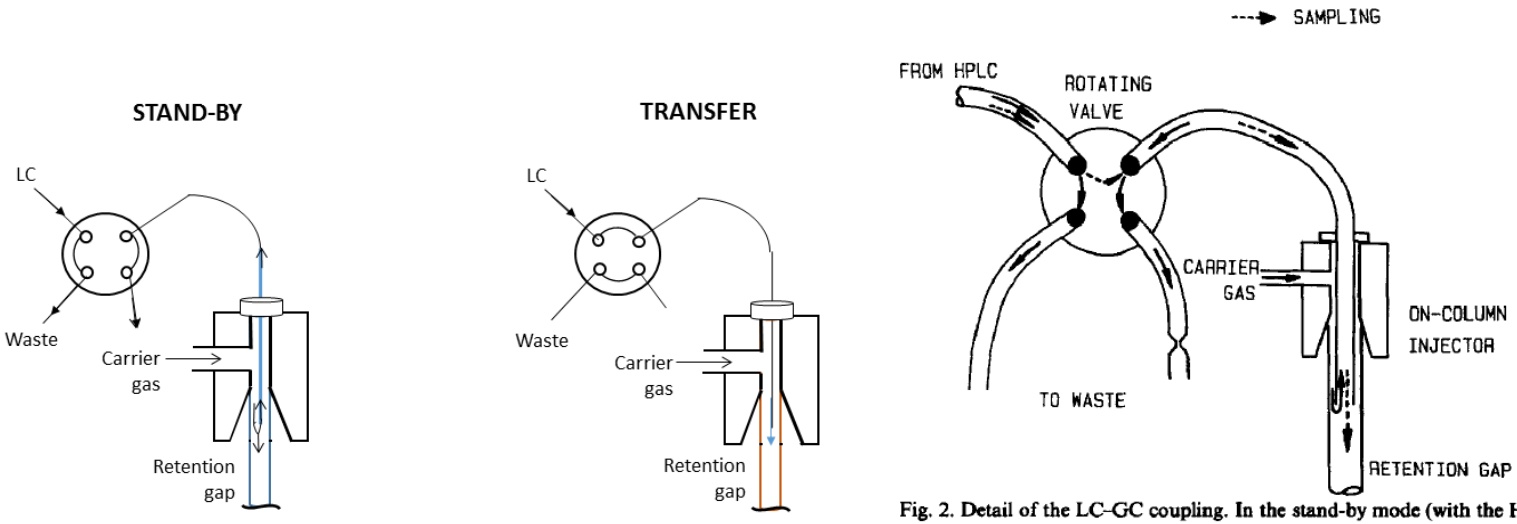


Fig. 2. Detail of the LC–GC coupling. In the stand-by mode (with the HPLC or GC systems running on their own) the effluent from the HPLC detector passes through the rotating switching valve to waste. The connection to the GC system is backflushed by the pressure of the carrier gas in the column inlet. In the sampling mode the HPLC pump pushes the effluents into the retention gap in the GC oven.

LC-GC: PTV injector

Programmed-Temperature Injector for Large-Volume Sample Introduction in Capillary Gas Chromatography and for Liquid Chromatography-Gas Chromatography Interfacing

Jacek Staniewski,*[1] Hans-Gerd Janssen, Carel A. Cramers, and Jacques A. Rijks
Eindhoven University of Technology, Faculty of Chemical Engineering
Laboratory of Instrumental Analysis, P.O. Box 513
5600 MB Eindhoven, The Netherlands

J. Microcol. Sep. **4**, 331-338 (1992)

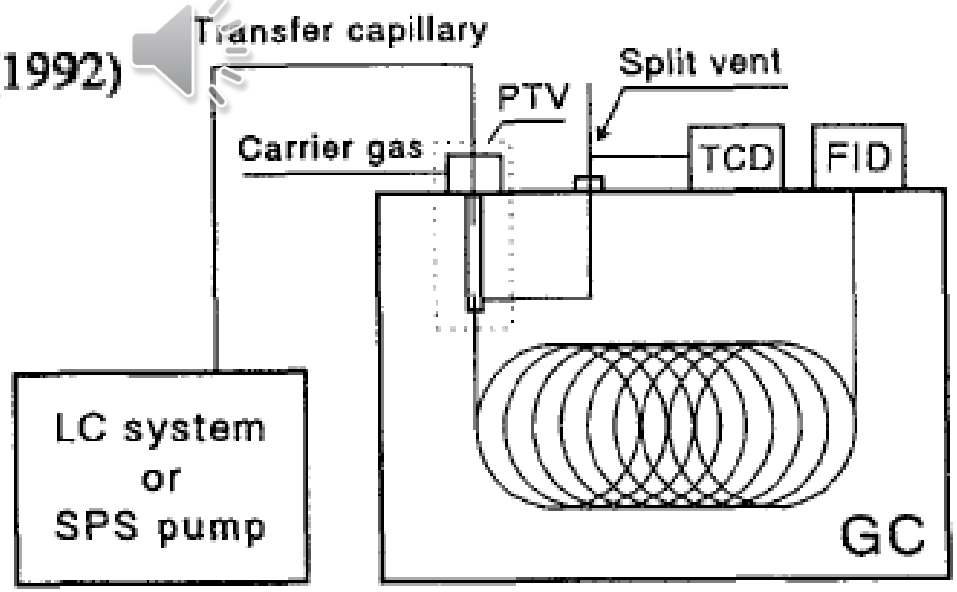


Figure 1. *Overall schematic of the instrumentation.*

LC Dimension

- sample preparation step: by simply separate the target compounds from the bulk of the matrix
- analytical separation

LC separation mechanisms

- Normal-phase LC (NPLC): polar column – apolar solvent
  common solvents used: e.g. hexane,
- Reverse-phase LC (RPLC): apolar column – polar solvent
  common solvents used: e.g. water, acetonitrile, methanol, etc
  (despite several attempts were done not easy to couple with GC)
- Size-excliusion LC (SEC-LC):
  involved usually high volume of solvents (rarely used)

Main limitations

- LC flow
- LC solvent

LC Dimension

LC eluent properties required for successful on-line LC/GC interfacing

To facilitate LC/GC interfacing, the LC eluent should

- solubilize the solutes and elute them in a narrow band;
- not be irreversibly retained by the LC stationary phase;
- be compatible with both LC and GC detectors;
- have a lower boiling point than that of the solutes of interest;
- have a high vapor pressure to promote rapid solvent evaporation;
- have a low polarity to efficiently wet the silanized RG surface under solvent-flooding conditions;
- have a polarity similar to that of the GC stationary phase to improve phase-soaking characteristics;
- be distilled, LC grade, degassed, filtered, and stored in glass vessels; and
- be free from contaminants such as buffer salts and particulate matter.

from: Davies et al., Anal Chem 60 (1988) A683

GC Dimension

- no particular attention or limitation for GC column,
- for the retention gap technique, the main issue is the proper selection of the pre-column

Retention gap

- wettable by the solvent to form a film of liquid
- inert
- retention power below the separation column
- internal diameter large enough to assure a sufficiently high vapor-flow rate for efficient discharge of the eluent
- length depends on :
  - volume of liquid;
  - transfer method employed;
  - type of solvent
  - temperature
  - flow rate of the carrier gas

rule of thumb: with 0.32 mm ID 12-30 cm per uL of liquid introduced

LC-GC Interface

Retention gap: On-column transfer

main drawback: sensible to involatile sample by-product, which affect the column performance

In-line vaporizer or wire interface

(intermediate between the retention gap and the vaporizing chamber> Not in use anymore, not discussed here)

Vaporizing chamber:

Split/splitless: very limited injection volume

PTV: thermally labile compounds since the injector has to be heated above the column temperature; more tricky to be optimized

TOTAD: Through Oven Transfer Adsorption Desorption
(less used, not reported in the next slides)

LC-GC Interfaces

Interfaces based on Retention gap-technique

- On-column interface
- Loop-type interface
- Y interface

In-line vaporizer or wire interface

Interfaces based on vaporiziong chamber

- PTV
- TOTAD: Through Oven Transfer Adsorption Desorption

LC-GC RETENTION GAP INTERFACE

Main approaches

Main interface types:

"on-column"

→ transfer T < *solvent boiling T → "***solvent flooding***"

→ transfer T= *boiling T → "**partially concurrent eluent evaporation**"

"loop-type"

