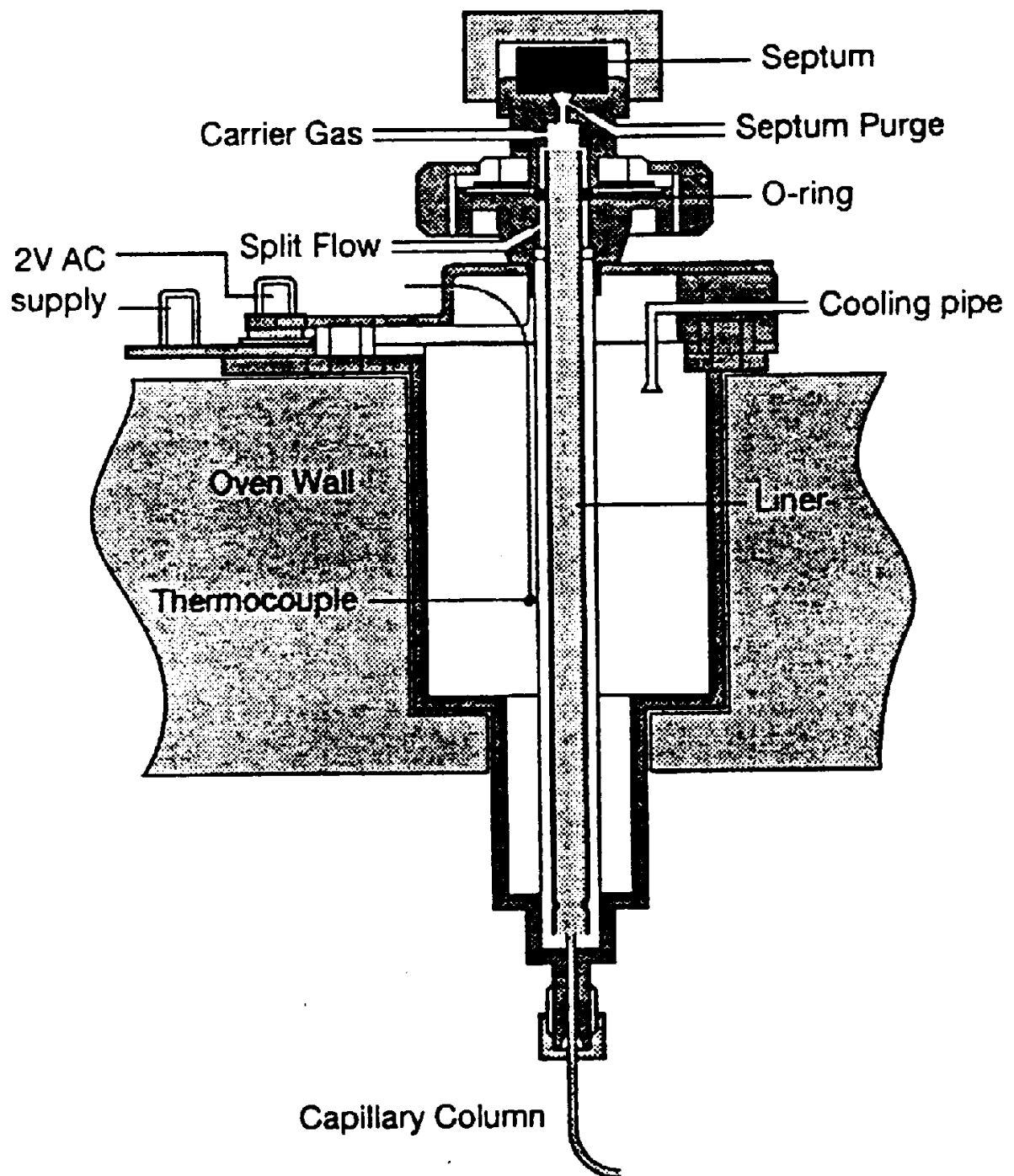


ATAS international B.V.

SAMPLE INTRODUCTION  
IN  
CAPILLARY GAS CHROMATOGRAPHY



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## **Preface**

The introduction of liquid samples in gas chromatography has been a problem ever since the introduction of this technique into routine laboratories. Especially in capillary gas chromatography, extraordinary demands are placed on the sample introduction device. An extremely small sample amount has to be introduced accurately and rapidly in a reproducible manner into the smallest possible gas volume. This gas plug must be transferred into the column without any losses by degradation, adsorption or discrimination. It is evident that this can only be achieved by employing very sophisticated inlet devices. For routine analysis of liquid samples four (groups of) injection techniques are available: split, splitless, on-column and programmed temperature vaporising injection. In this manual the principles, and the advantages and disadvantages of these four injection techniques will be discussed. Keywords in the comparison of these techniques are discrimination, thermal degradation and the applicability of the individual techniques for trace analysis. Special attention is paid to the selection of the most appropriate injection technique for a given application. The discussion is generally limited to liquid samples. The last chapter of this manual is entirely devoted to the introduction of large sample volumes in capillary GC using the PTV injection system. It is the personal feeling of the author that large volume injection techniques will become extremely important in the not too distant future.

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# **1. INJECTION TECHNIQUES FOR CAPILLARY GAS CHROMATOGRAPHY: SAMPLE INTRODUCTION WITH SPLIT, SPLITLESS AND ON-COLUMN INJECTION**

## 1.1. INTRODUCTION

The real chromatographic analysis starts with the introduction of the sample onto the column. The development of capillary gas chromatography resulted in many practical problems with the injection technique. The technique of on-column injection, often used with packed columns, is usually not possible with capillary columns. The injection system, in the capillary gas chromatograph, should fulfil the following two requirements:

1. The amount injected should not overload the column.
2. The width of the injected plug should be small compared to the spreading due to the chromatographic process. Failure to comply with this requirement will reduce the separation capability of the column.

Some general requirements, which a good injection technique should fulfil, are:

1. It should be possible to obtain the column's optimum separation efficiency.
2. It should allow accurate and reproducible injections of small amounts of representative samples.
3. It should induce no change in sample composition. It should not exhibit discrimination based on differences in boiling point, polarity, concentration or thermal/catalytic stability.
4. It should be applicable for trace analysis as well as for undiluted samples.

In the following sections split, splitless and on-column injection will be discussed in detail. These sections form the basis (or frame of reference) for the discussions of the various PTV sample introduction methods presented in chapter 2.

## 1.2. SPLIT INJECTION

The sample, in most cases a liquid, is introduced into a heated space, the liner, where fast evaporation takes place. As a result of the fast evaporation and the (required) turbulent flow, the sample vapour is mixed with the carrier gas in the liner. This diluted gas mixture flows with a high velocity past the column entrance where a small portion is introduced into the column, but most is carried away along the split line (Fig. 2.1). The splitting of the sample serves two purposes. Fast evaporation and a short residence time in the liner results in a small injection plug. Secondly, splitting reduces the size of the sample to an amount compatible with the sample capacity of the capillary column. To improve mixing between the vaporised sample and the carrier gas, packed liners containing a plug of glass wool are sometimes used. With such liners better reproducibility is normally obtained. Due to catalytic activity, however, even properly deactivated glass wool can result in serious degradation of unstable solutes.

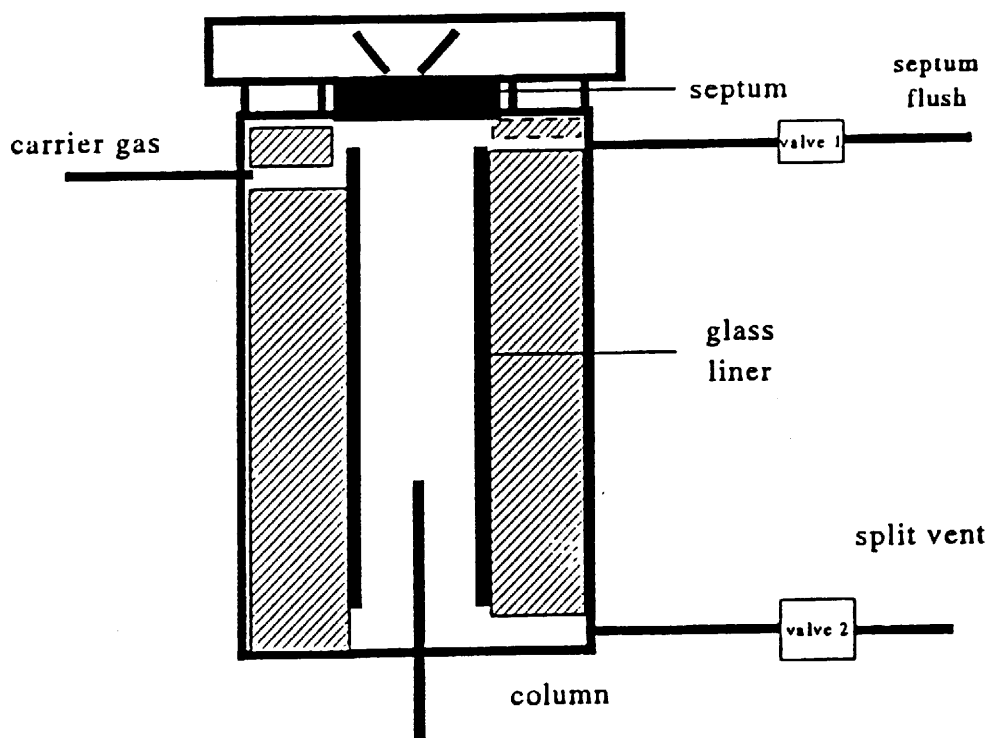


Figure 1.1. The Split-Splitless Injection System.

