PRACTICAL LABORATORY SKILLS TRAINING GUIDES

Gas Chromatography

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VAM
VALID ANALYTICAL MEASUREMENT

Practical Laboratory Skills Training Guides

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advancing the chemical sciences

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Setting standards
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Preface

Production of this set of five Training Guides and CD-ROMs was supported under contract with the Department of Trade and Industry as part of the National Measurement System Valid Analytical Measurement (VAM) programme.

The guides were written by staff at LGC in collaboration with members of the SOCSA Analytical Network Group whose assistance is gratefully acknowledged. They include liquid and gas chromatography, the measurement of mass, volume and pH.

Training has formed an essential part of the VAM programme since its inception in 1988. Many training courses on topics aimed at improving the quality of measurements have been developed. However, in working with groups of analytical scientists it has become clear that the basic skills required in an analytical laboratory are not covered on courses or readily available in paper format.

These guides are aimed at filling this gap and are aimed at those working at the bench. For each topic they include a limited amount of theory to explain the essential features but the main emphasis is on what to do to ensure reliable results. They contain references to further reading for those who wish to study the topics in more depth.

To help laboratory managers assess the competence of the trainee there are a limited number of exercises suggested. The chromatography modules also have a trouble shooting section.

The CD-ROMs cover Practical Laboratory Skills and have links to websites where more information may be obtained.

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Gas Chromatography

1 Basic Theory

1.1 Overview

Today, gas chromatography is the most widely used technique in analytical chemistry – a position it has held for over three decades. The popularity and applicability of the technique is principally due to its unchallenged resolving power for closely related volatile compounds and because of the high sensitivity and selectivity offered by many of the detector systems. The technique is very accurate and precise when used in a routine laboratory.

1.1.1 Principle

The sample is normally introduced as a vapour on to the chromatographic column. On the column, the solubility of each component in the gas phase is dependent on its vapour pressure, which is in turn a function of the column temperature and the affinity between the compound and the stationary phase. Differences in vapour pressure cause the molecules of each component to partition between the mobile gas phase and the stationary phase. In fact, as the molecules are continually moving rapidly between the two phases, it is the difference in residence time in each phase that affects the partition. Every time a molecule enters the gas phase it is swept towards the detector by the carrier gas flow. Consequently, compounds having different physical and chemical properties will arrive at the detector at different times. The stationary phase can be a solid or a liquid coating an inert solid support, this gives rise to two forms of gas chromatography; gas–solid (GSC) and gas–liquid chromatography (GLC) respectively. Figure 1 shows the basic components of a gas chromatograph.