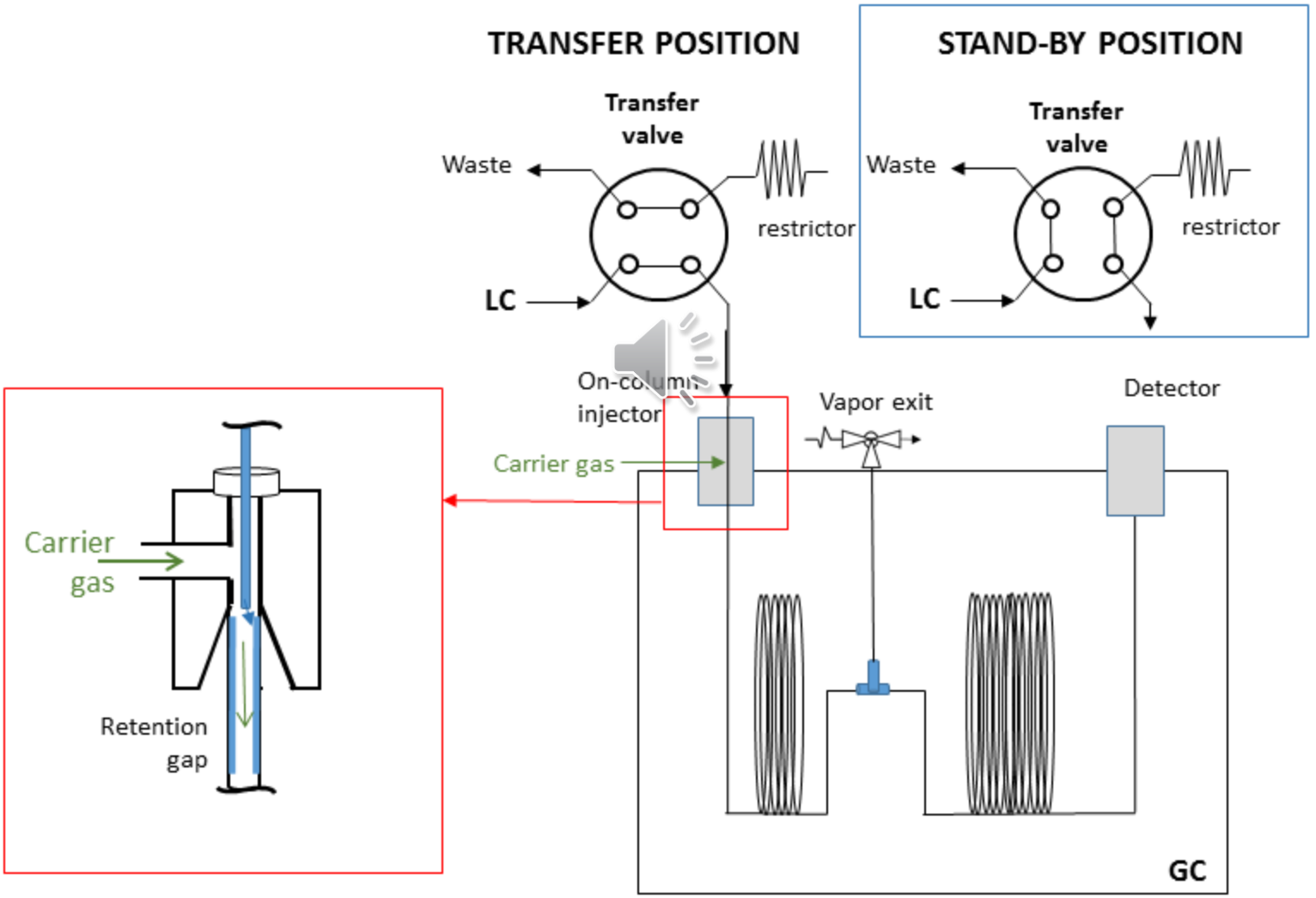
→ transfer T > *solvent boiling T → "**concurrent eluent evaporation**"

→ use of a co-solvent

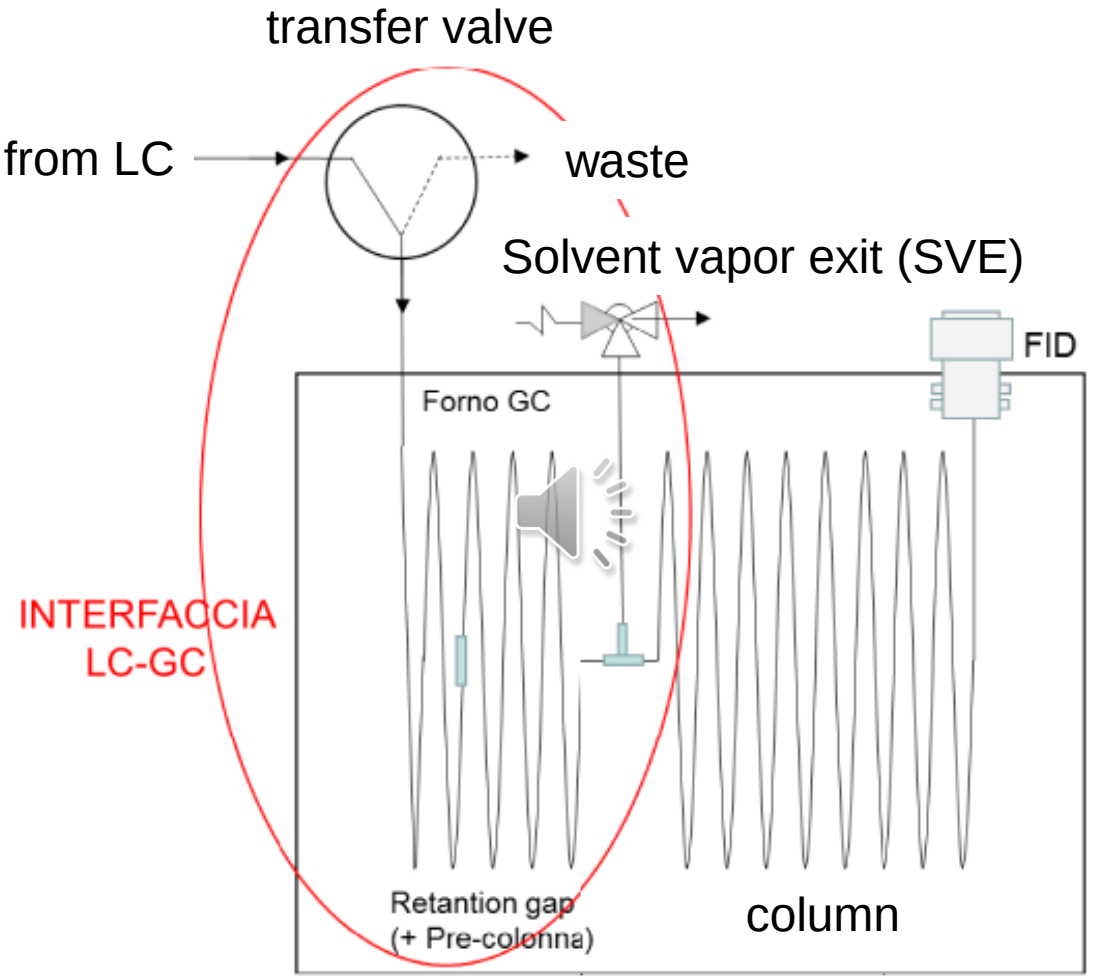
Table 1: LC–GC eluent evaporation techniques.	
Retention gap technique (on column interface)	- conventional - partially concurrent solvent evaporation
Concurrent eluent evaporation (loop-type interface)	- conventional - with co-solvent trapping
Vaporizers	- PTV solvent split - vapour overflow

**solvent boiling T is corrected for the GC inlet pressure*

LC-GC RETENTION GAP INTERFACE
On-column



LC-GC RETENTION GAP INTERFACE: ON-COLUMN INTERFACE



Solvent Vapor exit:

- Accelerate evaporation process
- Prevent passage of large volumes of vapors through the detector

RETENTION GAP INTERFACE: ON-COLUMN

solvent flooding

solvent trapping effect

very effective to trap volatiles compounds

phase focusing effect ⟶ phase-ratio focusing increased by

phase soaking effect

cold trapping

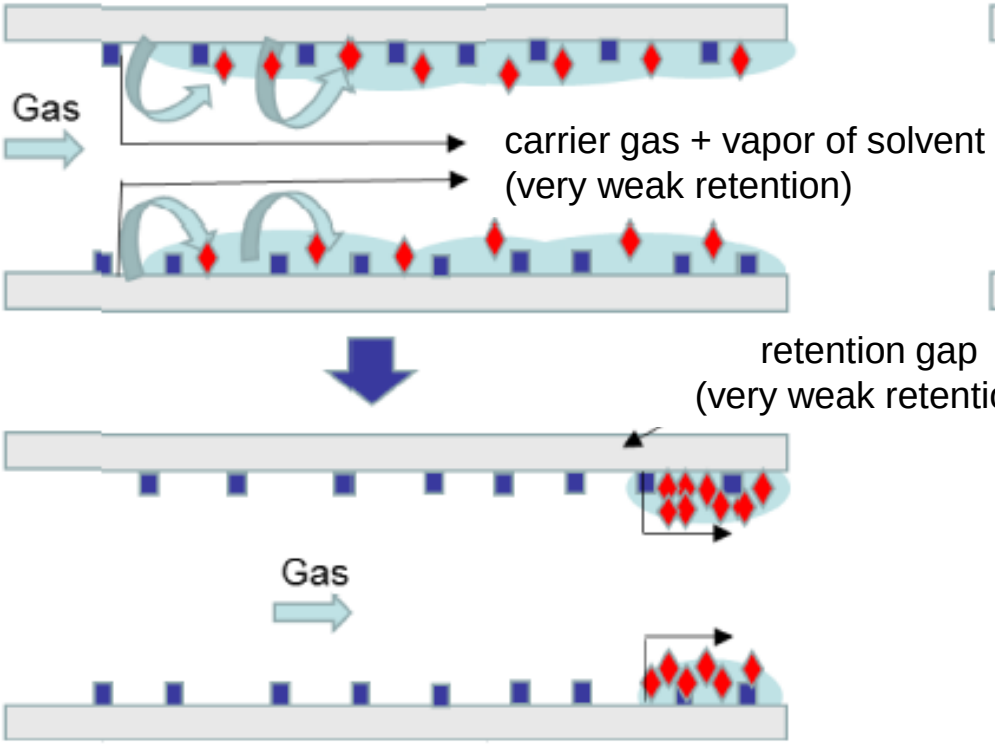
re-focusing of high boiling compounds

Limit of this tehnique are:

- volume of solvent transferred: lower than 100uL
- use of long retention gap (5-10 m)

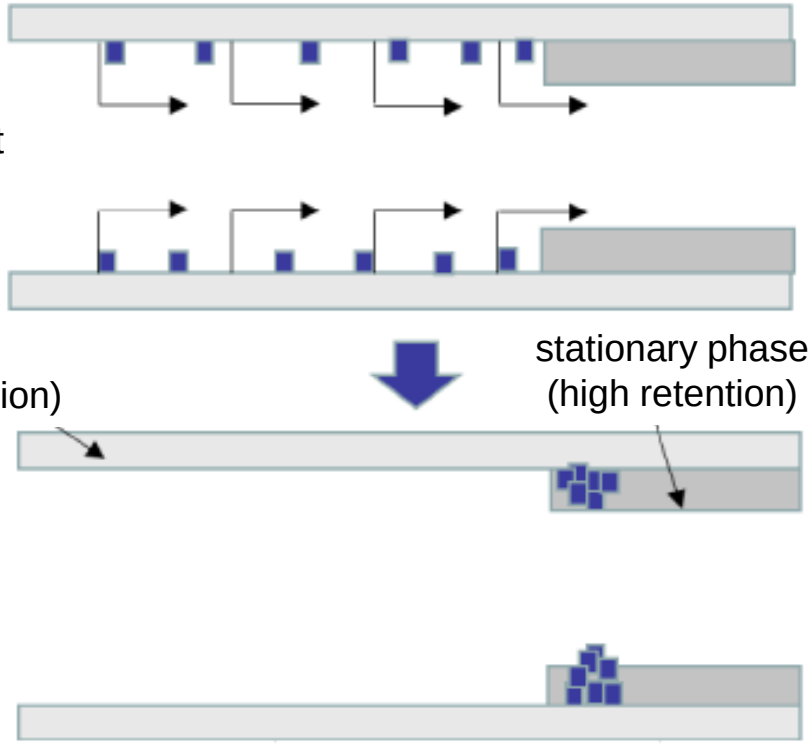
ON-COLUMN INTERFACE: solvent flooding

Solvent trapping



volatiles trapping

Phase-ratio focusing effect



high boiling compounds trapping

- volatiles
- High boiling components

phase soaking effect

cold trapping

ON-COLUMN INTERFACE:
Partially concurrent solvent evaporation

Advantages:

it is possible to reduce the length of the retantion gap or to increase the volume of the fraction to transfer

Disadvantages:

requires optimization of:

- "retantion gap" length
- LC flow rate (transfer rate)
- temperature (evaporation rate)
- closing of the vapor exit

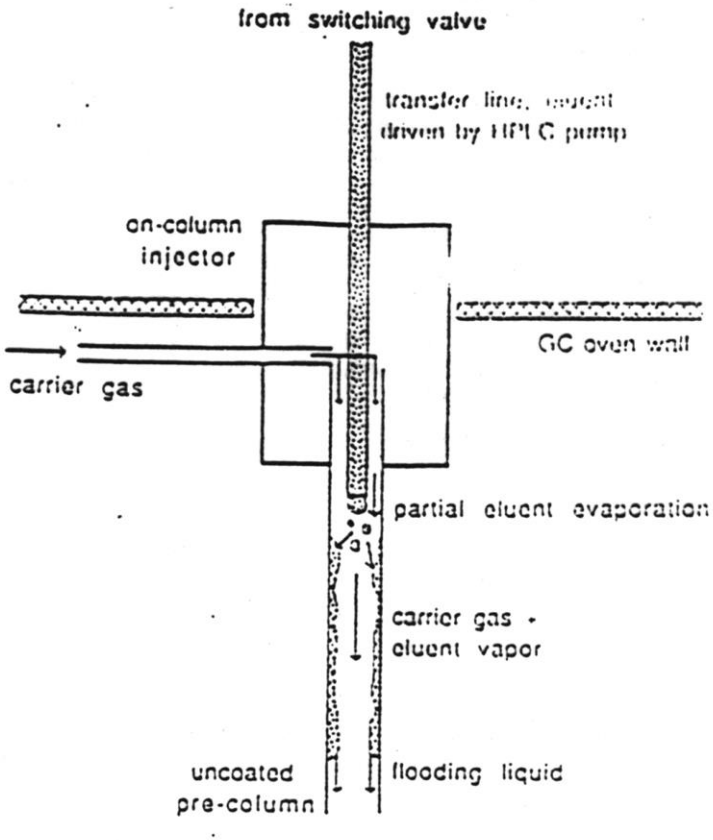


Figure 12
On-column interface during transfer involving partially concurrent eluent evaporation. Usually there is a rotary switching valve between the LC detector and the transfer capillary shown, allowing direction the LC effluent to waste or to GC. (From ref. 58)

transfer rate has to be slightly over the evaporation rate to minimize loss of volatiles

LC-GC RETENTION GAP INTERFACE

Main approaches

Main interface types:

"on-column"

→ transfer T < solvent boiling T → "*solvent flooding*"

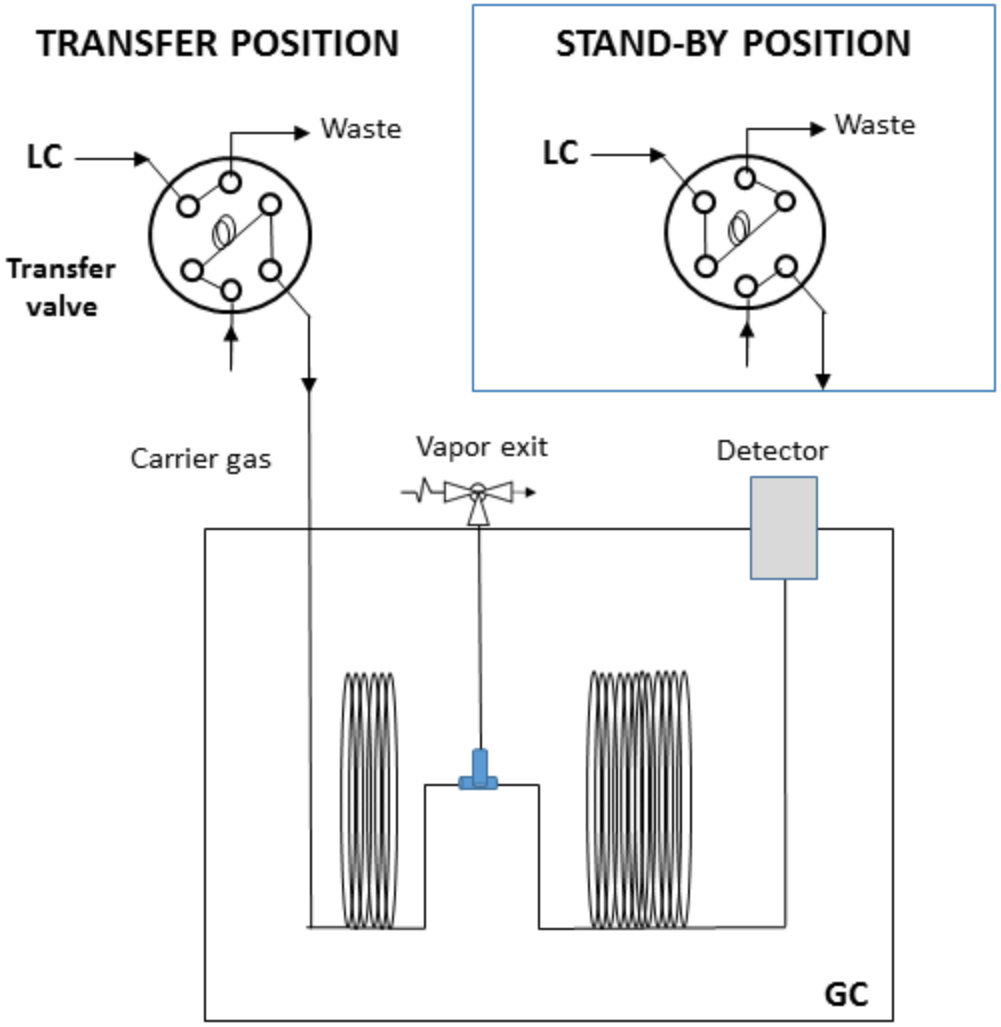
→ transfer T= boiling T → "partially concurrent eluent evaporation"

"loop-type"

→ transfer T > solvent boiling T → "**concurrent eluent evaporation**"