The ratio of the amount of material entering the column to the amount lost via the split can be calculated from the ratio of the column flow and the split flow. The split ratio is defined as:

$$\text{Split ratio} = \frac{F_{\text{column}}}{F_{\text{split}} + F_{\text{column}}} \quad 1$$

$F_{column}$  = column flow [ml/min]  
 $F_{split}$  = split flow [ml/min]

Because generally the column flow is much lower than the split flow, this equation can be rewritten as:

$$Split\ ratio = \frac{F_{column}}{F_{split}} \quad 2$$

The split flow can be easily measured with a bubble flow meter. The column flow can be calculated with equation 3. The actual measurement of the column flow via the detector outlet is often very inaccurate and is therefore discouraged. Especially if a FID is used as the detector.

$$F_{column} = \frac{\pi}{4} \cdot d_c^2 \cdot \frac{L}{t_0} \cdot \frac{273 + T_{out}}{273 + T_{column}} \cdot \frac{2}{3} \cdot \frac{\left[ \left( \frac{P_{in}}{P_{out}} \right)^3 - 1 \right]}{\left[ \left( \frac{P_{in}}{P_{out}} \right)^2 - 1 \right]} \quad 3$$

with:

$d_c$  = column diameter [mm]  
 $L$  = column length [m]  
 $t_0$  = dead time [min]  
 $P_{in}$  = inlet pressure (absolute) [bar]  
 $P_{out}$  = outlet pressure (usually 1 bar)  
 $T_{out}$  &  $T_{col}$  = ambient and column temperature respectively [°C]

Note : The dimensions of these parameters might look not consistent. The required correction factors, however, are already included in the equation.

An example of the calculation is given on the next page.

Example of a calculation:

Column: 25 meter, 0.32 mm.

Pressures:  $P_{in} = 0.7 \text{ bar (gauge reading)} + 1 \text{ bar} = 1.7 \text{ bar.}$

$P_{out} = \text{atmospheric} = 1 \text{ bar.}$

Temperatures:  $T_{out} = 25^{\circ}\text{C.}$

$T_{column} = 100^{\circ}\text{C.}$

Hold-up time: Measured using Methane = 1.21 minutes.

Result of calculation:  $F_{column} = 1.83 \text{ mL/min}$

Note: pressure gauges on most GC instruments give pressure. To this value Atmospheric pressure must be added.

If mass spectrometric detection is employed, equation 3 can no longer be used because in this case the value for  $P_{out}$  is not known accurately. In this particular case equation 3 can be approximated by:

$$F_{column} = \frac{\pi}{4} \cdot d_c^2 \cdot \frac{L}{t_0} \cdot \frac{273 + T_{out}}{273 + T_{column}} \cdot \frac{2}{3} \cdot P_{in}$$

### Practical aspects

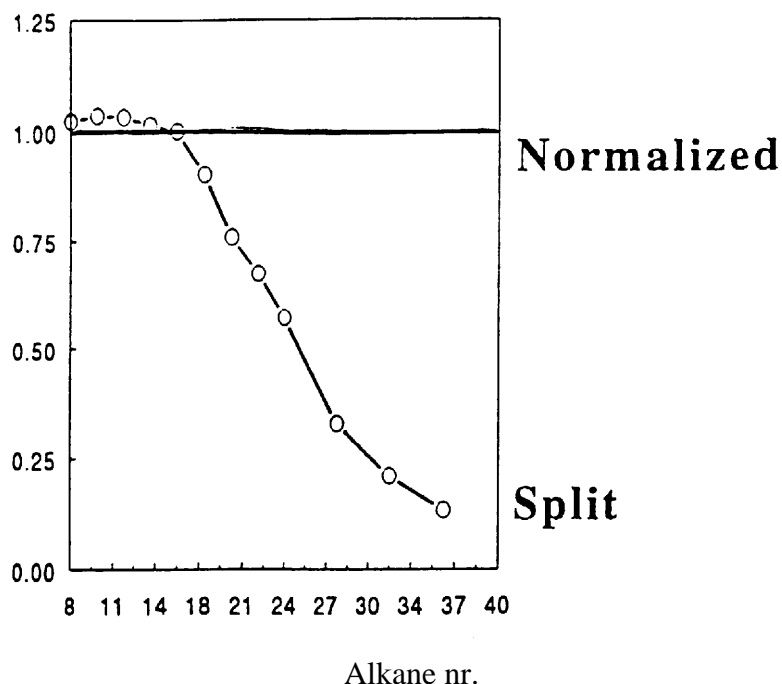
Normal split ratios are between 1:20 and 1:400. This means that only 1/20 or 1/400 part of the sample is introduced into the column. One must realise that sample size and split ratio must be adapted to the problem at hand. When the split ratio is too low a broad injection band will be introduced into the column resulting in broader peaks. Furthermore, column overloading can take place. This, usually results in asymmetrical peaks (peaks with leading fronts). A very high split ratio has a very unfavourable effect on the detection limit. For high capacity columns, such as wide bore columns and/or thick film columns, low split ratios (1:5 to 1:20) are commonly used. In high speed capillary chromatography where columns with inner diameters below 100  $\mu\text{m}$  are used, split ratios can exceed 1:1000.

### Advantages and disadvantages

Generally, the application of split injection is simple. Split injection has, however, a number of important disadvantages. Due to a large loss of sample, split injection is not suitable for trace analysis. The detection limit using a flame ionisation detector (FID) is about 25 ppm (=50 ng/ul). Depending on the injector temperature, thermal degradation can take place, especially when using liners packed with glass wool or liners containing glass frits. This means that split injection is not suited for the analysis of components liable to thermal degradation. Discrimination can not be avoided. In the heated liner since the less volatile sample constituents evaporate less readily from the syringe needle. Hence, the actual composition of the sample that enters the column is no longer a correct representation of the real sample. The reproducibility of split injection is strongly dependent on the geometry of the liner and "the style of injection". Despite the disadvantages summarised above, split injection nowadays is the most widely used injection technique. This is mainly due to its ruggedness and its ease of use.

The occurrence of discrimination during split injection is shown in figure 1. This figure shows the peak areas observed for a number of normal alkanes from a sample that contained equal amounts of the various compounds. The 'normalised' line gives the original composition of the sample. From the figure it is clear that the relative peak areas of the higher alkanes are too low. For these components the residence time of the injection needle in the heated inlet port is too short. Transfer of the components onto the column is not complete. Hence, the composition of the sample introduced onto the column is no longer equal to that of the original sample.

Discrimination can be minimised (but usually not avoided) by using a good method of handling the syringe. Discrimination is minimised if the sample is injected either extremely fast or extremely slow. If the syringe is inserted into the injector rapidly, the plunger is depressed rapidly and the syringe than immediately removed from the injector, there is not sufficient time for the needle to heat up and the sample is injected into the hot liner in the liquid state. Selective evaporation is avoided and, hence, discrimination is absent. Human operators can not carry out this very fast injection method. Only a few very fast autosamplers are fast enough. The second alternative is to inject very slowly. In this method the needle is left several seconds in the injector after injection. In this way also the higher boiling sample constituents are allowed sufficient time for evaporation.



**Figure 1.2.** Discrimination due to differences in boiling point. Sample: equal amounts of all components (normal alkanes) in hexane. Injection: split.

In the literature a number of other methods to reduce the discrimination effect have been described [1]. Schomburg, for example, developed an injection syringe with a cooled needle [2]. In this system evaporation of the sample from the syringe is suppressed, thereby preventing selective transfer of the more volatile components onto the column. The sample is introduced into the heated liner in the liquid state. Although discrimination is absent using this system (if carefully optimised), the system never gained widespread acceptance due to its technical complexity. Also various syringe manipulations have been studied (*e.g.* filled needle, hot needle, solvent flush, air flush, sandwich method). Although differences in the magnitude of the discrimination effect exist between these methods, none of them guarantees truly discrimination-free operation.

The occurrence of discrimination does not automatically precludes the use of split injection in daily practice. For *qualitative* analysis split injection is well suited. The only exceptions to this rule are highly unstable components that are completely degraded in the injector or very high boiling components that are not transferred to the column at all. If special care is taken, split injection can also be used for *quantitative* analysis of components for which discrimination occurs. In that case, however, poor reproducibility is generally found, both for absolute and relative peak areas.