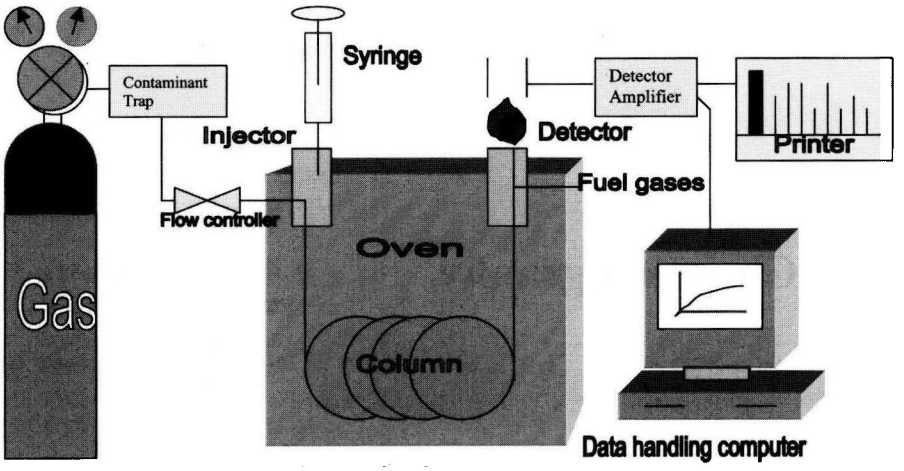

Figure 1 *Gas chromatograph*

1.2 Key Parameters

1.2.1 Retention Factor (k)

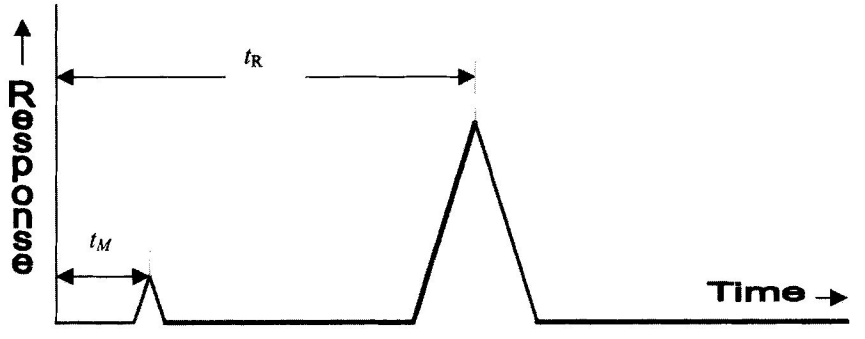

Figure 2 *Retention factor*

The retention factor (k) (see Figure 2) describes the ability of the stationary phase to retain a solute (Equation 1).

$$k = \frac{t_R - t_M}{t_M} \tag{1}$$

t_R retention time of the solute
t_M dead time (approximate time required for the mobile phase to pass through the column)

The longer the solute spends in the stationary phase the more likely it is to be separated from components of similar volatility. In gas chromatography, the

retention factor can be altered by changing the stationary phase or the temperature of the column.

1.2.2 Selectivity Factor (α)

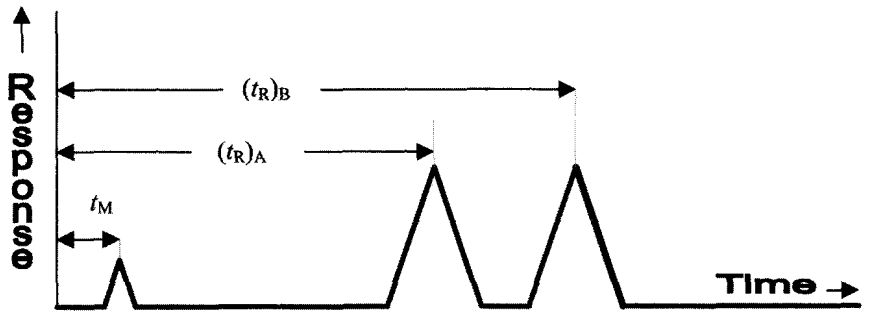

Figure 3 *Selectivity factor*

The selectivity factor (α) for two components provides a measure of how well they will separate on a particular column. Referring to Figure 3, the selectivity factor (α) is defined in Equation 2.

$$\alpha = \frac{(t_R)_B - t_M}{(t_R)_A - t_M} \tag{2}$$

$(t_R)_B$ retention time of component B which is more strongly retained
$(t_R)_A$ retention time of component A which is less strongly retained
t_M dead time

- When α = 1 then theoretically the two components cannot be separated on the column tested.
- When α > 1 then theoretically they can be resolved but this will depend on the resolution or performance of the column.

1.2.3 Column Resolution (R)

The resolution (*R*) of a column provides a quantitative measure of its ability to separate two components within a mixture.

Referring to Figure 4, the resolution is defined as shown in Equation 3.

$$R = \frac{2[(t_R)_B - (t_R)_A]}{W_A + W_B} \tag{3}$$

$(t_R)_B$ retention time of component B which is more strongly retained

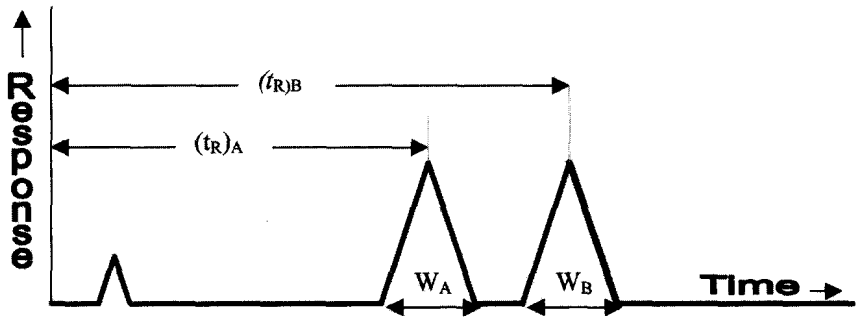

Figure 4 *Column resolution*

$(t_R)_A$ retention time of component A which is less strongly retained
W_A width of peak for compound A at the baseline
W_B width of peak for compound B at the baseline

- If R is less than 1, the component peaks are overlapping.
- If R is equal to or greater than 1, this indicates good separation.