→ use of a co-solvent

RETENTION GAP INTERFACE: LOOP-TYPE

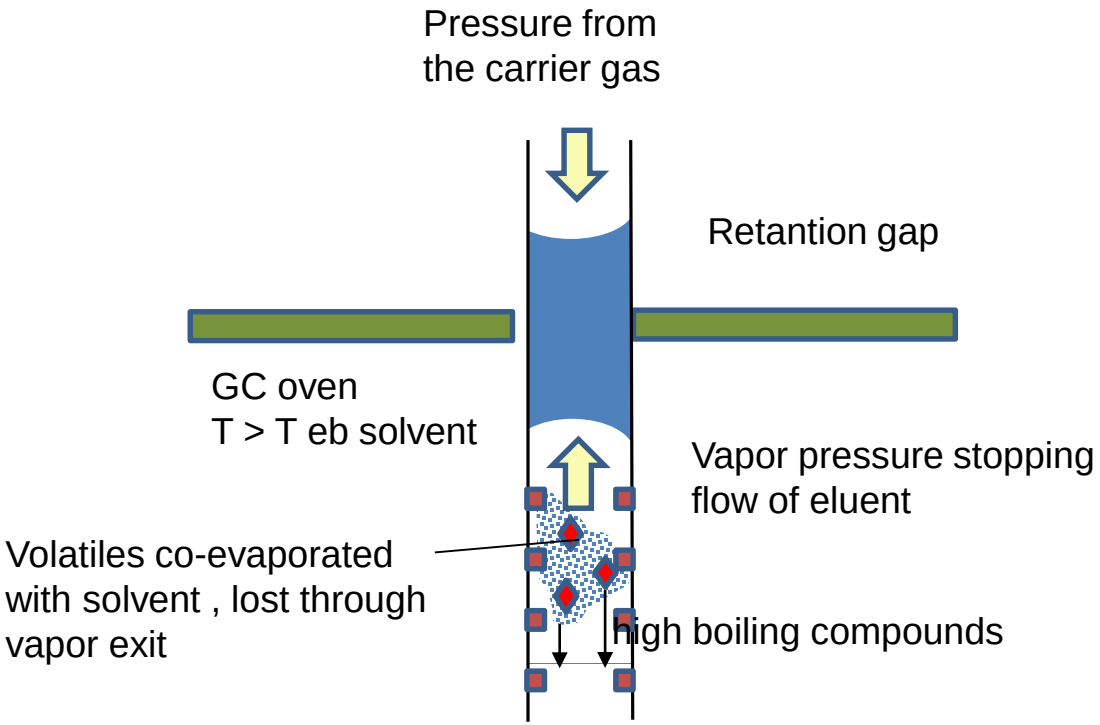


eluent from the LC is driven into the GC by the carrier gas

T>T eb

short retention gap : 2-3 m

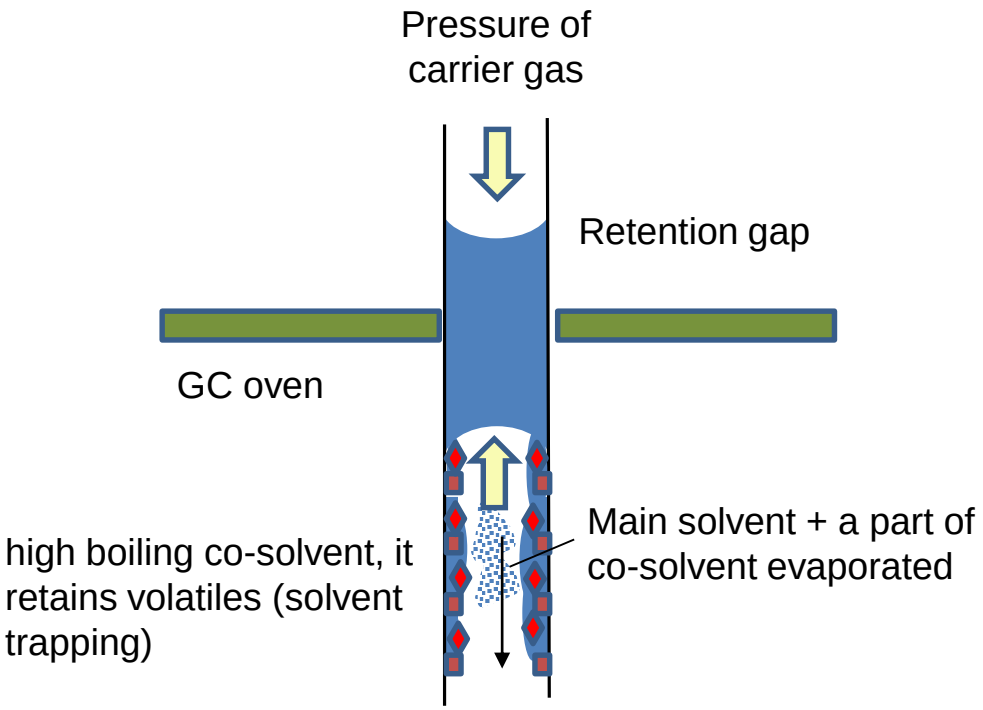
RETENTION GAP INTERFACE: LOOP-TYPE "Concurrent eluent evaporation"



Only the temperature during transfer needs to be optimized
The first sharp peak elutes at about 60-80° C above the transfer temperature

Drawback: the loss of volatiles; low repeatability since pushed by gas

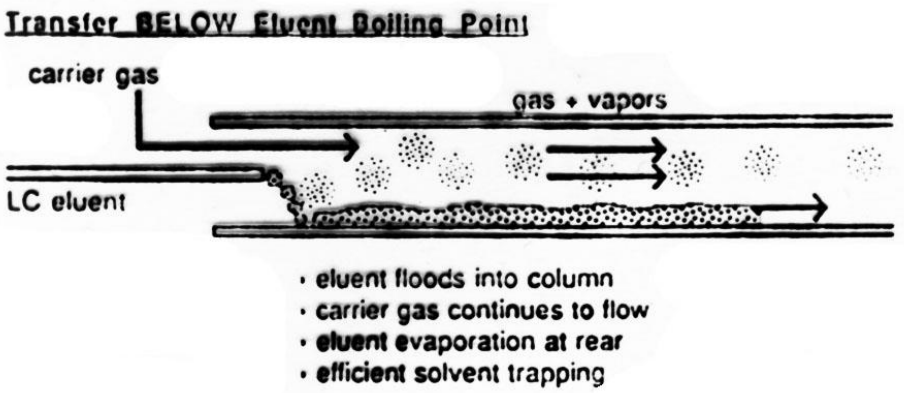
RETENTION GAP INTERFACE:
LOOP-TYPE
"Concurrent eluent evaporation" with solvent trapping



LC-GC RETENTION GAP INTERFACE

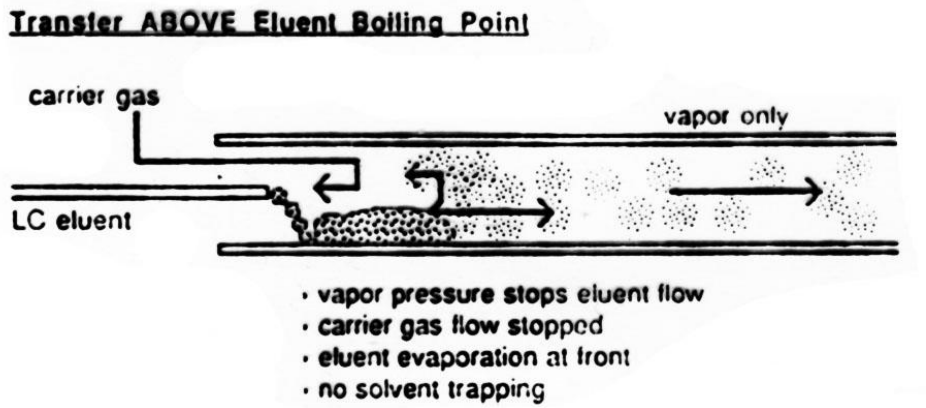
T below Tb

solvent flooding



T above Tb

concurrent eluent evaporation



T above Tb	no solvent trapping /broadening of early peaks	no flooding liquid/large capacity of retention gap
T below Tb	solvent trapping /sharp early peaks	band broadening in space needs for long retention gap