### 1.3. SPLITLESS INJECTION

The hardware required for splitless injection is very similar to that used for split injections. As in the case of split injection the sample is evaporated in a heated liner. The split line however, is now closed. Transport of sample vapours onto the column can only take place by means of the column flow. After the largest part of the sample has been introduced into the column, usually 10-40 secs. After the injection (*i.e.* the so-called splitless time), the split line is opened and the liner is quickly flushed. Sample is introduced onto the column during the entire splitless time. A very serious broadening of the peaks would result without reconcentration of the sample in the column. The use of a suitable initial column temperature ensures condensation and reconcentration of the sample takes place in the column. Two reconcentration mechanisms can be distinguished:

1. Cold trapping. Reconcentration of high boiling components takes place by a cold trapping mechanism (Fig. 1.3). In the first centimetres of the column there is a negative temperature gradient, where the temperature drops from the injection temperature ( $\pm 250^{\circ}\text{C}$ ) to the oven temperature (*e.g.*  $40^{\circ}\text{C}$ ). Due to this temperature drop the mobility of the heavy components reduces to virtually zero. The components remain in a small band and will only start to migrate when the oven temperature has risen sufficiently during a temperature programme. Optimal reconcentration takes place if the initial oven temperature is about  $150^{\circ}\text{C}$  or more, below the boiling point of the components.
2. Solvent effect. Reconcentration of low boiling components (b.p. less than roughly 50 to 100 degrees above the boiling point of the solvent), takes place by the so-called solvent effect (Fig. 1.3). When the starting temperature of the column is about  $20^{\circ}\text{C}$  below the boiling point of the chosen solvent, then the lighter components will condense in the column together with the solvent. The liquid film formed will start to evaporate from the back and the sample components will concentrate in a continuously shortening liquid film. This results in a very small band of reconcentrated sample components.

The two refocussing mechanisms ( cold trapping and solvent effect ) are schematically illustrated in figure 1.3.

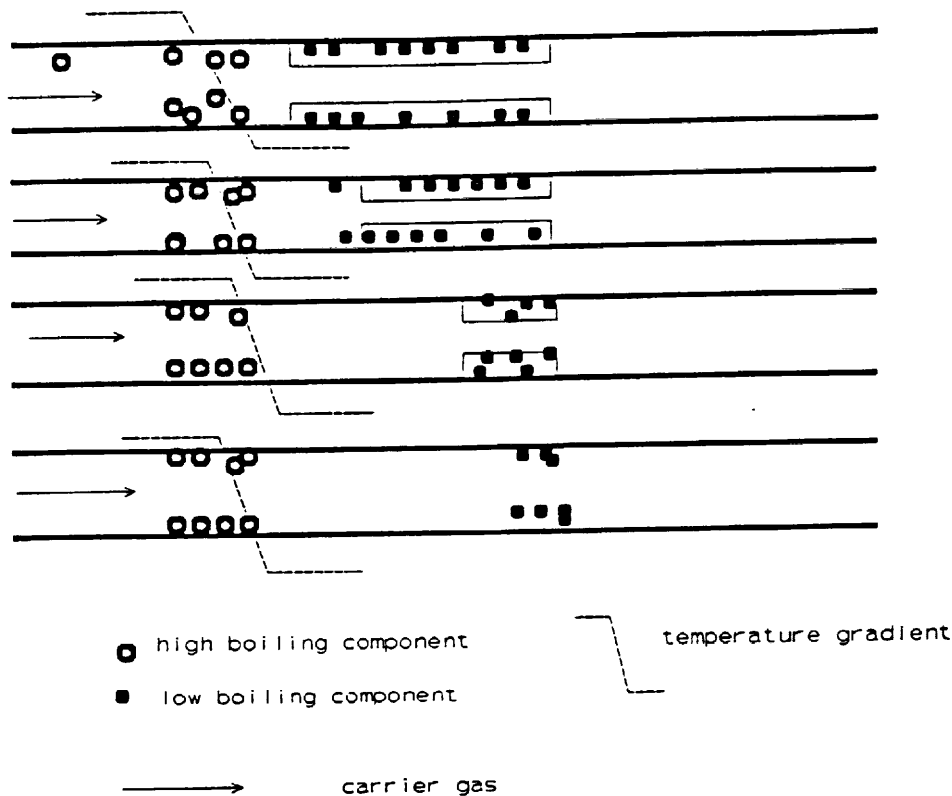


Figure 1.3. Cold trapping and Solvent effect for splitless injection.

For a proper reconcentration of the sample in the column, mixing of the sample vapour and carrier gas in the liner should be suppressed. This can be achieved by a combination of a small non-curved liner and a slow injection. In general, long and narrow inserts are preferred to obtain minimal sample dilution. This in contrast to split injection where mixing of the sample with carrier gas in the liner is a prerequisite. Therefore wide, baffled liners are used in split injection.

The initial temperature of the column is of the utmost importance. A fast condensation of the vapour can be achieved by selecting an oven temperature of about 20°C below the boiling point of the solvent. In contrast to what the name suggests, the split exit is open during most of the GC run. It is only a few seconds before and a short time after injection that the split flow is switched off. The time the split is closed is called the splitless time. Besides the correct choice of temperature and liner geometry the optimisation of splitless time is of crucial importance. If the split line is opened too soon losses of sample will occur, furthermore, reproducibility is often poor. If the splitless time is too long sample components and solvent will show severe tailing.

### Practical aspects

For splitless injection a conventional split injector with a solenoid valve in the split line is required. Prior to injection, the solenoid valve is closed. Very often also the septum purge is closed. After waiting a few seconds to give the system time to restabilise, the sample is introduced into the heated liner. Care has to be taken not to overfill the liner with gaseous sample. As a rule of the thumb one could say that 1  $\mu\text{L}$  of liquid sample upon evaporation gives about 500  $\mu\text{l}$  of vapour. If the amount of vapour exceeds the capacity of the liner, sample material might be forced back into the carrier gas lines (resulting in contamination of the carrier gas system) or lost through the septum purge line if this line is not closed.

A very important parameter in splitless injection is the splitless time. Optimisation can take place by injecting a sample using different splitless times. The optimal splitless time is achieved when the recovery of a certain peak is at a maximum. If too low injection times are used, significant losses of sample material occur which leads to unfavourable detection limits and a poor reproducibility. If times are used that are too long, traces of the solvent can give rise to a severely tailing solvent peak. In Figure 1.4A a plot of the peak area of n-C<sub>9</sub> as a function of the splitless time is shown. The optimum splitless time here is approximately 20 seconds. Figure 1.4B shows the retention time of the test component, again as a function of the splitless time. As can be seen from this figure, the retention time increases with increasing splitless time. The explanation for this at first glance surprising observation is relatively straightforward. In a splitless injection the solvent recondenses in the column inlet resulting in a very thick layer of a "temporary stationary phase". This layer of solvent will significantly retain the components present in the sample. When the splitless time is increased the amount of solvent that enters the column increases, which leads to an increased retention of the components eluting slightly after the solvent peak. The times of the later eluting compounds are not affected by the duration of the splitless time.

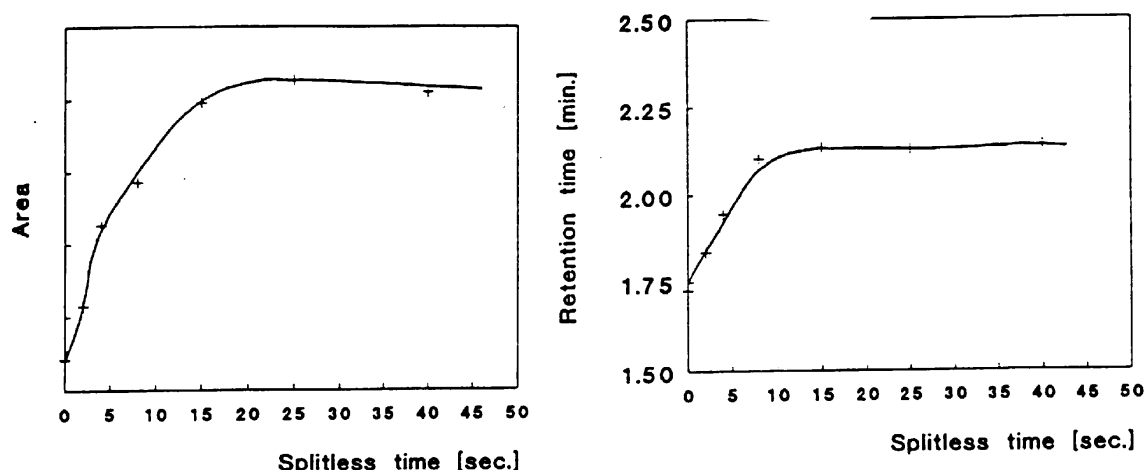


Figure 1.4. Peak area (A) and retention time (B) of n-C<sub>9</sub> as a function of the splitless time.

The solvent effect relies on the formation of a homogeneous solvent film at the inlet of the column. This is only obtained if the polarity of the solvent matches that of the stationary phase. If this is not the case, e.g. if a *polar* solvent is introduced on to a *non-polar* stationary phase,

droplet formation occurs if the volume exceeds a certain (low) critical limit (typically less than 0.5 to 1  $\mu\text{l}$ ). Each of these individual droplets tends to give rise to its own solvent effect. This can result in broadened peaks, peak splitting and the so-called 'Christmas tree effect'. These problems are generally most pronounced for the lower boiling components. Broadening or peak splitting due to droplet formation can be suppressed by using a retention gap (see below). The components that are not reconcentrated by the solvent effect, *i.e.* the high boiling constituents give good peak shapes. As their refocusing mechanism (cold trapping) does not rely on the formation of liquid film.

