Gas Chromatography

1. Introduction

2. Stationary phases

3. Retention in Gas-Liquid Chromatography

4. Capillary gas-liquid chromatography

5. Sample preparation and inlets

6. Detectors

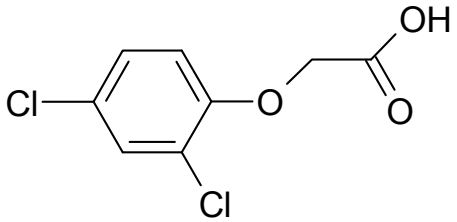
(Chapter 2 and 3 in The essence of chromatography)

Sample preparation and inlet

A. Sample Preparation:

1. **The prerequisite in GC separation is that all solutes being separated must be: (a) fairly volatile, and (b) thermally stable. (c) Usually, the solute should be dissolved in a non-aqueous matrix (H_2O changes column behevir).**

2. Lack of volatility prevents the direct use of GC for many solute. One way to overcome this difficulty is to *derivatize* the solutes into more volatile forms.



2,4-dichlorophenoxyacetic acid (A cancer suspect agent).

Silylation

3. Derivatization of a solute can be used for any of the following reasons

(a) To increase the volatility of the solute.

(b) To increase the thermal stability of solute

(c) To improve the response for the solute on certain detectors (e.g., incorporating halogen atoms into a solute so that it can be detected using an electron capture detector).

(d) To improve the separation of the solute from other sample components (i.e., changing the structure of a solute will also affect its retention on the column)

4. Most derivatization reactions can be classified into one of three group:
   (a) Silylation
   (b) Alkylation
   (c) Acylation

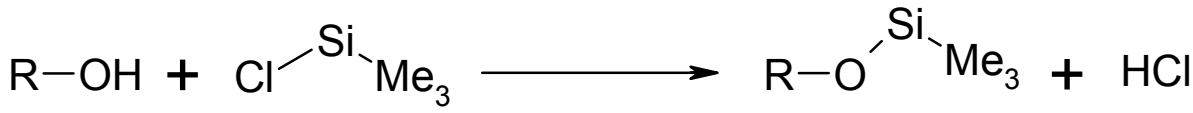
Most of these reactions are performed using minimal amount of sample and reagents (i.e., 0.1~2.0 mL) are typical carried out at room temperature. Some, however, do require heating to moderate temperatures (60 ~ 100 ^{O}C).

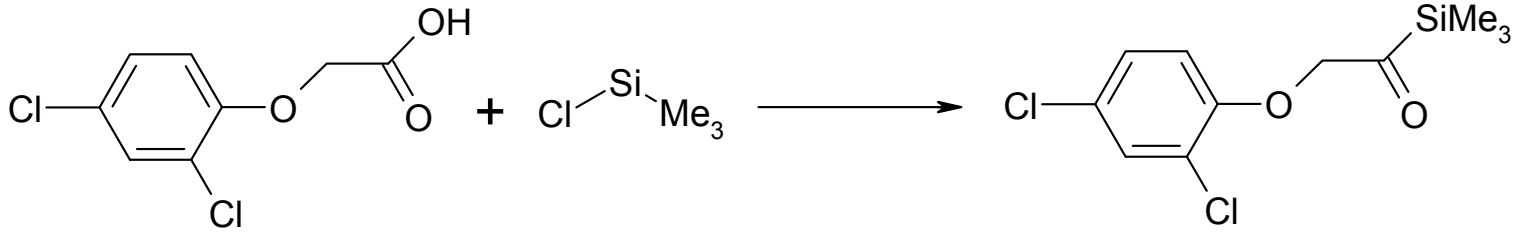
5. Silylation

(a) This is the most common type of derivation techniques used in GC.

(b) It involves replacing an active hydrogen on the solute (i.e. R-OH, RCOOH, R-NH_2, etc.) with an alkylsilyl group (usually –$SiMe_3$). The result of this reaction is that the solute is converted into a less polar, more volatile and more thermally stable form.

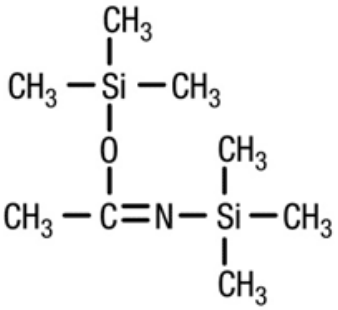
(c) The most common reagent used in silylation is trimethylchlorosilane (TMS). Examples of its use are shown below:





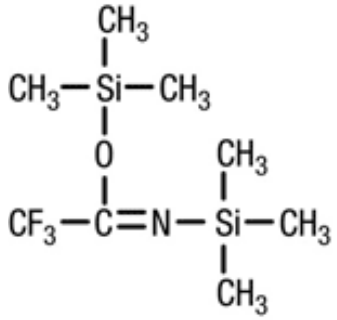
The resulting Product of this reaction is usually just referred to as a TMS-derivative.

