11 Gas chromatography

KEYPOINTS

Principles

A gaseous mobile phase flows under pressure through a heated tube either coated with a liquid stationary phase or packed with liquid stationary phase coated onto a solid support. The analyte is loaded onto the head of the column via a heated injection port where it evaporates. It then condenses at the head of the column, which is at a lower temperature. The oven temperature is then either held constant or programmed to rise gradually. Once on the column separation of a mixture occurs according to the relative lengths of time spent by its components in the stationary phase. Monitoring of the column effluent can be carried out with a variety of detectors.

Applications

- The characterisation of some unformulated drugs, particularly with regard to detection of process impurities.
- Limit tests for solvent residues and other volatile impurities in drug substances.
- Sometimes used for quantification of drugs in formulations, particularly if the drug lacks a chromophore.

- Characterisation of some raw materials used in synthesis of drug molecules.
- Characterisation of volatile oils (which may be used as excipients in formulations), proprietary cough mixtures and tonics, and fatty acids in fixed oils.
- Measurement of drugs and their metabolites in biological fluids.

Strengths

- Capable of the same quantitative accuracy and precision as high-pressure liquid chromatography (HPLC), particularly when used in conjunction with an internal standard.
- Has much greater separating power than HPLC when used with capillary columns.
- Readily automated.
- Can be used to determine compounds which lack chromophores.
- The mobile phase does not vary and does not require disposal and, even if helium is used as a carrier gas, is cheap compared to the organic solvents used in HPLC.

Limitations

- Only thermally stable and volatile compounds can be analysed.
- The sample may require derivatisation to convert it to a volatile form, thus introducing an extra step in analysis and, potentially, interferants.
- Quantitative sample introduction is more difficult because of the small volumes of sample injected.
- Aqueous solutions and salts cannot be injected into the instrument.

Introduction

The use of gas chromatography (GC) as a quantitative technique in the analysis of drugs has declined in importance since the advent of HPLC and the increasing sophistication of this technique. However, it does still have a role in certain types of quantitative analysis and has broad application in qualitative analysis. Since HPLC currently dominates quantitative analyses in the pharmaceutical industry, the strengths of GC may be overlooked. Capillary GC is capable of performing much more efficient separations than HPLC albeit with the limitation that the compounds being analysed must be volatile or must be rendered volatile by formation of a suitable derivative[1] and must also be thermally stable. GC is widely used in environmental science, brewing, the food industry, perfumery and flavourings analysis, the petrochemical industry, microbiological analyses and clinical biochemistry. Although packed column GC is still used in the pharmaceutical industry, this chapter will concentrate to a large extent on open tubular capillary GC, which is the more modern manifestation of GC.

Instrumentation

Figure 11.1 shows a schematic diagram of a GC system. The principles of the system are that:

(i) Injection of the sample may be made manually or using an autosampler through a resealable rubber septum.
(ii) The sample is evaporated in the heated injection port area and condenses on the head of the column.
(iii) The column may either be a capillary or a packed column, which will be discussed in more detail later. The mobile phase used to carry the sample through the column is a gas – usually nitrogen or helium.
(iv) The column is enclosed in an oven which may be set at any temperature between ambient and *ca* 400°C.
(v) The most commonly used detector is the flame ionisation detector (FID).

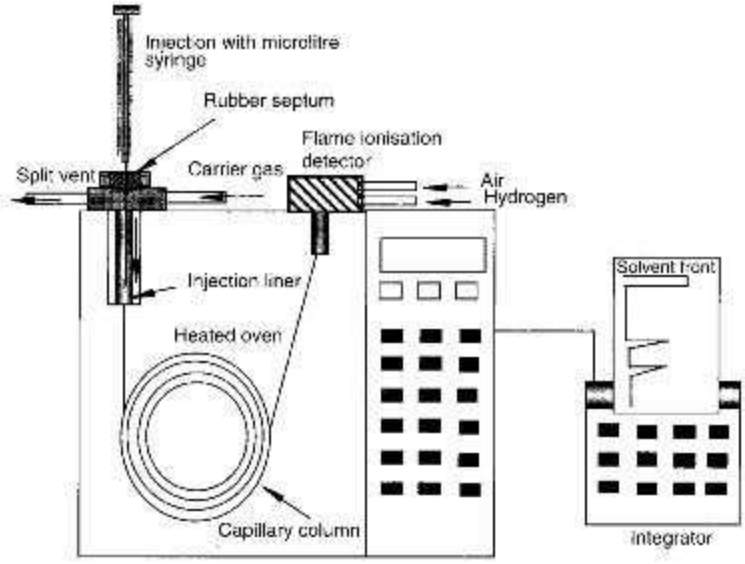


Fig. 11.1
Schematic diagram of a capillary GC system.