Figure 1.5 shows the splitless introduction of 2  $\mu\text{L}$  of a solution of normal alkanes in methanol on a non-polar OV-1 column. The poor peak shapes of the low boiling components clearly indicate that the solvent does not wet the stationary phase sufficiently well. Note the good peak shapes for the high boiling components.

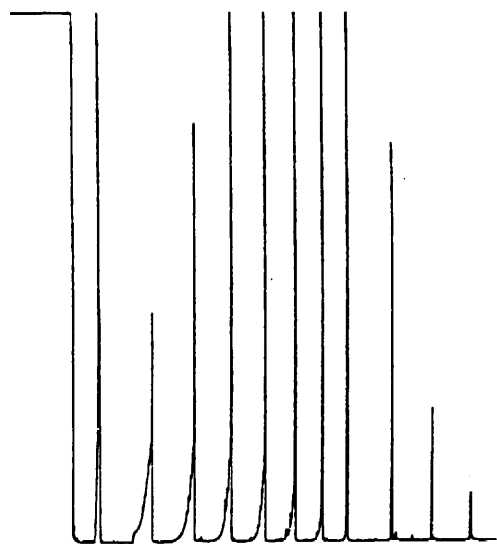


Figure 1.5. Introduction of a normal alkane standard mix dissolved in methanol on a non-polar stationary phase.

### Advantages and disadvantages

In contrast to split injection, the implementation of splitless injection is more complicated. The

oven temperature, the solvent and the splitless time have to be selected very carefully. Since the complete sample is introduced into the column, this technique is suitable for trace analysis. Trace analysis down to 0.5 ppm (FID) without pre-concentration is possible. Fractionation of the sample by expulsion of more volatile components from the syringe needle and retention of the less volatile components in the needle can occur. Thermal degradation is generally much more pronounced than in split injection because of a longer dwell time of the sample in the heated injector. The reproducibility of absolute peak areas is comparable to the values obtained in split injection. The technique is easy to use (once optimised), fairly rugged and easy to automate.

#### 1.4. ON-COLUMN INJECTION

With on-column injection a liquid sample is introduced directly into the column with a thin injection needle. During the course of 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.

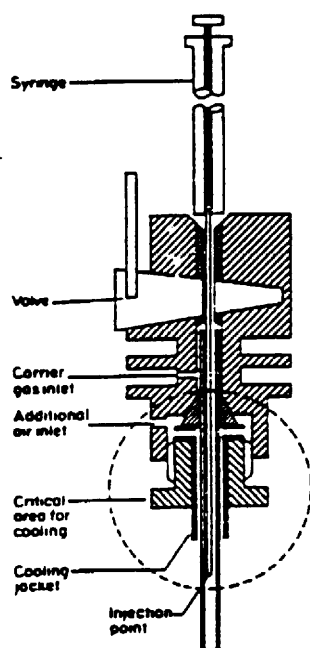
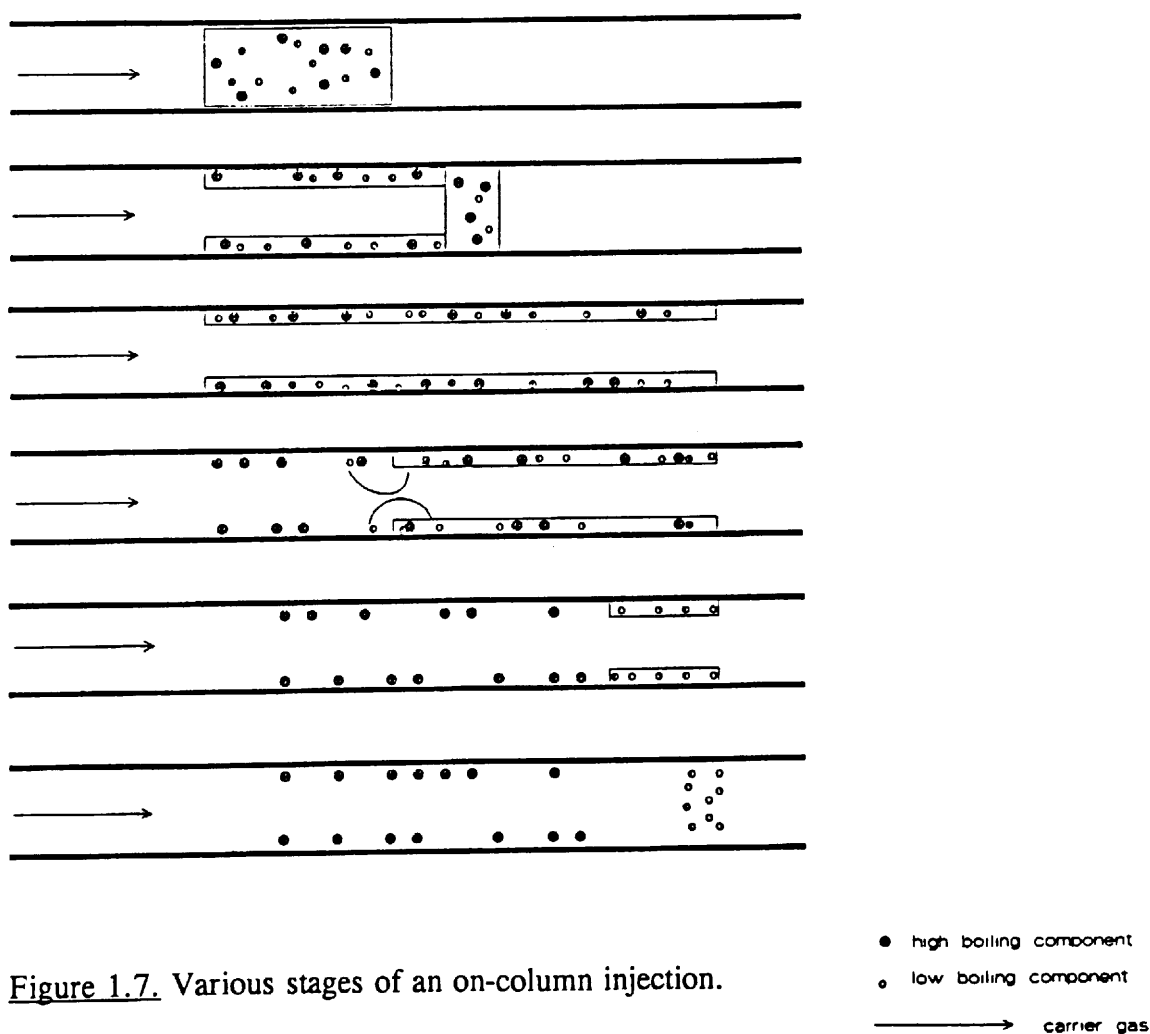


Figure 1.6. Schematic design of on-column injector

With regard to instrumentation, on-column injection is fairly difficult. The injector should contain a valve, which allows the introduction of the injection needle into the column without a

loss of carrier gas. Figure 1.6 shows a schematic representation of the Carlo Erba Instruments on-column injector. For injection, the injection needle is introduced into a port, which closely fits its external diameter. The valve, which closes the entrance to the column, is then opened. The carrier gas can not escape from the valve because the outside diameters of the injection needle exactly fits the hole drilled in the injector block. During injection the sample is deposited onto the inner wall of the column as a thin liquid film. After injection the needle is withdrawn to a position just above the valve and the valve is closed.

The sample injected onto the column, can wet a considerable length of the column. Without reconcentration this could give rise to considerable broadening of peaks, especially for the more volatile components. For instance: 1  $\mu\text{l}$  of sample can create a 30 cm wet zone! Reconcentration of volatile components takes place through the solvent effect, (see splitless injection & Fig. 1.3). The starting temperature of the oven should not be too high in order to prevent an evaporation of the liquid film occurring too rapidly. If this occurs, the liquid film can break up into separate parts, which results in the splitting of the peaks.



**Figure 1.7.** Various stages of an on-column injection.

There are many opinions on the selection of the optimal injection temperature (or initial oven temperature) in on-column injection. Good results are generally obtained with an injector at between 20°C below the boiling point and 10°C above the boiling point of the solvent. At higher temperatures selective evaporation from the needle can take place. With an on-column injection, no reconcentration of the higher boiling components takes place, since there is no negative temperature gradient between the injector and the column oven, as is the case in splitless injection.

When large volumes have to be injected (>2µl), use must be made of a so-called 'retention gap'. A retention gap is a few meters of deactivated uncoated capillary tubing connected in front of the analytical column. Care should be taken to avoid dead volumes in the coupling piece that is used for connecting the retention gap and the analytical column. Injections are made into the uncoated capillary. At the start of the temperature program, high boiling components will concentrate in the stationary phase of the analytical column, because retention in the analytical column is stronger than in the empty capillary.

Since injections are made directly into the capillary column or retention gap, the minimum column diameter that can be used is 250 µm. For these columns fused silica needles with an outside diameter of about 200µm have to be employed. For 320 µm i.d. GC columns more rugged stainless steel injection needles can be used. Columns with inner diameters below 250 µm can only be used in combination with a wide bore retention gap.