1.3 Quantitative Measures of Column Efficiency

In Figure 2, Figure 3 and Figure 4 the peaks have been exaggerated to illustrate that the solute elutes from the chromatographic column as a band of material. This occurs because each molecule of the solute has a unique diffusion path through the liquid and gas phases. Consequently the molecules elute from the column at slightly different retention times. The effect is not only influenced by the chemical interaction of solute and stationary phase but it is also highly dependent on the physical dimensions of the column, *i.e.* length, internal diameter or particle size, and coating thickness of stationary phase. This spreading (banding) effect increases in proportion to the length of time the solute molecules stay on the column; this explains why the later eluting peaks in a chromatogram are broader and shorter than the earlier peaks.

It is clear from this discussion that the ability of a column to separate similarly eluting components is related to how the peak or solute band spreads as it passes along the column, *i.e.* the efficiency of the column. Analysts use the plate model in chromatography to measure column efficiency; the higher the number of theoretical plates the higher the efficiency of the column.

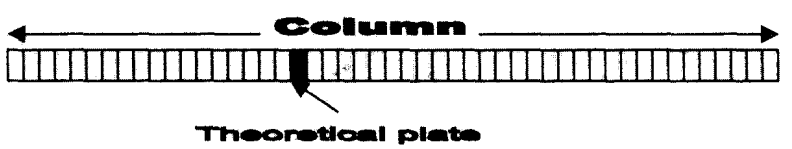

Figure 5 *Column as a series of theoretical plates*

This compares the chromatographic process with distillation and quantifies the efficiency of the column in terms of the number of theoretical plates. This comparison

is much easier to understand for gas chromatography than other forms of chromatography. Figure 5 shows the column as a series of theoretical plates.

Under 'simulated distillation' conditions – for example the hydrocarbon components of an oil elute from the chromatographic column in the same order as their boiling points – the components appear to boil off the chromatographic column as its temperature is increased in the same way as from a distillation column.

1.3.1 Number of Theoretical Plates (N)

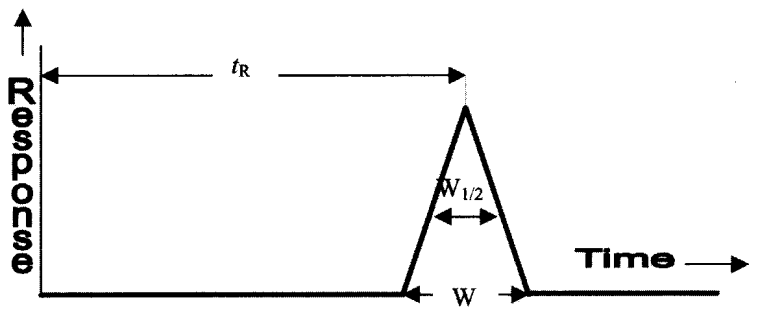

Figure 6 *Measurement of column efficiency*

t_R	*retention time*
$W_{1/2}$	*width of the peak at 50% of the peak height*
W	*width of the peak at the baseline*

From Figure 6 the number of theoretical plates (N) is given by Equation 4.

$$N = 5.54 \times (t_R/W_{1/2})^2 = 16 \times (t_R/W)^2 \quad (4)$$

where 5.54 and 16 are constants

Columns are available in different lengths (L) and so it is normal to express the efficiency of the column as the number of theoretical plates per metre. Alternatively the plate height (H) or height equivalent to a theoretical plate (HETP) is commonly used (Equation 5).

$$\text{HETP} = L / N \quad (5)$$

Note that the value of HETP will be a minimum when the best efficiency for the column is achieved. The value of N and HETP will change as a column becomes older and less efficient. The analyst can use these parameters as a quantitative measure to determine when a column is no longer 'fit-for-purpose' and when it needs to be changed.

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2 Carrier Gas

The carrier gas constitutes the mobile phase in the gas chromatographic system. During the chromatographic process component molecules from the sample are continually interchanging between the stationary and mobile phases (Figure 7). Every time these molecules enter the gas phase they are swept towards the detector by the flow of carrier gas.