Syringes

The volumes injected in GC are routinely in the range of 0.5–2 μl. The most commonly used type of syringe is shown in Figure 11.2; the usual syringe volumes are 5 and 10 μl. A recommended technique for injection into a capillary GC is to fill the syringe with about 0.5 μl of solvent and draw this solvent into the barrel slightly before filling with sample. The sample is also drawn into the barrel to leave an air gap below it. The syringe needle can then be introduced into the injector and left for a couple of seconds to warm up before the plunger is depressed. The syringe is then withdrawn immediately from the injection port.

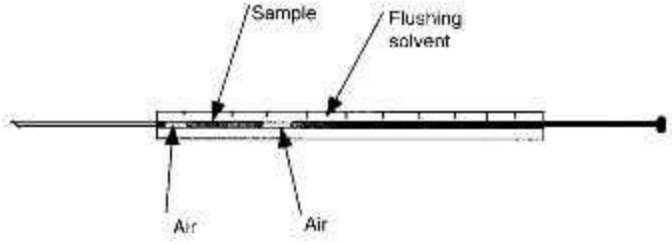


Fig. 11.2
10 μl sample in barrel syringe.

Injection systems

Packed column injections

Injection generally occurs through a resealable rubber septum. The injector port is held at 150–250° depending on the volatility of the sample and direct injection of 0.1–10 μl of sample is made onto the head of the column. The amount of sample injected onto a packed column is *ca* 1–2 μg per component. Injection into packed

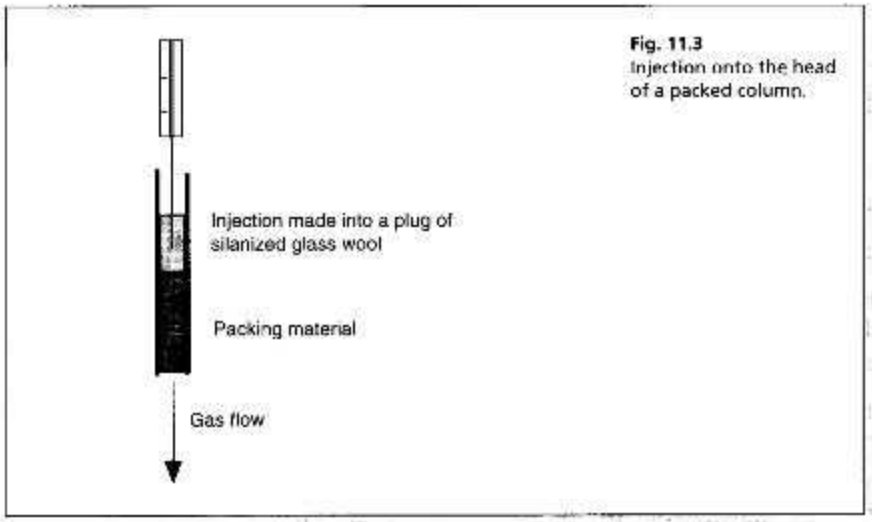


Fig. 11.3
Injection onto the head of a packed column.

columns presents less of a problem than sample introduction into a capillary column since all of the sample is introduced into the packed column (Fig. 11.3). Thus, although packed columns do not produce high resolution chromatography, this is their strength.

Split/splitless injection

This type of injector is used in conjuction with capillary column GC. Capillary columns commonly have internal diameters beteen 0.2 and 0.5 mm and lengths between 12 and 50 m. Injection takes place into a heated glass or quartz liner rather than directly onto the column.

In the split mode, the sample is split into two unequal portions the smaller of which goes onto the column. Split ratios range between 10:1 and 100:1, with the larger portion being vented in the higher flow out of the split vent. This technique is used with concentrated samples. In the splitless mode, all of the sample is introduced onto the column and the injector purge valve remains closed for 0.5–1 min after injection. The difficulty faced with split/splitless injection onto a capillary column is in obtaining good injection precision.[2] Attention has to be paid to certain points:

(i) Since injection is made at high temperatures into an injection port, a lack of precision resulting from decomposition of some of the components in a mixture before they reach the column has to be considered. Thus it is important to ensure that the sample has minimal contact with metal surfaces during the injection process since these can catalyse decomposition.

(ii) If a split injection is used, care has to be taken that there is no discrimination between more and less volatile components in a mixture in terms of the proportion lost through the split vent.