As is the case with splitless injection, the formation of homogeneous solvent film in the column inlet is of utmost importance. When polar solvents are introduced on to non-polar stationary phases the incompatibility of solvent and stationary phase will again result in droplet formation in the column giving rise to peak broadening and peak splitting. In contrast to the situation in splitless injection this will now no longer only occur for the low boiling components but will be observed throughout the entire chromatogram. The use of a deactivated retention gap in general will be sufficient to circumvent these problems. A striking example of the problems that can occur if the solvent does not form a homogeneous liquid film is given in figure 1.8. This figure shows the analysis of a trimethyl-silyl derivative of the linear surfactant Triton X-100 with hexane (A) and methanol (B) as the solvent. Poor, significantly broadened peaks are observed when methanol is used as the solvent.

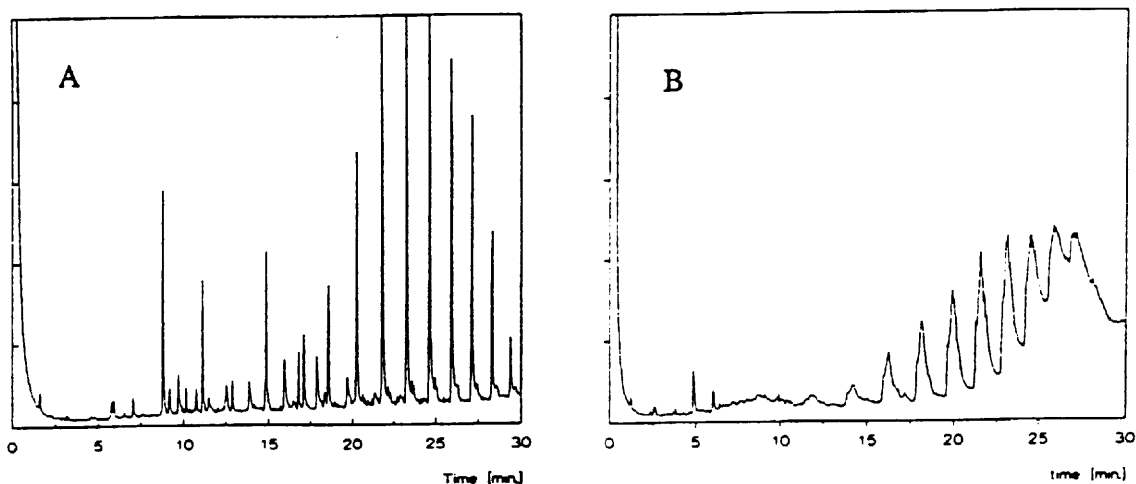


Figure 1.8. Introduction of non-polar and polar solvents on to a non-polar stationary phase. A: Non-polar solvent (hexane), B: Polar solvent (Methanol).

### Practical aspects

When using low boiling solvents, the injection port has to be cooled; preventing selective evaporation and consequently discrimination. A number of on-column injectors are equipped with a cooling system, the so-called secondary cooling, to reach a sufficiently low temperature and to rapidly cool the hot injector.

### Advantages and disadvantages

With an on-column injection, very reproducible, absolute volumes can be injected greatly reducing the chance of discrimination occurring. On-column injection is very suitable for high boiling components because no evaporation takes place during the injection period. In figure 1.9 the peak areas obtained in the analysis of a sample containing equal amounts of normal alkanes are shown as a function of the carbon number. In the figure both the lines for split and on-column injection are given. It is interesting to see the large difference between the lines for the two injection techniques. As in on-column injection no intermediate evaporation step is used, the composition of the sample that is introduced on to the column must be exactly equal to the original composition. Indeed the on-column data show a much better agreement with the actual amounts of the various n-alkanes in the sample.

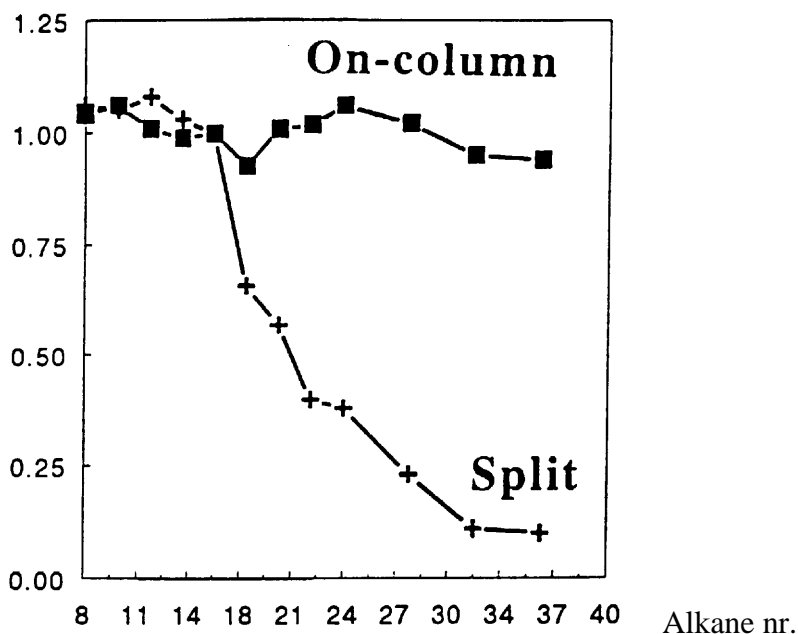


Figure 1.9. Analysis of an alkane standard with on-column and split injection. The 'normalised' line gives the actual composition of the sample.

For an FID detector, the detection limit obtained with on-column injection is similar to that of the splitless injection, about 0.5 ppm. The use of a suitable retention gap permits the injection of large volumes [4]. Another important advantage of on-column injection is that thermal degradation of solutes during injection can not occur. The solutes are directly introduced into the well deactivated column. On-column is therefore the technique of choice for the introduction of unstable solutes such as modern pesticides and many food and flavour components. From these characteristics it is evident that on-column injection is clearly superior to both split and splitless injection. It is advisable to use a retention gap when highly contaminated samples have to be injected. As in the case of splitless injection, the initial oven temperature has to be carefully selected. Despite its significant advantages, on-column injection has not gained wide-spread acceptance in routine analysis. This is mainly due to the less rugged nature of the technique and the difficulties encountered in automation. In addition, there is a serious risk of column contamination as the entire contents of the syringe (including any involatile material) is directly injected into the column or retention gap.

### 1.5. COMPARISON OF CONVENTIONAL SPLIT, SPLITLESS AND ON-COLUMN INJECTION

In the previous paragraphs, the principles and the advantages and disadvantages of split, splitless

and on-column injection have been treated in detail. In this paragraph the selection of the most appropriate injection technique for a given application will be discussed. Very often this is considered to be the most difficult step in method development for capillary gas chromatography.

In selecting the proper injection technique for a particular application a number of factors must be considered. Apart from the concentration levels of the components in the sample, their boiling points and thermal stability's, the nature and properties of the solvent can also affect the ultimate choice of the injection technique. Moreover it appears that the ultimate choice of the injection system is often also at least partly determined by the mere availability of the various injection systems. In deriving this decision tree type graph it is assumed that all samples are clean, i.e. do not contain components or solid particles that cannot be eluted from the GC column.

Figure 1.10 shows a decision diagram that can be used for selecting the proper injection technique for a given application. In constructing this diagram only conventional split, splitless and on-column injection are considered. Furthermore, it is assumed that all samples are clean, *i.e.* do not contain dirt, dust or very high boiling components that can not be eluted from the chromatographic column. The discussion is generally limited to liquid samples. A question mark in the diagram indicates that that particular type of sample can not be analysed using one of the conventional injection techniques. In chapter 2 it will be shown that programmed temperature sample introduction is a good alternative for a significant number of the sample types identified in figure 1.10, -including those that can not be handled using conventional sampling techniques.

The first important parameter in the process of selecting the proper injection technique for a particular application is related to the concentration of the components in the sample. High concentrations (> 50 ppm, FID detection) can be analysed directly with hot split injection or, after dilution, with hot splitless or cold on-column injection. Concentrations between approximately 0.3 and 50 ppm require the use of hot splitless or on-column injection. Split injection of these samples is only possible after a suitable pre-concentration step.

A second important parameter to consider is the boiling point of the solutes. For example, if the sample contains high boiling components, hot injection techniques cannot be used. For these types of sample, only cold on-column injection is suitable. Apart from the concentrations and boiling points of the components, their thermal stability should also be considered when selecting an injection technique. For components liable to thermal degradation, cold on-column injection should always be the method of choice. Finally, the polarity of the solvent can affect the ultimate choice. Large volumes of polar solvents introduced onto non-polar or intermediate polarity columns will give rise to distorted peaks as severe flooding of the column inlet occurs.

From Figure 1.10 it is evident that the analysis of highly diluted samples using one of the conventional injection modes is impossible. As there is a continuous need for lower detection limits this is a serious shortcoming of the conventional injection techniques. Apart from problems in the low concentration range, a few other question marks appear in the figure. These question marks are mainly related to the analysis of relatively large volumes of polar solvents. With programmed temperature vaporising injectors these two problems can be solved in an elegant and easy way, as will be discussed in chapter 2 of this course manual. A final remark about the analysis of dirty samples: These types of sample can best be analysed using split and splitless injection. In both cases, however, frequent cleaning/replacement of the injector liner will be necessary. If on-column injection has to be used for dirty samples, it is advisable to work with a retention gap. Here very frequent replacement of the retention gap will be necessary because of the limited capacity of the retention gap to accommodate involatile material and the fact that deposited dirt will affect the chromatographic retention process.