On-column interface

Loop-type interface

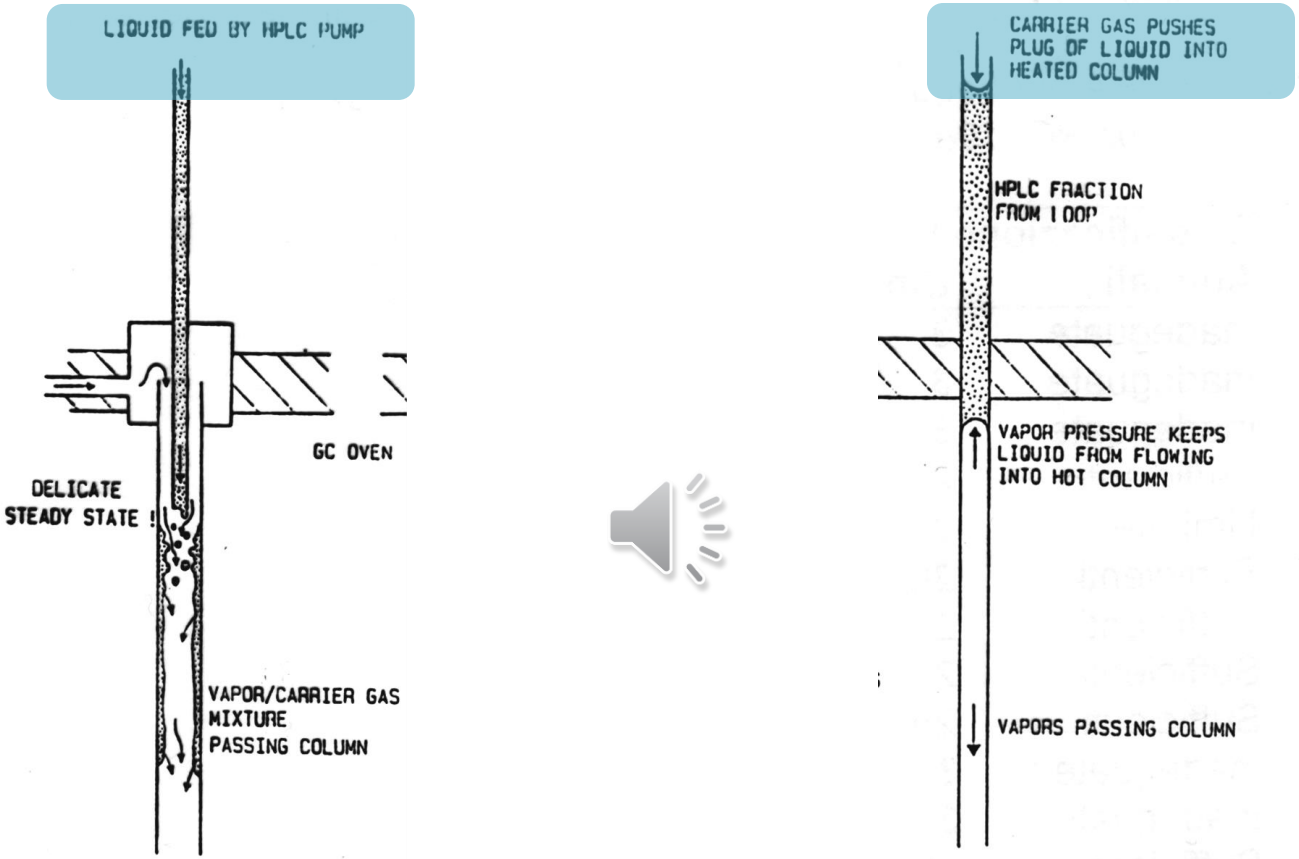
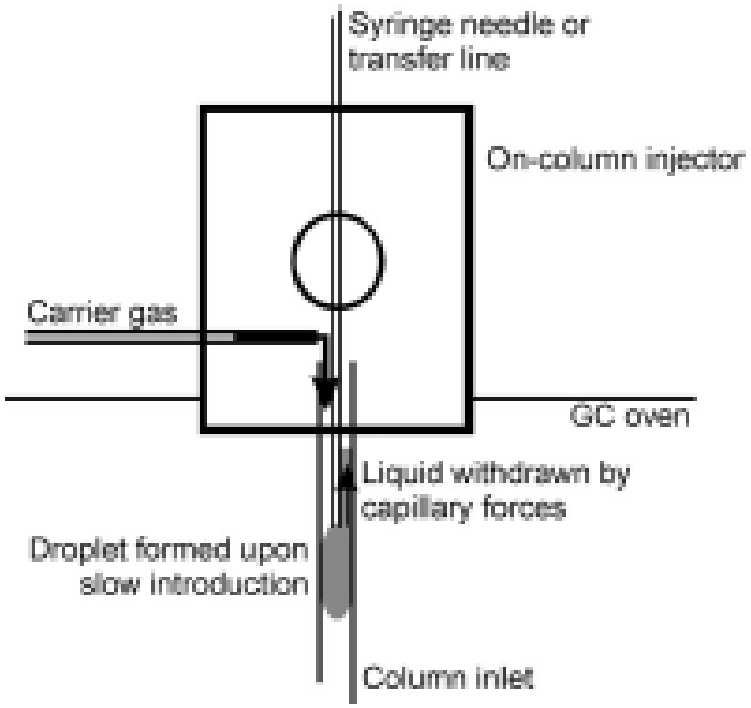


Figure 27
Comparison of the on-column and the loop-type interface for concurrent eluent evaporation. Using the on-column interface (left), the eluent is introduced into the GC column by the LC pump. To achieve concurrent eluent evaporation, input flow rate, carrier gas inlet pressure and GC column temperature must be adjusted to each other. Using the loop-type interface (right), the carrier gas pushes the eluent against its own vapor pressure built up in the oven-thermostated capillary column. Only the oven temperature must be adjusted. (From ref. 23).

Y-INTERFACE:
Partially concurrent evaporation

Introduced to solve the memory problem of on-column interface of about **0.5-3%**

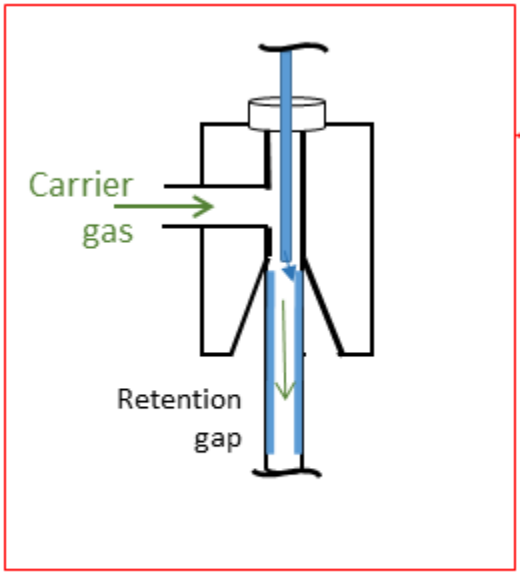
a slow transfer cause the droplet on the exit of the transfer line to touch the column wall and be pulled backwards for capillary force. When the transfer stop, a low portion is driven back to the transfer line until the next transfer

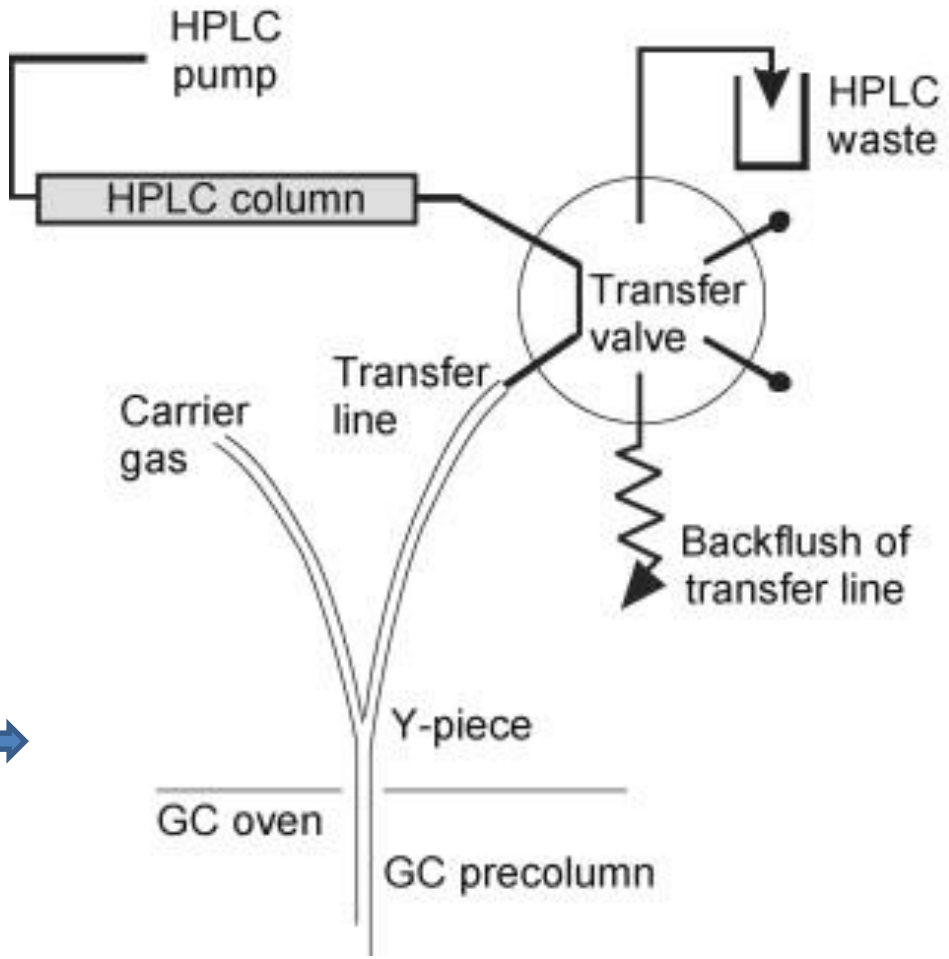


Y-INTERFACE:
Partially concurrent evaporation

Biedermann M, Grob K, J Chromatogr A 1216 (2009) 8652

On-column





VAPORIZING CHAMBER INTERFACE: PTV

Employing a PTV injector

1. Analyte are retained in a packed chamber (injector liner)
2. The solvent is vaporized (splitting valve open)
3. Analytes are desorbed at high T (splitting valve closed)