(d) Besides trimethylchlorosilane, a number of other silylation reagents can also be used. These reagents have slightly different reactivity from trimethylchlorosilane.



BSA
M.W. 203.4
bp 71-73°C/35mm
d_4^{20} 0.832

N, O-Bis(trimethylsilyl)acetamide

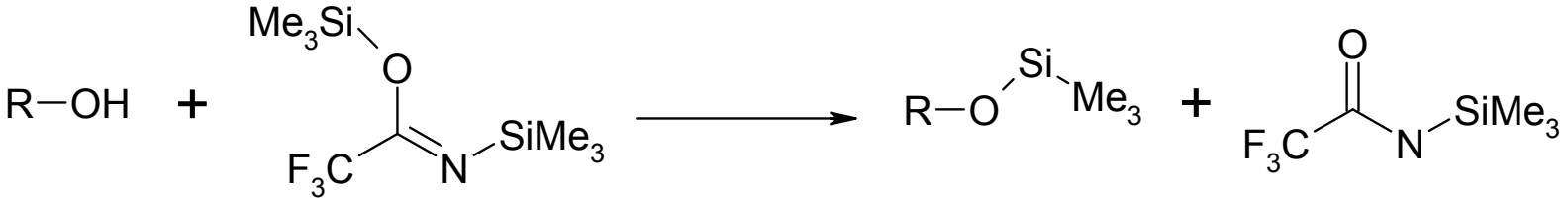


BSTFA
M.W. 257.4
bp 40°C/12mm
d_4^{20} 0.961

N,O-bis(Trimethylsilyl)trifluoroacetamide

The byproduct of BSTFA is highly Volatile.

BSA and BSTFA are highly stable TMS derivatives, with most organic functional groups, under mild reaction conditions.



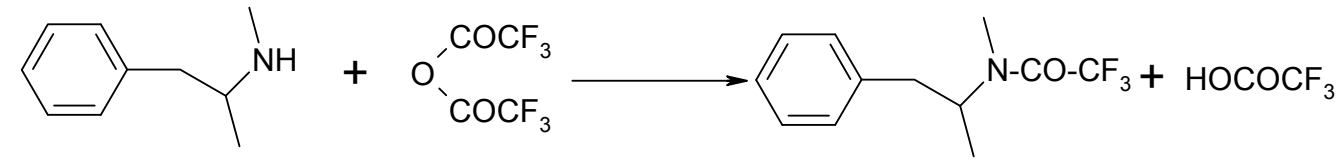
(e) Alylation

i. Alkylation involves the addition of alkayl group to some active function group on the solute. A common example is esterification of a carboxylic acid, forming a volatile methyl ester. This is commonly done using borontrifluoride in methanol as the reagent.

(f) Acylation

i. Acylation involves the conversion of a solute into an acylate derivates. This is often used to improve the volatility of alcohols, phenols, thiols and amine (e.g., -OH, -SH and -NH) containing compounds. As is true for other GC derivations, acylation can also be used to increase the response of a solute to a given detector (e.g., allowing the use of electron capture in solute's detection by including fluorine atoms in the derivitizing agent.

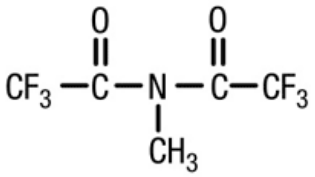
ii. Trifluoroacetic anhydride (TFAA) is one common reagent used for acylation.



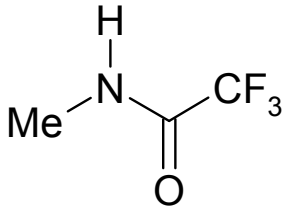
methamphetamine

Drug-of-abuse confirmation testing by GC/MS

iii.Anther set of reagents used for solute with primary and secondary amines, as well as hydroxyl and thiol groups are N-Methyl-bis[trifluoroacetamide] (MBTFA). The reaction is under mild nonacidic conditions.