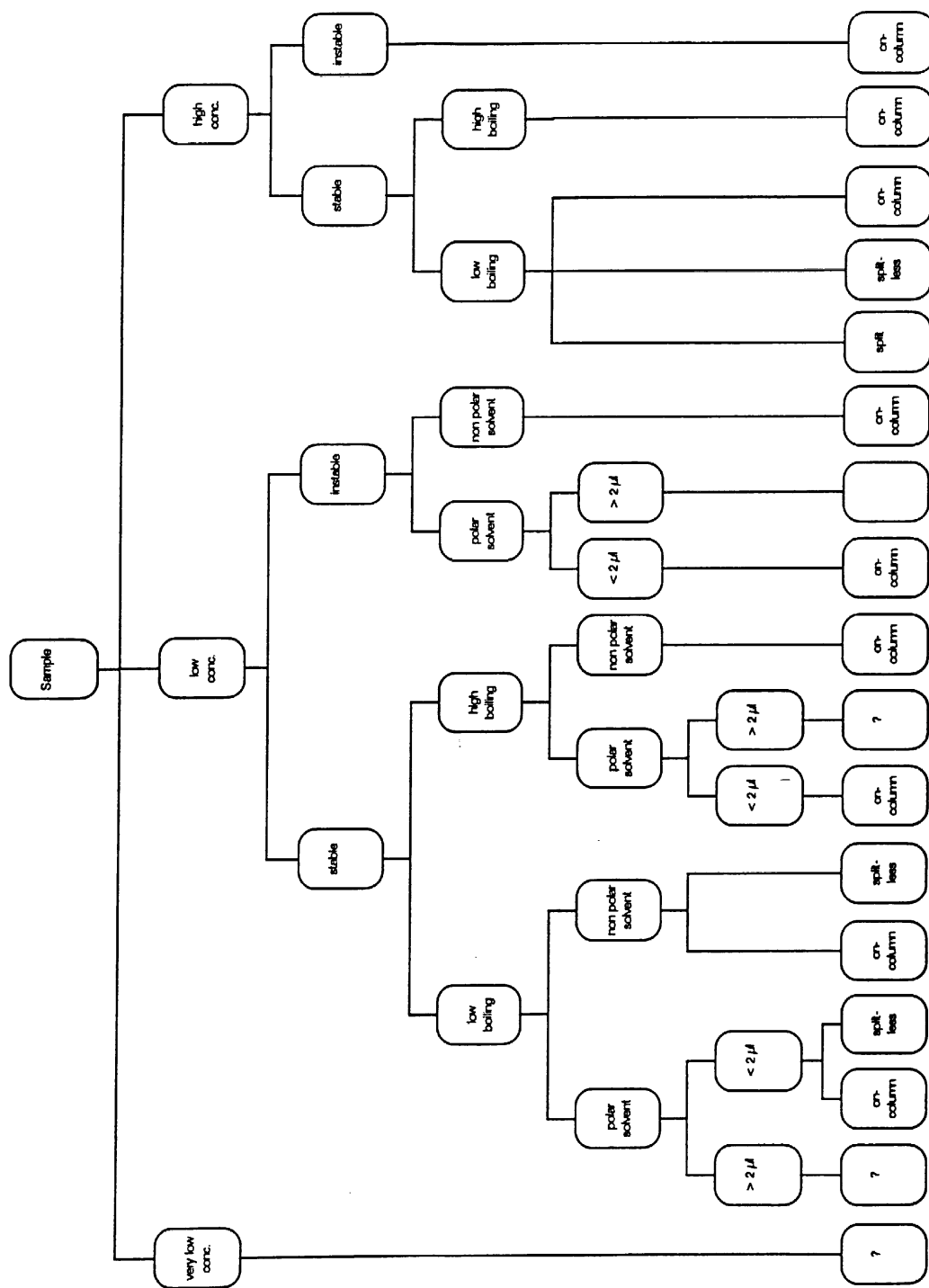


Figure 1.10. Decision diagram for the selection of the most suitable injection technique. Guidelines: Concentration ranges: very low: < 0.5 ppm, low: 0.5 - 50 ppm, high: > 50 ppm, (FID detection). Boiling points: high: above approx. n-C<sub>20</sub>, low: below approx. n-C<sub>20</sub>.

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## 2. TEMPERATURE PROGRAMMED SAMPLE INTRODUCTION

### 2.1. INTRODUCTION

Temperature programmed sample introduction was first described by Vogt in 1979 [1,2]. Originally Vogt developed the technique as a method for the introduction of large sample volumes (up to 250  $\mu\text{L}$ ) in capillary GC. Vogt introduced the sample into the liner at a controlled rate. The temperature of the liner was chosen slightly below the boiling point of the solvent. The low-boiling solvent was continuously evaporated and vented through the split line. Based on this idea Poy developed the Programmed Temperature Vaporising injector; PTV [3]. By introducing the sample at a low initial liner temperature many of the disadvantages of the classical hot injection techniques could be circumvented. Nowadays PTV injection is generally considered to be the most universal injection technique available.

In this chapter the principles and the possibilities and limitations of PTV type injectors will be discussed.

### 2.2. PROGRAMMED TEMPERATURE VAPORISATION

It was Poy who first realised that the technique of temperature programmed sample introduction that was developed by Vogt for the introduction of large sample volumes, had a number of advantages over conventional split, splitless and on-column injection. For example, discrimination, due to differences in boiling point can be minimised by introducing the sample in the liquid state into a cold liner and subsequently raise the temperature of the liner to the normal temperature of a conventional hot injector. It was Poy who proposed the name "programmed temperature vaporising" injection, abbreviated to PTV. Although names as "Cold Injection System" or "Cold/Programmable Injector" better reflect the main principle of the injection system, "PTV" is now a widely accepted description in chromatographic nomenclature.

A schematic drawing of a PTV injector is given in figure 2.1. As can be seen from this figure the programmed temperature vaporiser injector closely resembles the classical split/splitless injector. The heart of the system again is the liner. Of course also here various liner geometries can be used. The carrier gas enters the liner at the top. The split flow leaves the liner at the bottom. In the split line there is again a solenoid valve that allows switching from split to splitless operation.

The primary difference between conventional split/splitless injectors on the one hand and PTV injectors on the other is the temperature control. In PTV injectors the vaporisation chamber can be heated or cooled rapidly. The heat can be provided electrically by resistive heating, by using heater cartridges or by means of preheated compressed air. The heating is controlled using a

sophisticated control unit. Depending on the construction of the device and the software, heating can be performed ballistically or linearly at a pre-selected rate. Cooling can be performed using cold air, expanding CO<sub>2</sub> or liquid nitrogen. In order to facilitate rapid heating and cooling the thermal mass of the liner is minimised. For that reason liners for PTV injectors are normally smaller than those used in conventional injectors.

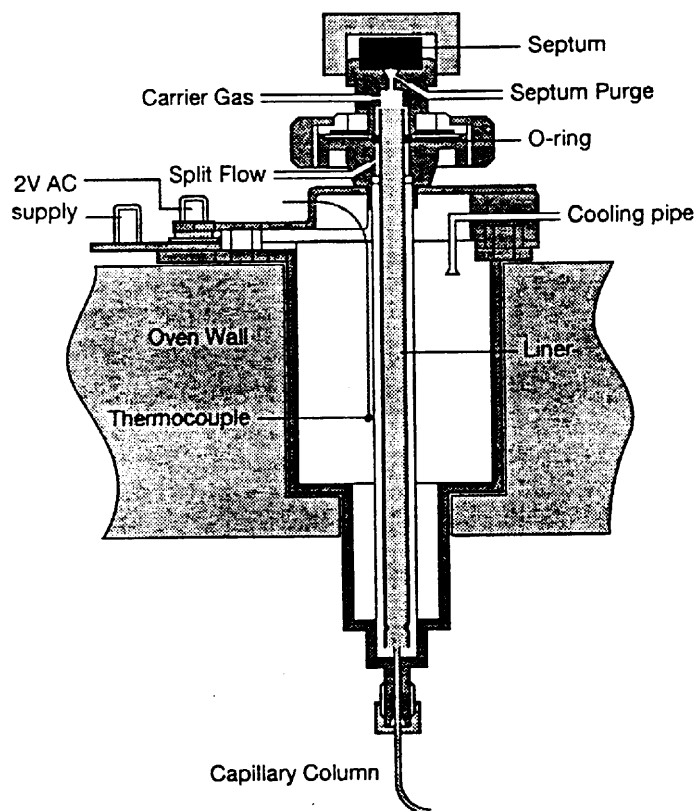


Figure 2.1. Schematic representation of a PTV injector.