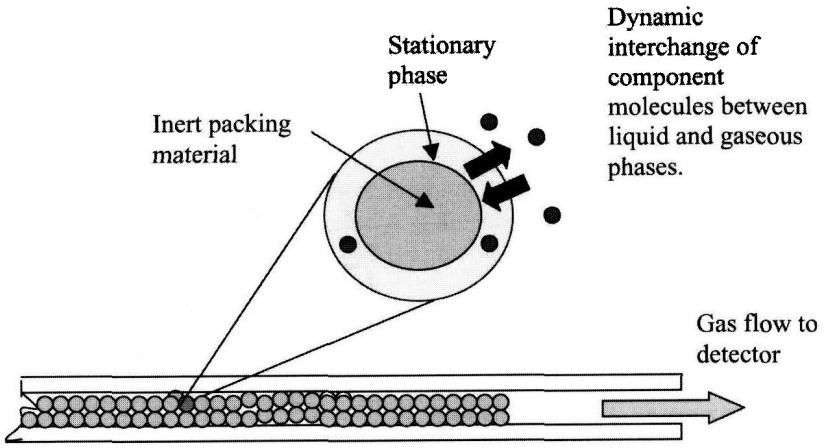

Figure 7 *Chromatographic processes on a packed column*

Consequently the carrier gas flow is an important variable in controlling the retention times of the sample components, the overall analysis, and the sample turn around times – *doubling the carrier gas flow halves the retention times of all the components on the column.*

Once a new chromatographic method is set up the carrier gas flow needs to be optimised to obtain the best chromatographic resolution from a particular column. The flow rate that provides the greatest resolution (HETP minimum) can be determined by simple experiment or by consulting a prepared van-Deemter plot.

The van-Deemter plots for a capillary column using three different gases are shown in Figure 8. The plots show that the curve for nitrogen is highly peaked and that the best resolution for this gas occurs over a narrow range of low flow rates (average linear velocity for capillary columns). In contrast the plots for helium and hydrogen are very flat and the best resolution is seen to occur at much higher flow rates. Higher flow rates mean much faster analyses and so there is a significant performance gain on using these gases in preference to nitrogen. Hydrogen often is regarded as the 'ideal' carrier gas, however its use has safety implications so most chromatographers use helium as it is non-flammable and therefore safer. For low resolution, *i.e.* packed column work, the van-Deemter curves for these gases are similar in shape and there is little advantage in using helium or hydrogen. In these cases the choice of carrier gas is governed by the availability, safety and cost of the gas, or its compatibility with the detector that is to be used.

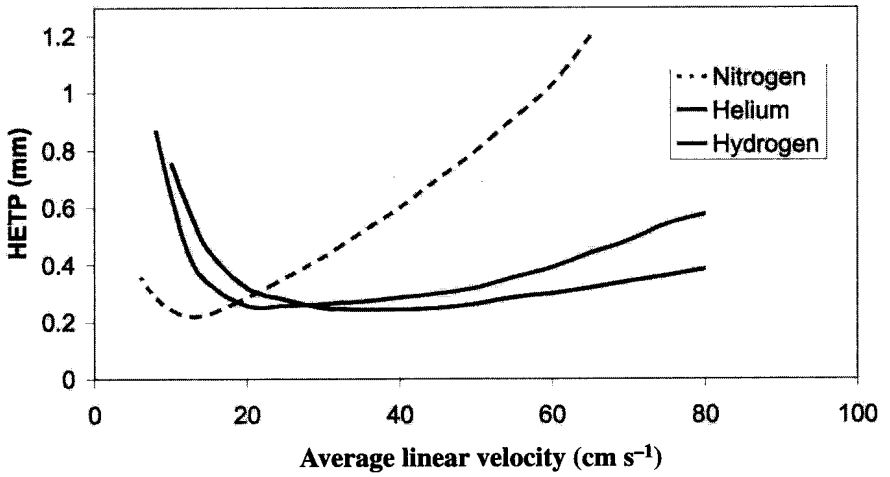

Figure 8 *Column HETP* v *average linear velocity for three gases*

2.1 Technical Requirements

All the gases used in GC analysis need to be of very high purity and steps must be taken to remove trace levels of moisture, oxygen and hydrocarbons from the gas before it enters the instrument. Oxygen is a particularly important contaminant to remove because it promotes column degradation and can also cause problems with the more sophisticated detectors such as electron capture and mass spectrometer detectors. Contaminants are removed by passing the gas through cartridges of absorbent. These are often 'self indicating' so the analyst can easily check if the absorbent bed is exhausted.

Gases are supplied in cylinders at high pressures so, unlike HPLC, there is no requirement for pumps to facilitate the movement of the mobile phase through the instrument. Cylinder pressures are attenuated and regulated precisely to ensure a constant pressure at the front end of the column. Mass flow controllers ensure that the pre-set flow-rate of gas is always constant during the analysis and is independent of the column oven temperature.