MBTFA
M.W. 223.08
bp 123-124°C
d_4^{20} 1.55

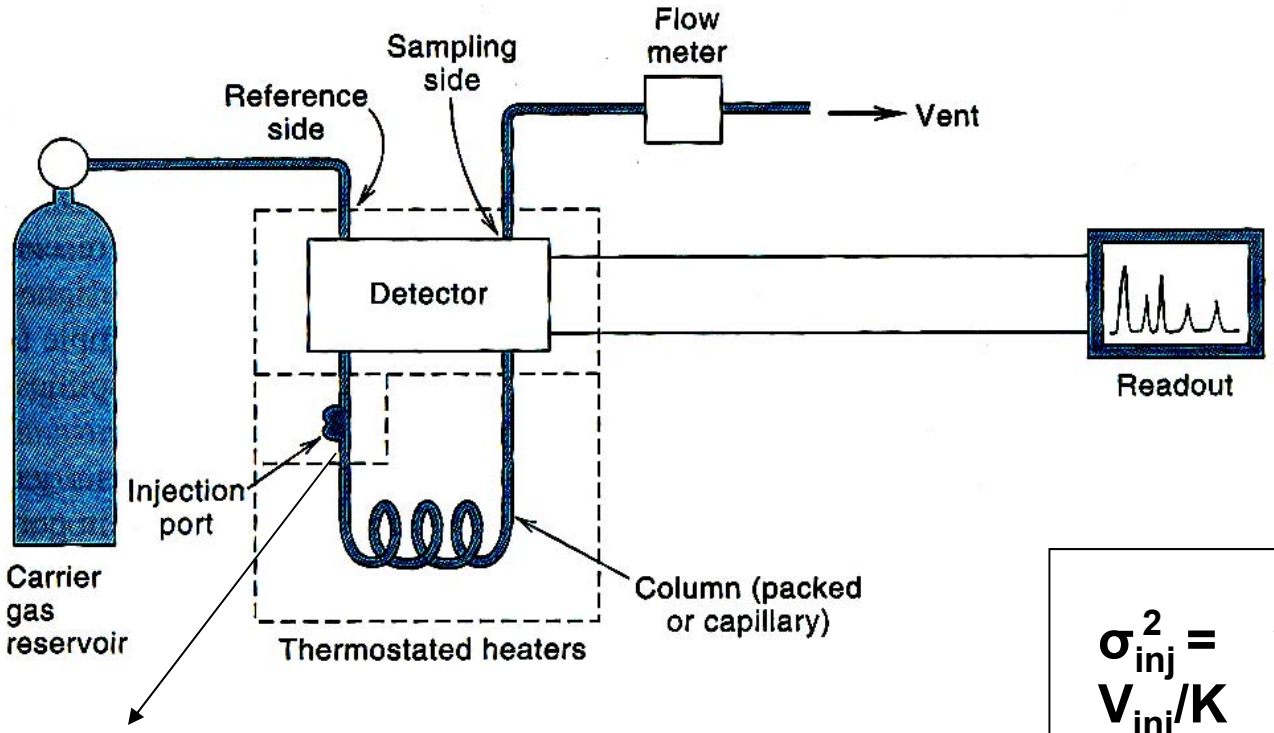


Byproduct is volatile

Sample preparation and Inlets

A. Sample Preparation:

B. Sample Inlets:



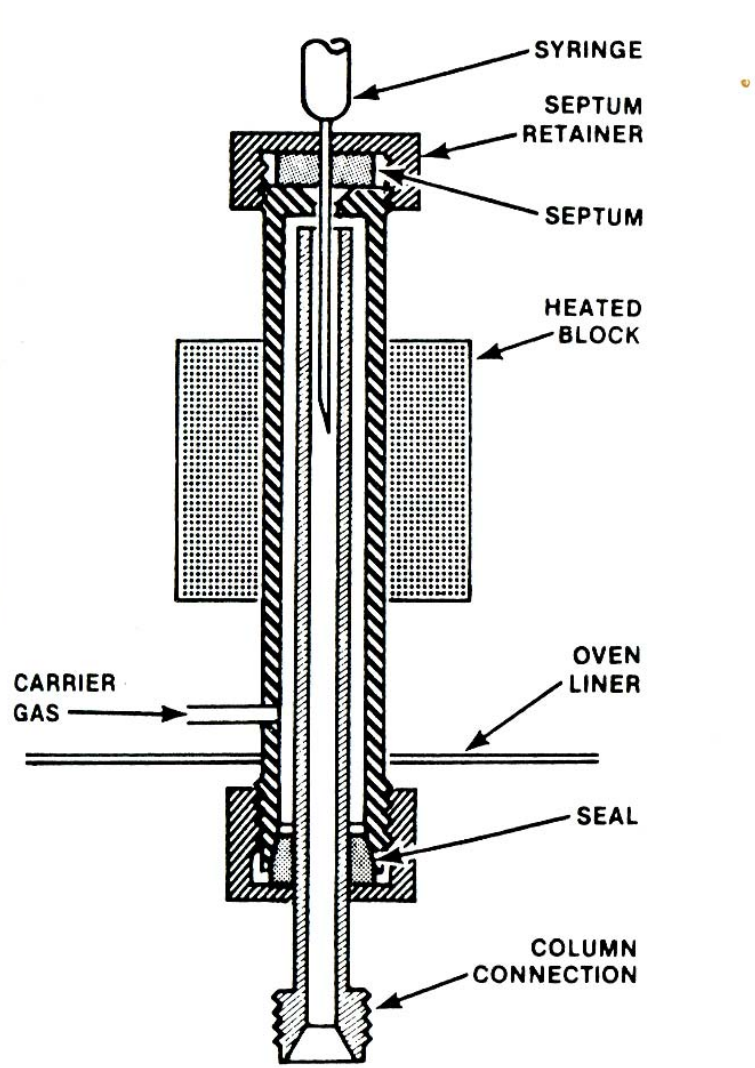
Sample inlet provide means by which the sample is vaporized and mixed with carrier gas.

$$\sigma^2_{inj} = {}^{2}$$
$$V_{inj}/K$$
$$H_{inj} = \sigma^2_{inj}/L$$

1. Direct Injection

a. *Gaseous solutes* can usually be directly injected onto a GC.

b. *Volatile liquid and solid solutes* can also be applied directly to a GC system as long as they are dissolved in a solvent that does not interfere with solute peaks and does not contain other nonvolatile materials that may be deposited in the injector or on the column.