Not for thermo-lable analytes

Some problem of discrimination if not properly optimized

VAPORIZING CHAMBER INTERFACE: PTV

Main parameters to be optimized in PTV injector

1. LC flow rate (transfer rate)
2. Volume to be transfer
3. Liner selection (volume and packing material)

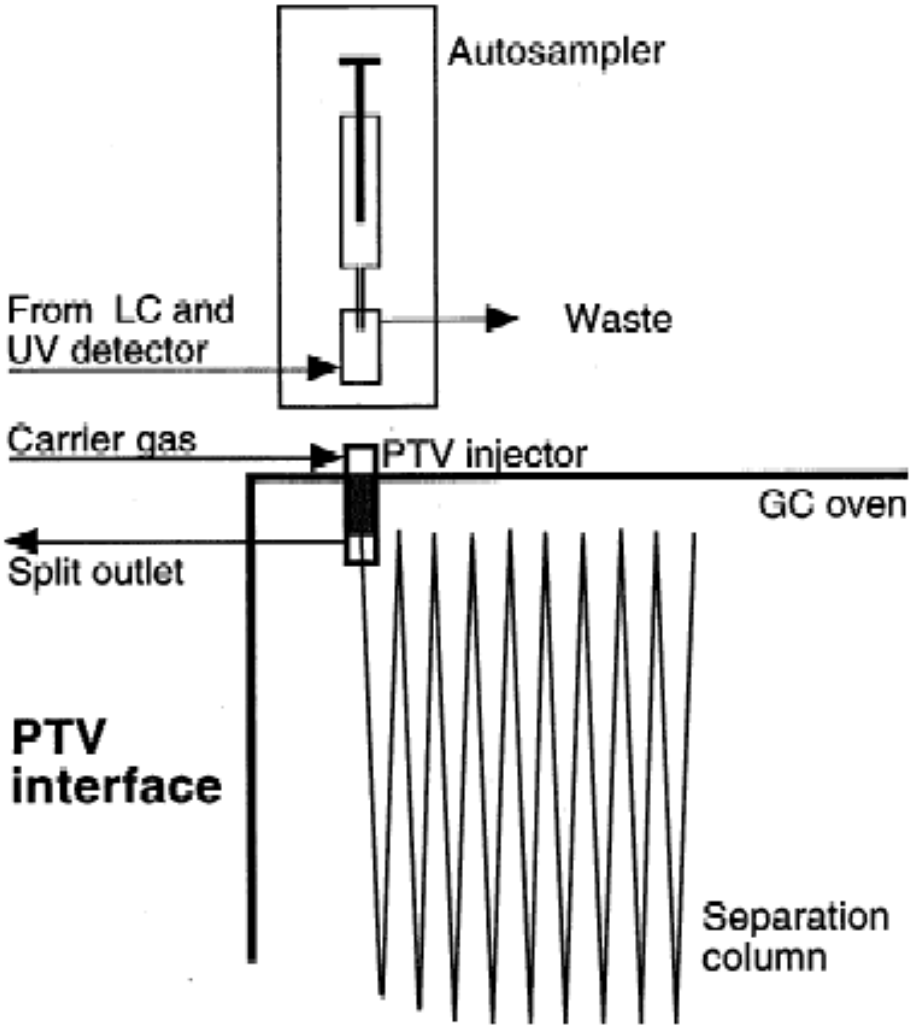
Different transfer mode

1. PTV solvent split
2. PTV large volume splitless
3. PTV vapour overflow with or without splitting

A problem when performing LVI using PTV is the recondensation in the split line and/or in the split valve, which cause an increase in flow resistance and an increase in the pressure in the injector.

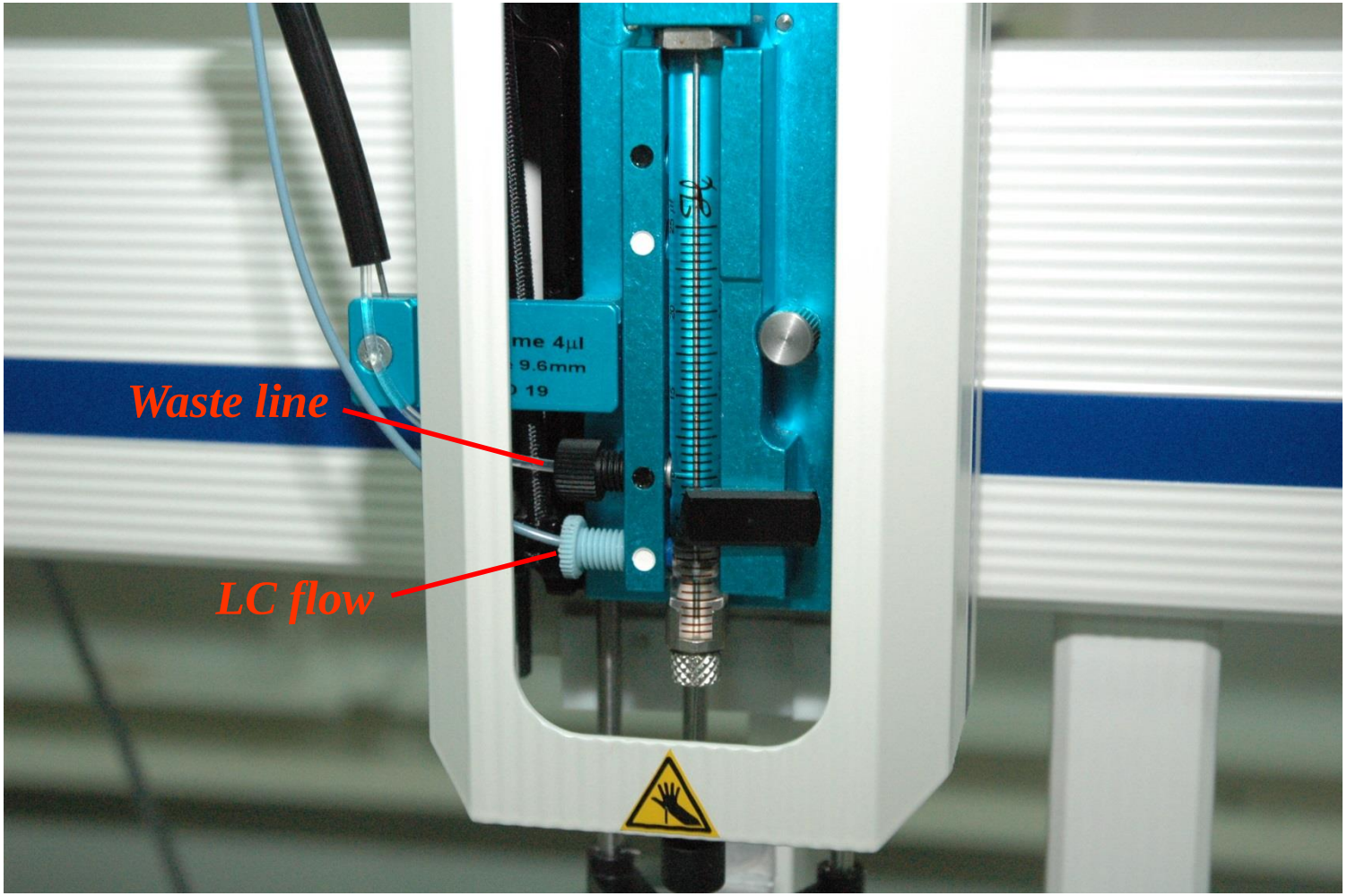
Conseguently, both back-flow of solvent into the carrier gas and flow, as well as split change, make quantification impossible