In the PTV injector different independent temperature plateaux, iso-times and programming rates can be used which makes this injector a very flexible inlet device. Hot split and splitless, cold split or splitless injection and, after the introduction of a special insert, cold on-column injection can all be performed using only one injector. Combining a cool injection step with a controlled vaporisation eliminates a number of important disadvantages associated with the use of conventional hot sample inlets.

In the cold injection modes, discrimination is absent, as transfer of the sample into the chromatographic system is performed in the liquid state. Additionally, thermal degradation is less likely to occur. Apart from the five possible injection modes described above, PTV injectors can also be used for the introduction of very large solvent volumes. This type of injection is called the solvent vent injection. In this injection mode a large volume of sample is introduced

into the liner. The initial inlet conditions are arranged so that the solvent is vented via the split line while the components are trapped and pre-concentrated. At the end of the solvent elimination the split vent is closed and the liner is heated. In this way very large sample volumes up to 250 µl have been introduced onto 320 µm capillary columns [4]. An in-depth discussion of PTV large volume sampling is presented in chapter 3 of this manual. The solvent vent injection can also be used for the introduction of polar solvents. As the solvent is vented prior to introduction in the GC column no band distortion occurs.

One of the major advantages of PTV injectors is their great flexibility. Up to 5 (liquid) injection modes are possible without making changes to the hardware. The choice between these 5 injection modes is represented schematically in figure 2.2. This figure illustrates that the injection of a liquid sample using a PTV injector consists of three successive steps. In the first step, the actual injection, the sample is transferred from the syringe needle into the liner of the injector. In the third step the components are then transferred to the column. In an intermediate step it is possible to selectively remove the solvent. The PTV injector offers many other injection modes such as thermal desorption, pyrolysis, combined thermal desorption/pyrolysis, reaction injections etc. In this chapter the liquid injection techniques will be discussed.

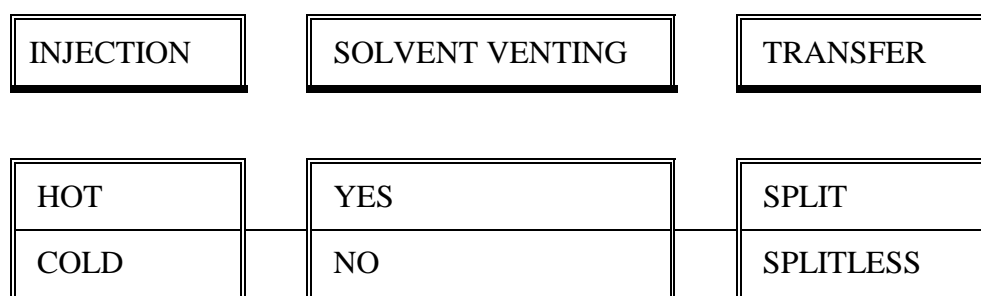


Figure 2.2. Choosing the PTV injection mode.

When selecting the PTV injection mode the first decision that has to be made is what should be the temperature upon introduction of the sample. Stated more precisely, the temperature at the moment the syringe is inserted into the injector.

The next question is whether it is necessary to vent the solvent prior to transferring the components to the column. This could be advantageous if, for example, the solvent is polar and the column non-polar or when the solvent is incompatible with the detector used in the instrumental set-up. Finally, it should be decided whether the sample be transferred to the column in the split or in the splitless mode. The five useful injection modes that result from these three decisions are summarised below:

1. Hot split injection: Split vent open continuously, sample is introduced in to hot injector. This mode is comparable to conventional split injection.

2. Cold split injection: Split vent open continuously, injection into cold liner. Injector is heated directly after injection of sample.
3. Hot splitless injection: Injector is hot at the injection of the sample. Split exit is closed first and opened after splitless time. This mode is equal to conventional splitless injection.
4. Cold splitless injection: Split initially closed. Sample is injected into cold liner. Injector is heated directly following sample introduction. Split vent is opened after splitless time.
5. Cold splitless/solvent vent injection: Split is initially open. Sample is introduced into cold liner. Before the injector is heated the solvent is vented at a low temperature. After removal of the solvent the split exit is closed and the injector is heated.

In the hot split and hot splitless mode PTV injectors are comparable to conventional (hot) split/splitless injectors. The other three injection types are unique for PTV injectors.

An important advantage of cold sample introduction is the absence of discrimination on the basis of differences in boiling points. The sample is introduced into the liner in the liquid state without an intermediate evaporation step. As a result, fractionation of the sample due to additional evaporation of the more volatile constituents is absent. Evaporation of the components, and thus transport of the components from the liner to the column, starts as the injector is heated. As the evaporation process now occurs in a controlled manner, the reproducibility and accuracy of the sampling process is improved in comparison to the hot injection techniques. Moreover, relatively slow heating of the components reduces the thermal stress that is applied to the molecules. This in general, results in a reduced thermal degradation as compared to the corresponding hot injection technique. In subsequent paragraphs the three "cold" temperature programmed injection modes of PTV type injectors will be discussed. For a discussion of the corresponding "hot" modes the reader is referred to chapter 1.

### 2.3. COLD SPLIT INJECTION

In the cold split injection mode the liquid sample is introduced directly into the cooled liner of the PTV injector. The temperature of the liner is below the boiling point of the solvent. Because the liner is cold, selective evaporation from the syringe is precluded. This means that discrimination is absent. Moreover, it allows the analyst to accurately introduce known amounts of liquids into the system. In hot injection techniques this is virtually impossible. Here, the actual amount of sample introduced into the system is generally larger than that displaced from the barrel of the syringe. This is because part of the sample present in the needle also evaporates. After the syringe has been withdrawn from the injector the liner is heated. The final temperature should be sufficiently high to transfer high boiling components present in the sample to the

column. The split flows used in cold split injections are similar to those in hot split injection. In figure 2.3, a comparison of the peak areas of a series of normal alkanes obtained using hot split/splitless, cold PTV split and on-column injection is made. The sample contained equal amounts of the various alkanes in hexane. Figure 2.3 clearly shows that discrimination is absent in the cold split mode.

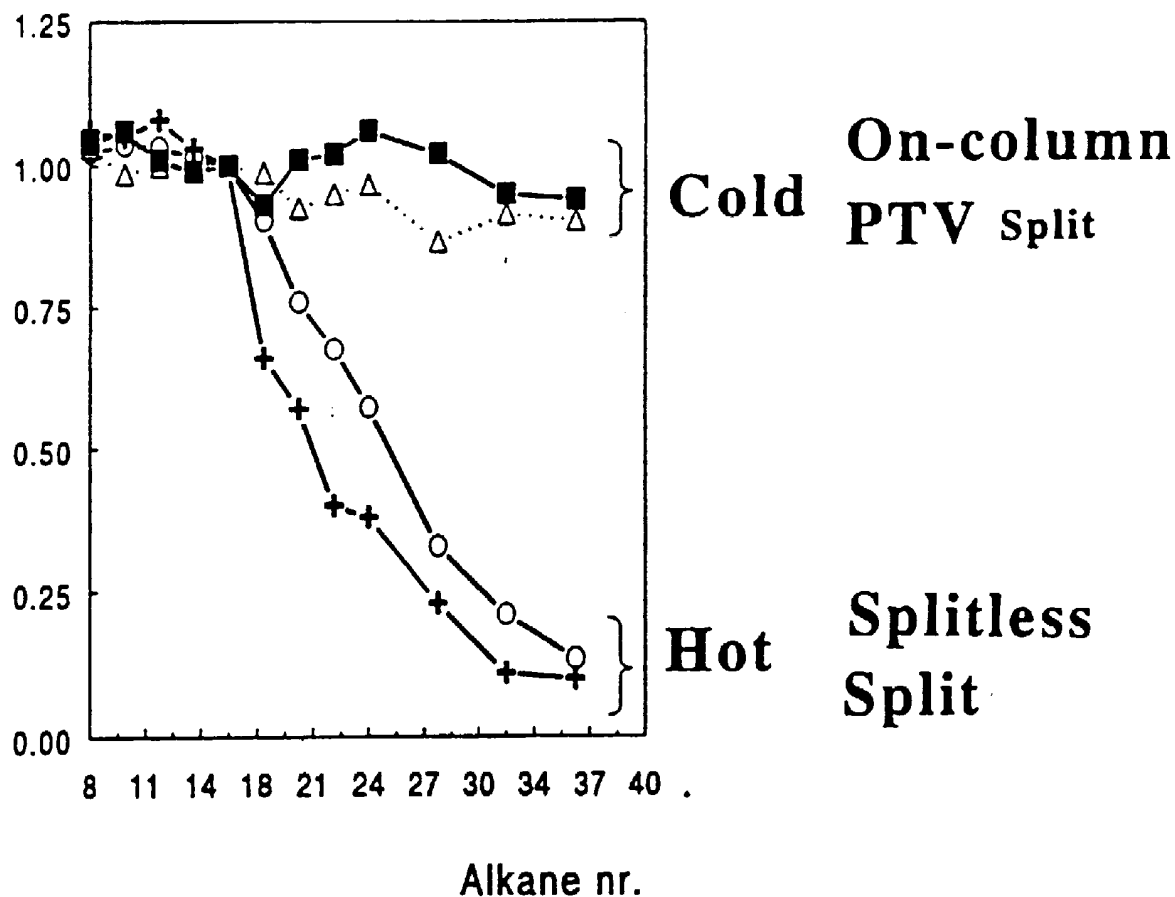


Figure 2.3. Discrimination of high boiling components. Comparison of cold and hot injection techniques.