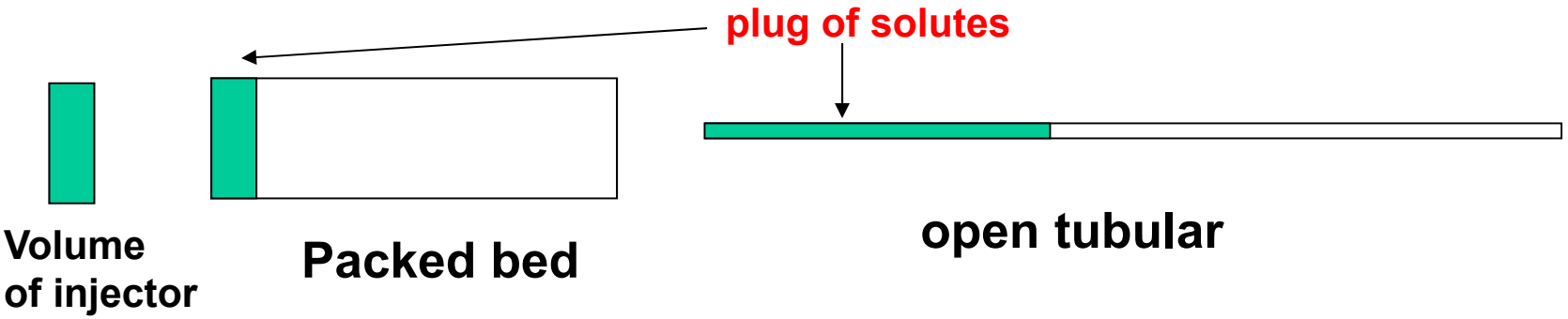
2 Inlets for Packed column

a. The solutes are injected by using a micro-syringe placed into a heated injection port. As a solute is injected into the port, it is quickly volatilized and taken by the carrier gas to the column.

b. For thermally labile solute, the heated injected port may cause decomposition of the sample. For these solutes, direct injection of the sample onto the column is sometimes used, which allows a lower injection temperature.

3. Inlets for open tubular (capillary) columns

a. Open tubular columns usually have a much smaller cross-section area than that of packed columns. This makes them more subject to extra-column band-broadening, requiring that special low volume injection techniques be used with them.