Multidimensional liquid-gas chromatography: present



David F, , Huffman P, Sandra P, LC GC Eur 9 (1999) 550

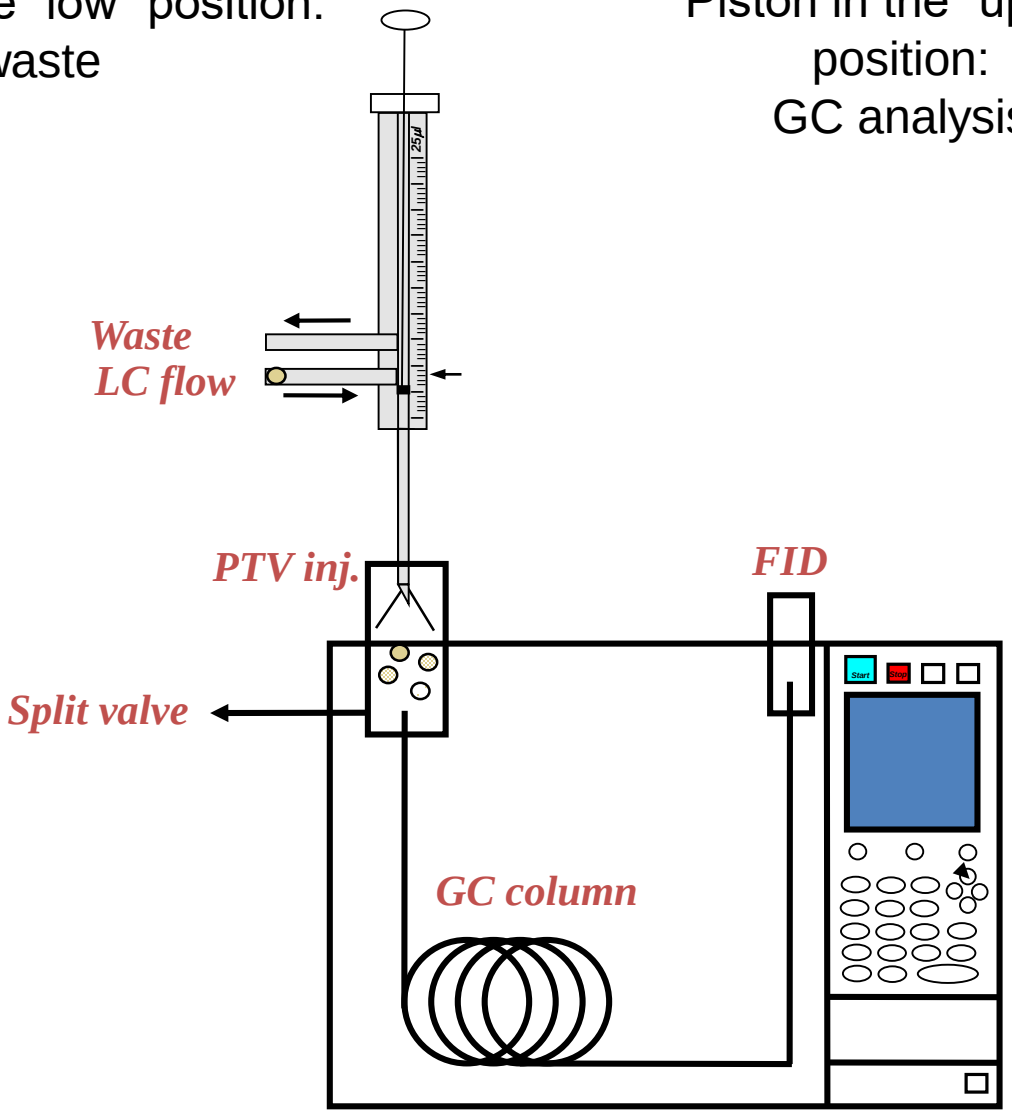
Transfer device: a modified syringe



Transfer mechanism

Piston in the “low” position: waste

Piston in the “upper” position: GC analysis



PTV-INTERFACE

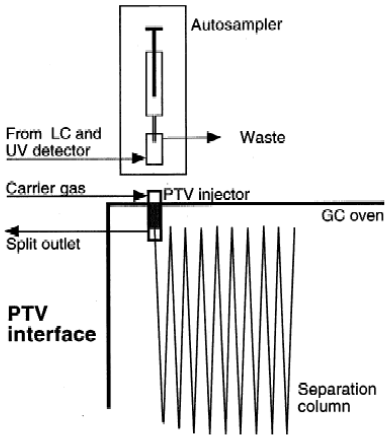
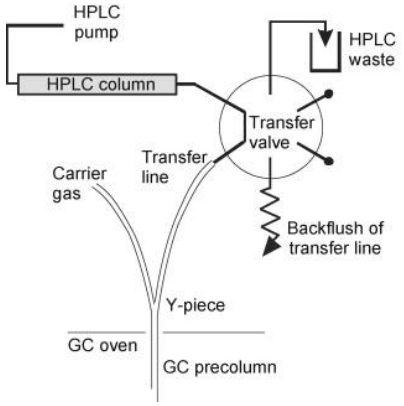
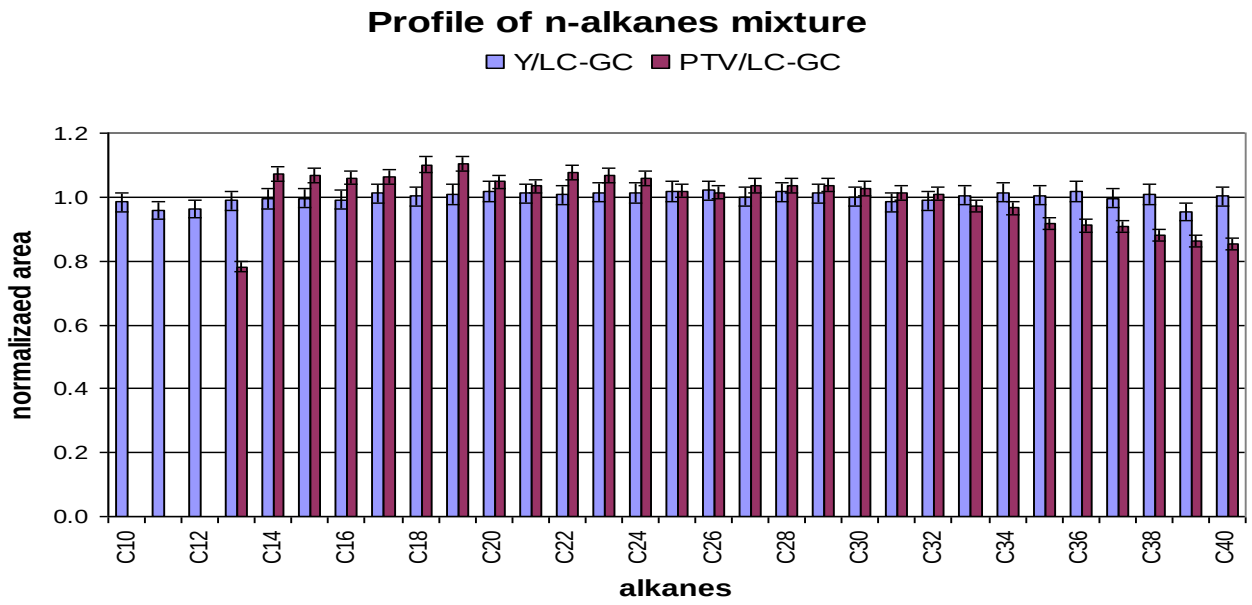


Fig. 4. Coupling of LC to GC through PTV solvent splitting using an autosampler as interface.