2.2 Tips

- The provision of gases for gas chromatographic analyses is the responsibility of the user. Always use high purity carrier gases, typically with purity of 99.995% or better, and note the specific recommendations of manufacturers and suppliers.
- Follow the guidance of manufacturers and suppliers to select the most effective system for removing trace contaminants from gases. Check regularly that gas-trap components are working effectively and replace the absorbent cartridges whenever necessary.
- Prevent the ingress of air into the instrument gases by leak-testing all connectors and joints in the gas delivery system. This is not only important when the

instrument is first set up, but also when the column or detectors have been changed.

- Always check that the reserves of gas are sufficient to allow the instrument to be left running overnight and at weekends.

3 Injection and Sampling Methods

3.1 Injection Methods

The function of the injector is to introduce a representative portion of the sample as a narrow band on to the chromatographic column. Because most samples that are analysed by gas chromatography are liquids, an essential feature of the injection stage is that the sample and solvent are vaporised prior to reaching the column. To accomplish this, injectors are equipped with dedicated heaters that control the temperature of the injector zone to a pre-set value.

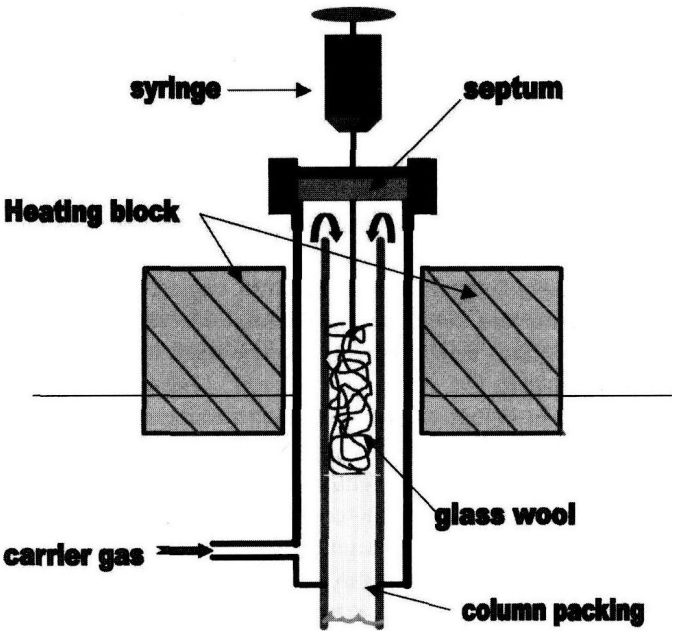

Figure 9 *Schematic-injector for packed column*

One of the earliest and simplest injector designs, developed for packed column systems, is shown in Figure 9. The sample is injected through the septum directly on to a glass wool plug at the top of the column. This region of the column is at high temperature and on injection the sample is vaporised and the flow of carrier gas ensures the vapour is pushed downwards in the direction of the analytical column.

3.1.1 'On-column' Injection

The packed column injector design is an example of 'on-column' injection where the whole of the sample passes through the chromatographic column. 'On-column' injection is also possible with capillary columns but here the sample is normally injected on to a cold column. The column temperature program is then activated to facilitate the separation and elution of the sample components. This cold 'on-column' technique is most suitable for samples that contain labile components or components having a wide range of volatility. To avoid overloading and droplet formation at the front of the capillary column, the injection volume is normally kept quite small, in the region of ≤0.3 μL. Consequently, detection limits are relatively high and the technique is not readily suited to trace analysis. Highly accurate quantitation is possible with this injection technique.

3.1.2 Split/Splitless Injector

This is a common injector system for use with capillary columns and has two modes of operation: split and splitless.

Split mode – Only a small fraction of the sample is introduced on to the column, the remainder is directed to waste according to a pre-set 'split ratio'. A high split ratio ensures that the column is not overloaded with sample at the beginning of the analysis. Some sample discrimination can occur to the detriment of the higher boiling components of the sample. For accurate quantitation the solvent and injection conditions must be carefully selected and controlled accurately.

Splitless mode – The sample is injected into a hot injector but the column is initially held at a temperature 15 °C to 30 °C below the boiling point of the solvent. Solvent and thermal focusing techniques can be used to concentrate the sample into a narrow band on the front of the column. This technique is useful for the determination of involatile analytes in low boiling solvents at very low concentrations. Commonly, a sample volume of 1 μL to 2 μL is injected into the heated injector.