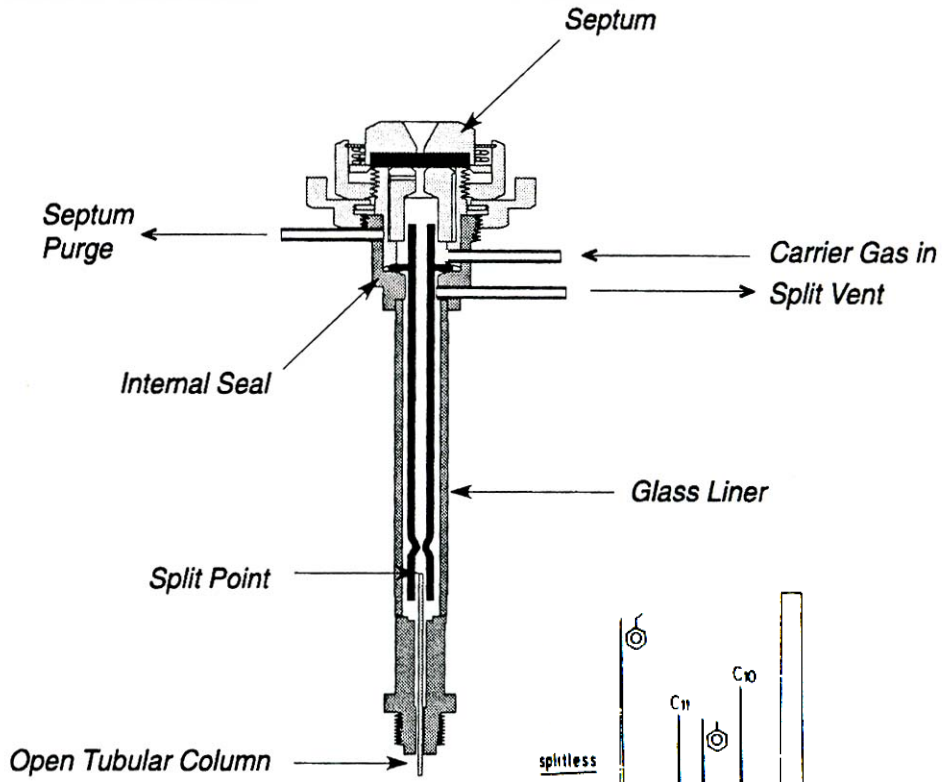


b. Injection techniques used on open tubular columns include inlet splitters/splitless, cold on-column injectors and programmed temperature vaporizers. The aim of each is to apply a narrow plug of solutes to the column that is representative of the original sample.

c. *Inlet splitters* are commonly used if the solute are reasonably volatile, thermally stable and each make up between 0.001 and 10% of the sample composition.

(i) In this technique, the sample is first placed into the injection port and is vaporized.

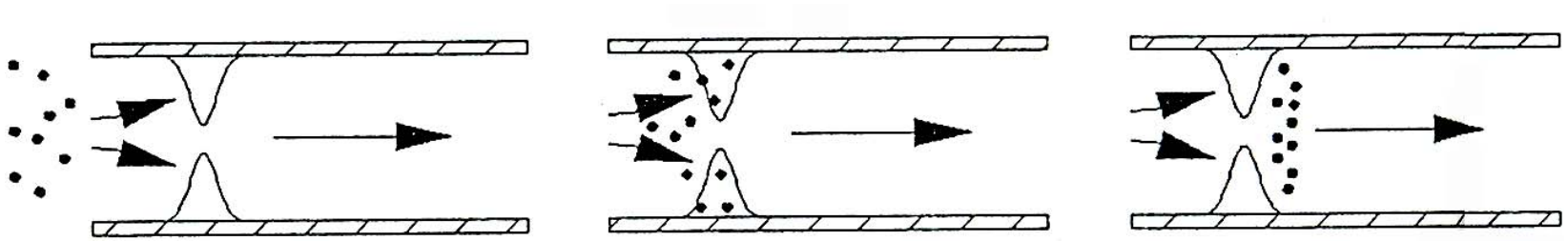
(ii) As the sample leaves the inject port, only a small portion of the vaporized samples is applied to the column (usually 1/20 to 1/200), with the remainder going to waste. This is splitting of the sample is used along with rapid injection, high carrier gas flow rate through the injectors, and high injector temperature to minimize the time that sample spends in the injector, which also minimizes extra-column band-broadening.