Y-INTERFACE

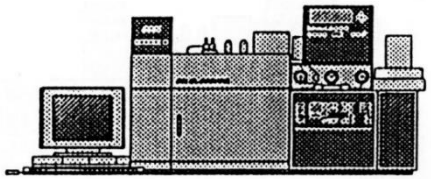


Comparison: Discrimination



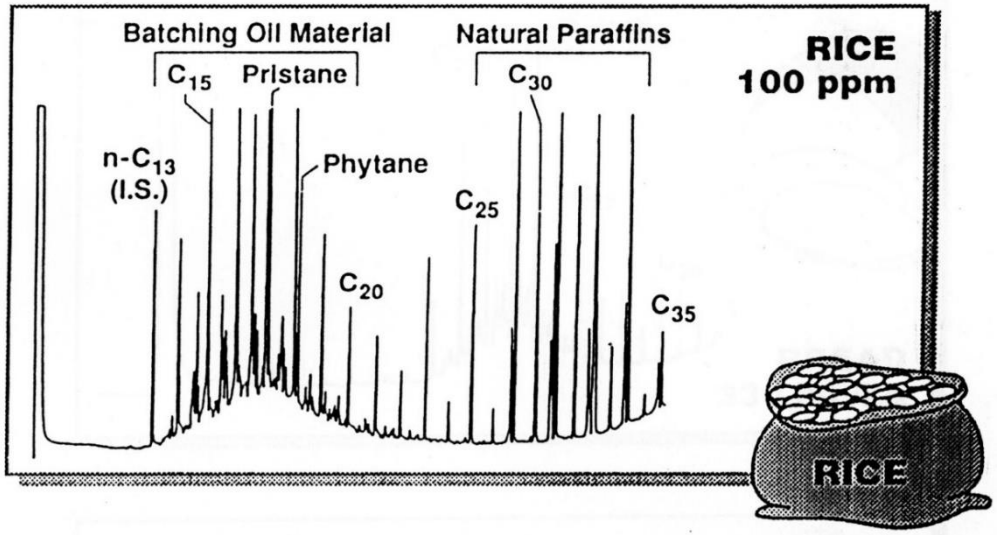
LC-GC Main applications
NPLC-GC

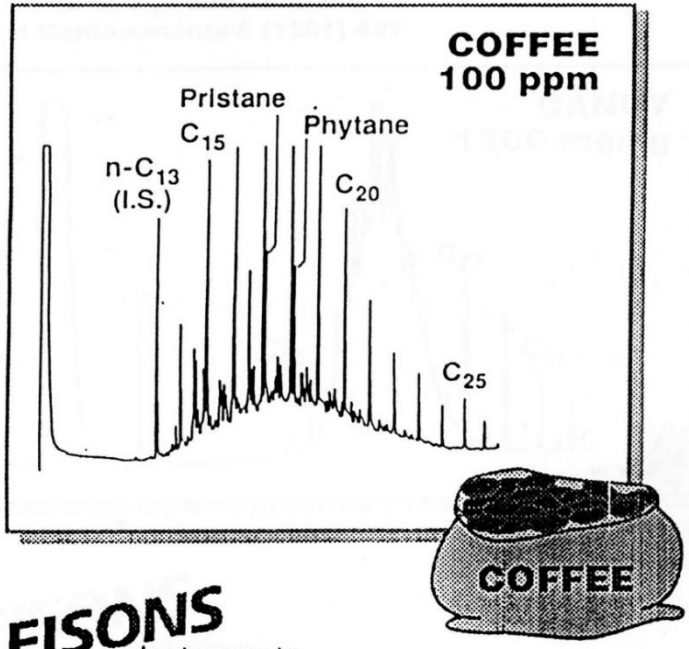
- Mineral oil contamination in food

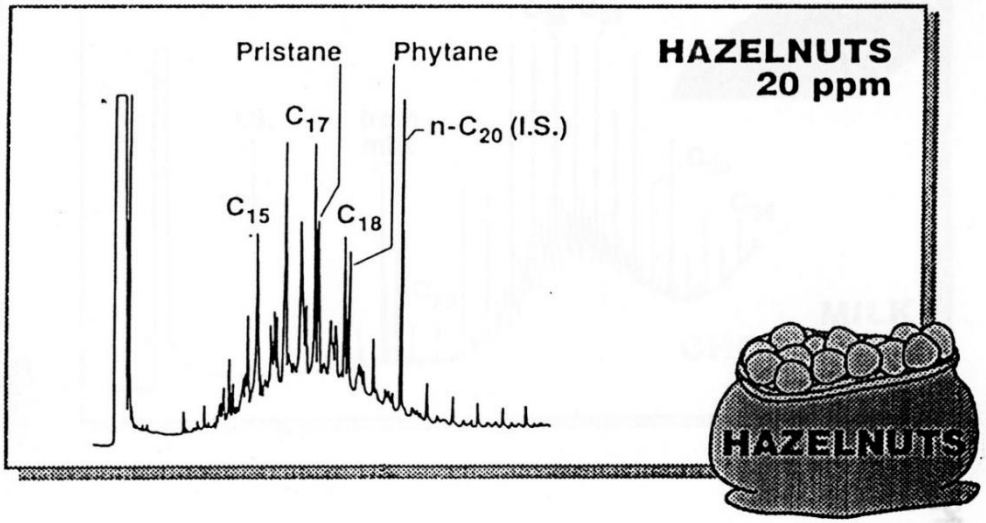


FOOD CONTAMINATION: MINERAL OIL FROM JUTE SACKS

K. Grob et al, J. AOAC 74 (1991) 506

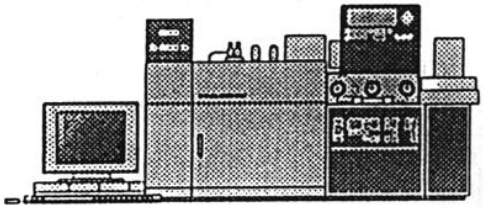






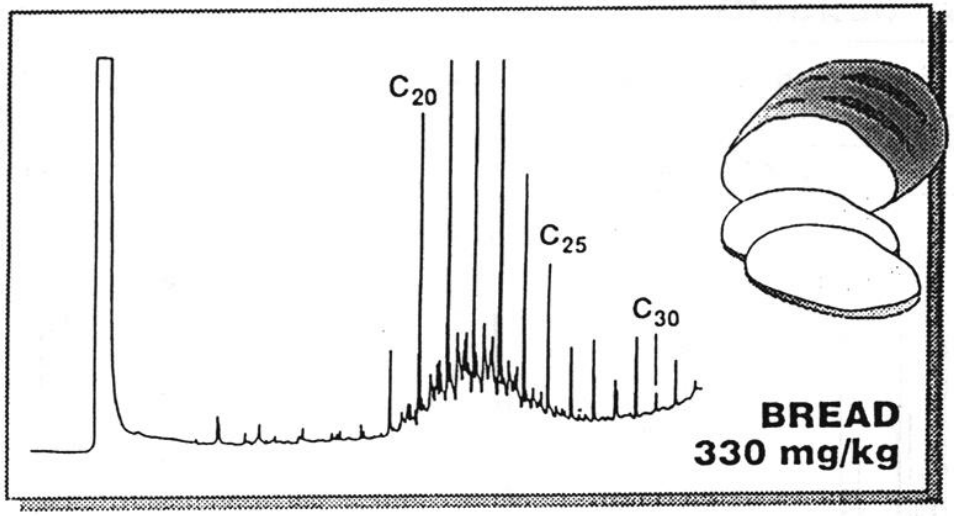
FISONS Instruments

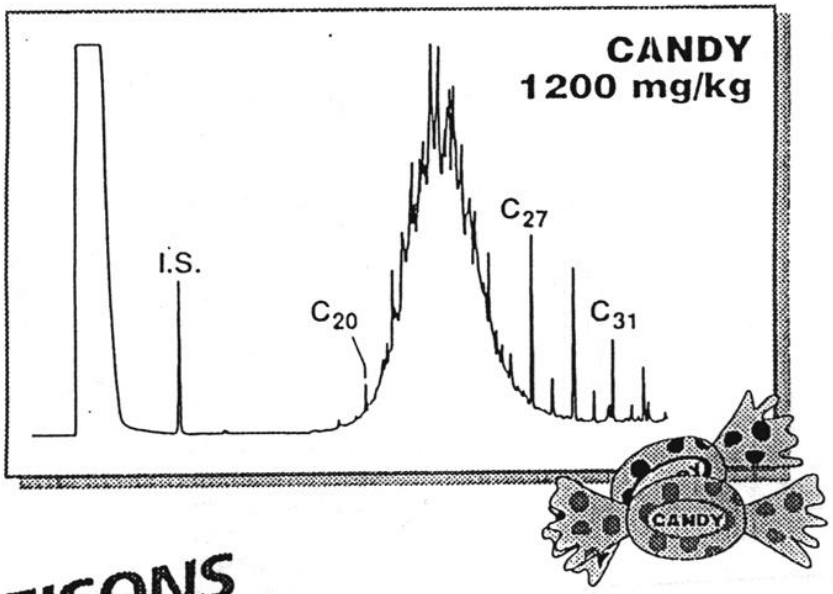
LC-GC Main applications

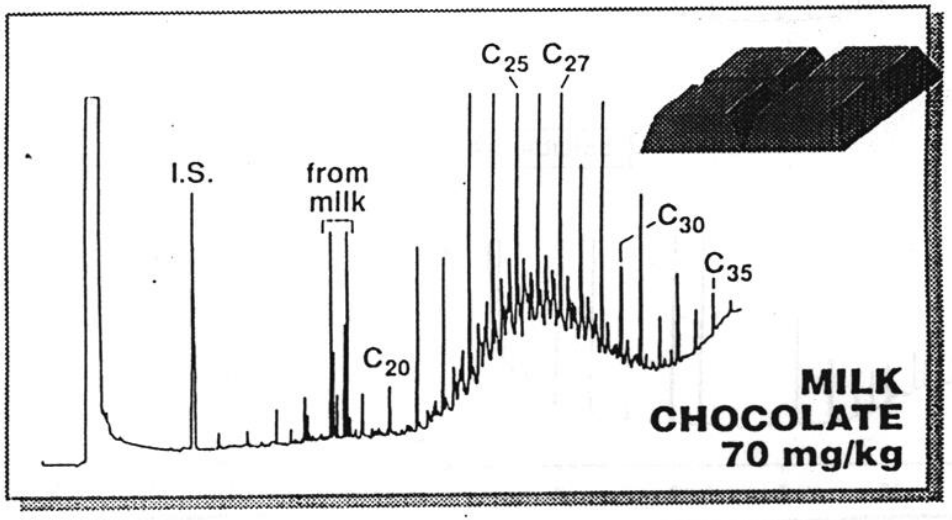


FOOD CONTAMINATION: LUBRICANTS & RELEASE AGENTS

K. Grob et al, Food Additives and Contaminants 8 (1991) 437



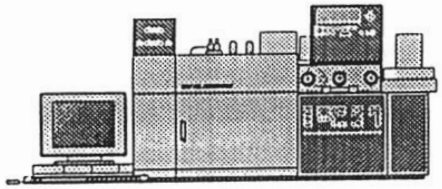




FISONS Instruments

LC-GC Main applications
NPLC-GC

- Olive oil analysis for quality and authenticity assessment



FOOD SOPHISTICATION: EXTRA-VERGIN OLIVE OIL

K. Grob et al, J. AOCS 67 (1990) 626

	Sitosterol concentration	
	mean (ppm)	RDS (%)
7 Consecutive injections	673.6	0.2
Re-injection over a period of 1.5 months (n=5)	670.6	0.17
10 complete analysis	666.1	0.71

REPRODUCIBILITY

FISONS Instruments