3.1.3 Programmed Temperature Vaporiser

This injector has 'universal' features in that it is possible to operate it in hot or cold, split or splitless, total sample introduction or solvent elimination modes. The advantages and disadvantages of each mode have already been mentioned but the use of a temperature-programmed facility helps maintain the optimum conditions for the successful sample injection, *e.g.* for thermal focusing. The injector temperature can be programmed to follow column oven temperature changes. In the on-column mode the retention times of components can be controlled to ensure precision that provides a coefficient of variation between 0.1% to 0.5%. This is highly desirable, particularly when components are identified by their retention times alone.

3.2 Sampling Methods

3.2.1 Headspace Sampling

When a solid or liquid sample is placed in a closed container an equilibrium is formed between the volatile components in the bulk of the material and the headspace above the material. In the *static* headspace method small samples are taken from the sample headspace using a gas syringe and these are injected directly on to the gas chromatography column. This technique is quick and simple and, because samples are free of a matrix, the analysis is fairly straightforward. The main disadvantage of the technique is that for low sample concentrations, *e.g.* analytes of low volatility, the method is very insensitive. The situation can be improved using a *dynamic* headspace method, an example of which is the purge and trap technique.

3.2.2 Purge and Trap

In the purge and trap system, a stream of inert gas is used to strip components from an aqueous sample. The components are trapped and concentrated on an adsorbent trap and any water vapour passes through the trap unaffected. The trap is then thermally desorbed and the components are carried on to the column in a stream of carrier gas. Because the water matrix is almost entirely removed by the process, the sample is greatly concentrated and the technique allows the detection of parts per billion levels of volatiles in aqueous samples.

3.2.3 Thermal Desorption

In this technique trace contaminants in air are trapped on adsorbent tubes. The tubes are then thermally desorbed into a stream of helium carrier gas and passed to the column. To help focus the samples a low volume cryogenic trap is often employed prior to gas chromatographic analysis, *i.e.* two stage desorption. Because the traps do not adsorb the permanent gases (*e.g.* nitrogen, oxygen, *etc.*) in the original sample the method involves significant concentration of the analytes and is capable of detecting trace concentrations of contaminants in air.

3.2.4 Pyrolysis

Many large molecules, particularly polymers, cannot be analysed directly by gas chromatography due to their low volatility. In the pyrolysis method thermal energy is used to decompose the materials into simpler, lower molecular weight, fragments which can be analysed by gas chromatography. A number of different thermal sources are in use such as 'ribbon-type', 'curie point' and 'laser pyrolysers'. The technique is suitable not only to identify or determine the initial composition of the polymer but also to provide an insight into the thermal stability and thermal decomposition processes relevant to the material.

3.2.5 Derivatisation

For involatile compounds it is necessary to produce a volatile derivative. Derivatisation may be employed in chromatography to change the chromatographic behaviour or to improve detectability, and involves chemical modification of functional groups in the compound. Specific improvements in volatility, hydrolytic and thermal stability, peak shape, chromatographic resolution, detection selectivity and sensitivity can be achieved if a suitable derivatisation reaction is used. Typical functional groups that can be readily derivatised are; alcohols, phenols, carboxylic acids, amines, amides and thiols. Typical derivatising agents are trimethyl silyl, benzyl, benzoyl and phenoxyacyl halides. The presence of halogen atoms in the derivative ensures enhanced sensitivity and low detection limits using the electron capture detector.

3.3 Technical Requirements

It is suggested that operators should consult the manufacturer's manual to familiarise themselves with injector characteristics. Run standards and check for unusual or distorted peaks that might indicate the injector is not functioning correctly.

3.4 Tips

- Distorted or split peaks are an indication that too large a sample has been injected.
- Too low an injector temperature can lead to tailing peaks.
- Too high an injector temperature can lead to component degradation.
- Broad peaks at the beginning of a chromatogram are an indication that the solute has not been focused tightly into a band by the sampling and injection techniques. Check that the parameters associated with these techniques have been optimised and set correctly.
- Injector contamination can lead to memory effects – extra peaks in the chromatogram. Clean or replace the injector liner regularly.

4 The Chromatographic Column

The chromatographic column is the heart of the gas chromatograph, providing the necessary separation of the sample components before they are detected, identified or quantified. Early commercial columns consisted of long glass or metal tubes, 1/4 inch diameter, packed with minerals, brick dust, molecular sieves (for GSC) or inert particles coated with gums, waxes and polymers (for GLC). The use of packed columns has declined as the higher performance open tubular capillary columns have become available.

Performance criteria demand that open tubular columns should be long and of narrow bore with an even distribution of stationary phase. Modern methods of extrusion, similar to those used in the manufacture of fibre optics, together with

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