(iii) The main difficulty with inlet splitters is that solute with different volatilities may not be divided between the column and waste streams in the same ratios, affecting their quantitation.

d. *Splitless injectors;* Samples injected along with a large volume (about 5 µL) of a more volatile solvent.

i. As this combination is applied to the column, the volatile solvent travels ahead of the solutes. Due to its large volume, however, this solvent soon forms a thick liquid layer around the greatly increases retention of other solutes as they reached that region and concentrate them. The result is a narrower sample plug and less band-broadening.



"Cold-trap" focusing

ii. Since this method tends to concentrate solute, one application for it is in trace analysis or work with dilute samples.

e. Cold on-column injectors

(i) Cold on-column injectors involves direct injection of a sample onto a column at low temperature.

(ii) No heated injection port is used. The low initial column temperature increases the retention of all solutes and concentrates them at the top of the column in a narrow plug. The column temperature is then increased, allowing the solutes to volatilize and be separated.

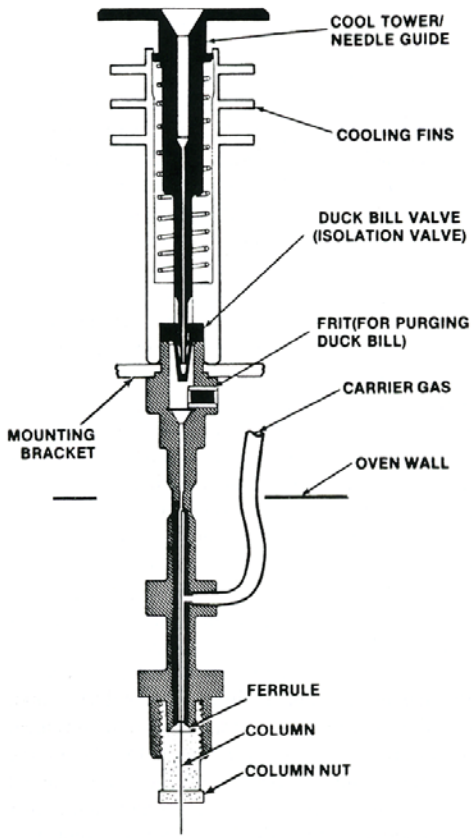
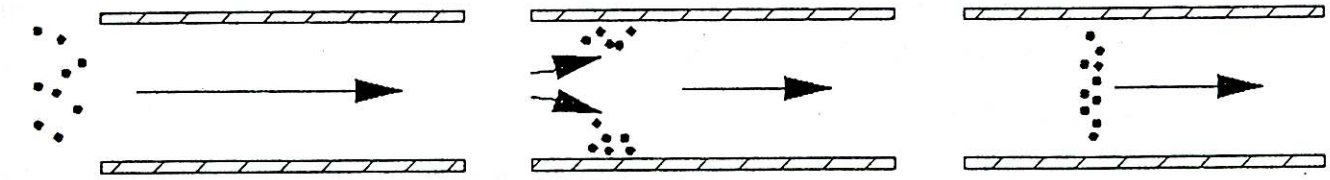
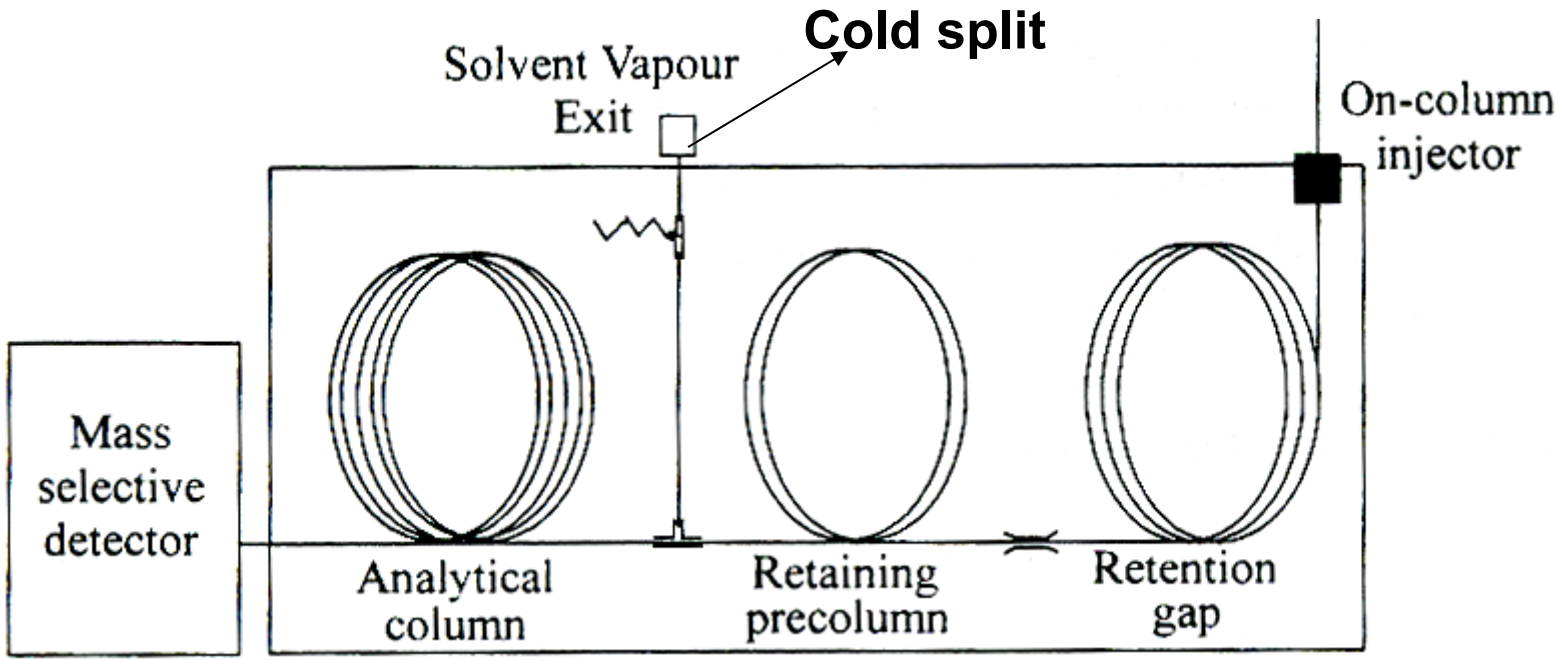