Experimental data indicate that discrimination is absent up to at least n-C<sub>90</sub> or n-C<sub>100</sub>. It is needless to say that in this case a final injector temperature should be chosen that is high enough to obtain sufficient vapour pressures for these high boiling components. As an example, rapid and quantitative transfer of C<sub>100</sub> to the column requires final PTV temperatures between 500 and 600°C. For even higher components discrimination can in some cases occur as these components have insufficient vapour pressure at the final temperature of the injector. This means that they will remain in the injector. To avoid band broadening for components that evaporate slowly, peak focusing in the GC column is necessary. This can be achieved by using low initial GC temperatures followed by temperature programming.

As has already been explained earlier in this chapter, PTV liners generally are smaller than liners for conventional split/splitless injectors. Because the amount of liquid that can be retained in the injector is proportional to the available surface area, only relatively small sample volumes can be injected. For 2 mm i.d. liners with a length of 9 cm the maximum volume is approximately 4 to 6  $\mu\text{L}$ . Packing the liner with deactivated glass wool is an efficient means of increasing the available surface area and, hence, the sample capacity of the liner. As even deactivated glass wool is not fully inert, this might give rise to increased thermal degradation. An alternative might be the use of baffled liners. Both with regard to sample capacity and inertness these liners take an intermediate position between empty (straight) and glass wool packed liners. Nowadays also more inert packing materials are commercially available. An example of such a material is Supelcoport, a material originally developed for use as a support material in packed column GC.

#### 2.4. COLD SPLITLESS INJECTION

Similar to the situation in conventional hot splitless injections, in cold splitless injection the split valve remains closed when the sample is injected into the liner. A distinct difference is of course that the liner now is cold. After the syringe is removed from the injector the liner is heated and the components are transferred to the column. The temperature of the column should be some 10 to 20°C below the boiling point of the solvent so that a film of recondensed liquid is formed in the column inlet. In this liquid film, the more volatile sample constituents are refocused by the solvent effect. The high boiling components are sharpened by cold trapping. When transfer of the components to the column has reached completion, the split vent is opened to remove the remaining traces of solvent vapour from the liner. The procedure for optimisation of the splitless time is the same as used in the optimisation of conventional splitless injection. Due to the low volume of the liner only small sample sizes can be introduced (*e.g.* < 4  $\mu\text{L}$ ). As the sample is again introduced in a cold liner discrimination is absent. As in cold split injection discrimination might be observed for very high boiling components that are not transferred quantitatively to the column due to the limited maximum temperature of the injector. If carefully optimised, these problems do not occur for components with a boiling point equal to or below that of approximately n-C<sub>90</sub>. For SimDist applications, however, where components up to C<sub>120</sub> have to be analysed, discrimination might still occur.

Table 2.1. Thermal degradation during cold splitless injection. The sample contained equal amounts of all components. The liner was packed with deactivated glass wool. The numbers

between brackets give the relative peak areas found using on-column injection.

Component	Areas relative to n-C <sub>16</sub>
C <sub>10</sub> OOTMS	0.857 (0.943)
C <sub>16</sub>	1.000 (1.000)
C <sub>14</sub> OOTMS	0.367 (0.921)
C <sub>20</sub>	0.984 (1.056)
C <sub>18</sub> OOTMS	not found (0.961)
C <sub>24</sub>	0.934 (0.949)
C <sub>22</sub> OOTMS	not found (0.905)
C <sub>28</sub>	0.890 (0.966)

Analogous to the situation in conventional (hot) splitless injection techniques, thermal degradation can occur due to the long residence times of the components in the liner. A frequently used sample for evaluating the degradation characteristics of injection systems in capillary GC is the test mixture proposed by Donike [5]. This mixture contains a number of thermally unstable trimethylsilylestere of high molecular weight fatty acids and a set of normal alkanes that serve as reference components. In table 2.1 the results of the Donike test sample analysed on a non-polar column and introduced in the cold-splitless mode are given.

## 2.5. COLD SPLITLESS INJECTION WITH SOLVENT ELIMINATION

In the cold splitless/solvent elimination mode the solvent is selectively removed from the PTV liner prior to transferring the sample to the GC column. The conditions are chosen such that the components are retained in the liner by cold trapping while at the same time the solvent is eliminated through the split line. At the end of the solvent elimination process the split vent is closed and the injector is heated.

Cold splitless injection with solvent elimination has a number of interesting application areas. Firstly it can be applied for the introduction of large sample volumes. This is an attractive means of reducing the detection limits. It is discussed in great detail in Chapter 3. Apart from the application in large volume sampling, solvent vent injection techniques can also be used to remove small quantities of a solvent prior to introduction. This could be advantageous for the introduction of polar solvents on non-polar columns or for removing solvents that are not compatible with the detection system used. Typical examples of solvent/detector systems that are not compatible are for example chlorine containing solvents and Nitrogen/Phosphorus detection or halogen containing solvents and Electron Capture Detection. If vulnerable, non-chemically-

bonded stationary phases are used, solvent elimination might have a favourable effect on the lifetime of the chromatographic column. Figure 2.4 illustrates the effects of solvent elimination on peak shapes observed when a polar solvent is introduced onto a non-polar column without and with solvent elimination. The experiment with solvent elimination clearly gives better peak shapes, as is evidenced by figure 2.4.

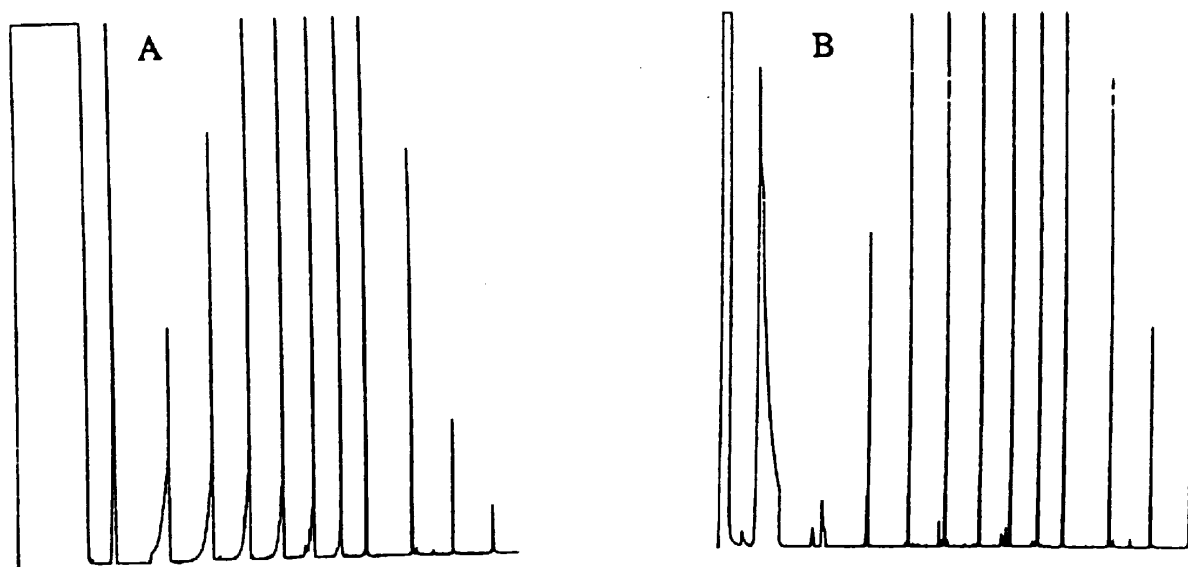


Figure 2.4. Introduction of 2  $\mu\text{L}$  of an alkane standard in methanol on a non-polar column. A: without solvent venting, B: with solvent venting.

## 2.6. SUMMARY PTV INJECTION TECHNIQUES

In previous paragraphs the flexible nature of PTV type injectors has been demonstrated. Apart from the five different injection modes that can be performed on all commercially available PTV injectors, a number of PTV's can be reconfigured easily in order to allow use in the on-column mode. A special insert that can be inserted in the liner allows introduction of the sample directly into the column. Hence, up to six different injection modes can all be performed using just one injector. Several types of samples, as for examples highly diluted samples or samples containing

polar solvents, that can not be analysed using one of the conventional injection techniques can conveniently be handled using PTV injectors.

Figure 2.5 gives a summary of the temperature program of the liner and the status of the split valve in the five different PTV injection modes.

Mode	Temperature	Split
hot split	↓ ————— 300 °C	-----
cold split	↓ ——— / ————— 300 °C	-----
hot splitless	↓ ————— 300 °C	—————
cold splitless	↓ ——— / ————— 300 °C	—————
cold splitless + solvent vent	↓ ——— / ————— 300 °C	-----

Figure 2.5. Temperature profile of the PTV injector and status of the split valve in the 5 injection modes possible with all commercially available PTV injectors. Initial temperatures and programming rates of the injector, splitless times and solvent vent times must be optimised experimentally. Split valve: drawn line = closed, dashed line: open.

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