Figure 3.8. Cold-on column injector with a duckbill valve sealing mechanism (©Hewlett-Packard Co.).



(iii) For larger amount samples, retention gaps are used. Retention gaps are column inlets with reduced retention power compared the separation column. They function as guard column to protect the separation column from contamination of involatile residues.



"Cold-trap" focusing

f. Programmed temperature vaporizer (PTV)

A programmed temperature vaporize involves placing sample into a cold injection port, where it is then heated and applied to column at any desired temperature. This technique is gaining popularity as a "universal" injector for open-tubular columns since it temperature program may be changed so that it can be used either in cold injectors, splitless injectors, or split injectors.

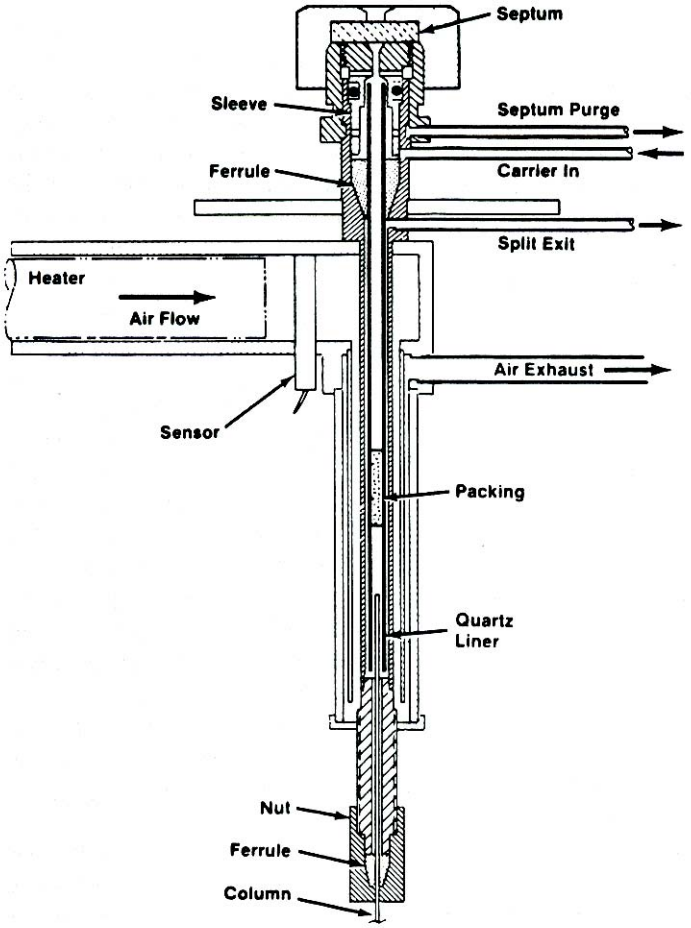


Figure 3.6. Schematic diagram of a PTV type injector. (From ref. [66]; ©Wiley-VCH).

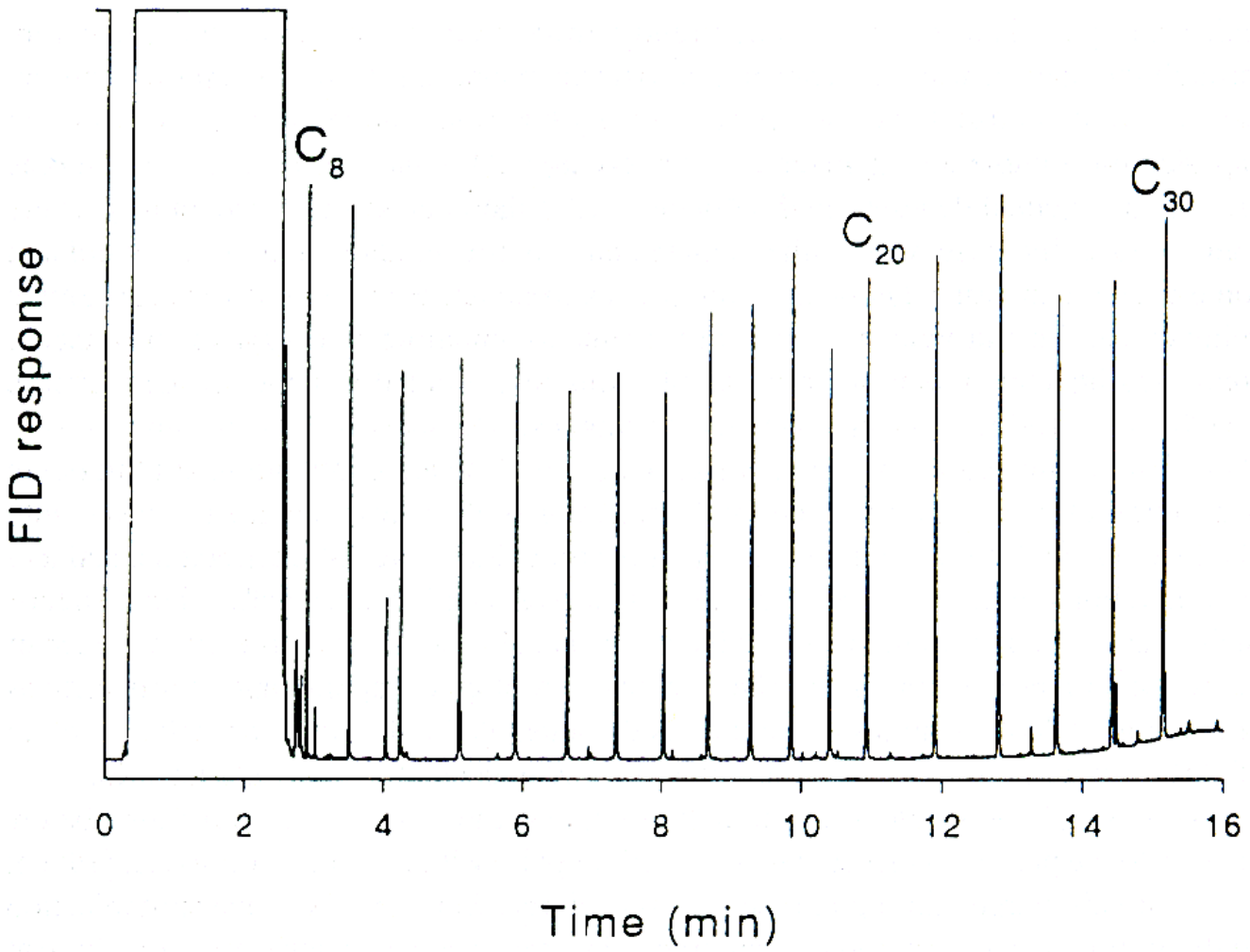


Figure 3.7. Large volume injection of 100 µl of a mixture of n-alkanes of wide volatility using the PTV injector in the cold split solvent elimination mode. The vaporizing chamber was thermostated at 0°C, split flow 250 ml/min with the split vent closed after 2.5 min. For splitless transfer the vaporizing chamber was heated at 4°C/s to 325°C with the purge flow started after 1.5 min. (From ref. [68]; ©Wiley-VCH).

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