(iii) If a splitless injection is made, volumes have to be kept below *ca* 2 μl in case the sample backflashes through rapid expansion of the solvent in which it is dissolved, into either the gas supply lines or the purge lines. Each 1 μl of solvent expands greatly upon vapourisation, e.g. methanol *ca* 0.66 ml/μl or ethyl acetate *ca* 0.23 ml/μl at atmospheric pressure.

(iv) Even if an internal standard (p. 259) is used to compensate for losses, the possibility of it being discriminated randomly against either through differences in volatility or decomposition compared to the sample has to be considered.

(v) In the splitless mode the sample must be efficiently trapped at the head of the column. For this to occur, it must be sufficiently involatile, i.e. have a boiling point > *ca* 50°C higher than the column starting temperature. If the sample is relatively volatile it has to be injected into the GC in a low volatility solvent which will condense at the head of the column, trapping the sample in the process.

(vi) Sample transfer may be slow and it is important to take this into account when setting purge valve times, e.g. for a typical 1 ml/min flow of helium through a capillary column, about 0.5 min would be required to transfer a 2 μl injection volume of ethyl acetate onto the column.

(vii) Injection precision is greatly improved by the use of an autosampler to carry out injection since it can achieve much better precision in measuring volumes of *ca* 1 μl than a human operator.

Cool on-column injection

Direct on-column injection into the capillary column may be carried out in a manner analogous to injection into a packed column. This technique requires a syringe with a very fine fused silica needle. The technique has the advantages of: (1) reduced discrimination between components in mixtures; (2) no sample degradation in a hot injector; and (3) no backflash, hence quantitative sample transfer. It also has the following disadvantages: (1) samples have to be clean otherwise residues will be deposited on the column; (2) the injector is mechanically more complex and requires more maintenance than a septum injection system; and (3) the syringe needle may damage the head of the column.

GC oven

GC ovens incorporate a fan which ensures uniform heat distribution throughout the oven. They can be programmed to either produce a constant temperature, isothermal conditions or a gradual increase in temperature. Oven programming rates can range from 1°C/min to 40°C/min. Complex temperature programmes can be produced involving a number of temperature ramps interspersed with periods of isothermal conditions, e.g. 60°C (1 min)/ 5°C/min to 100°C (5 min)/10°C/min to 200°C (5 min). The advantages of temperature programmes are that materials of widely differing volatilities can be separated in a reasonable time and also injection of the sample can be carried out at low temperature where it will be trapped at the head of the column and then the temperature can be raised until it elutes.

Types of column

Packed columns

The columns are usually made from glass which is silanized to remove polar silanol Si–OH groups from its surface that can contribute to the peak tailing of the peaks of polar analytes. These columns have internal diameters of 2–5 mm. The columns are packed with particles of a solid support which are coated with the liquid stationary phase. The most commonly used support is diatomaceous earth (mainly calcium silicate). This material is usually acid washed to remove mineral impurities and then silanized as shown in Figure 11.4 to remove the polar Si–OH groups on the surface of the support, which can lead to tailing of the analyte peak.

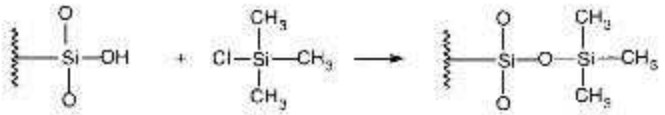


Fig. 11.4
Silanization of free silanol groups.

The support can then be mechanically coated with a variety of liquid stationary phases. The mobile phase most commonly used in packed column GC is nitrogen with a flow rate of *ca* 20 ml/min. Packed column GC affords a relatively low degree of resolution compared to capillary GC; typically 4000–6000 plates for a 2 m column compared to > 100 000 plates for a 25 m capillary column. The high temperature limit of packed columns is *ca* 280°C; beyond this temperature the liquid stationary phase evaporates at a rate which creates a large background signal. However, for many routine quality control operations, they are quite adequate.

Capillary columns

Capillary columns are made from fused silica, usually coated on the outside with polyamide to give the column flexibility. Coating on the outside with aluminium has also been used for high temperature (> 400°C) work. The internal diameter of the columns ranges between 0.15 and 0.5 mm. The wall of the column is coated with the liquid stationary phase, which may have a thickness between 0.1 and 5 μm. The most common type of coating is based on organo silicone polymers, which are chemically bonded to the silanol groups on the wall of the column and the chains of the polymers are further cross-linked. These types of phases have more or less replaced the wall-coated open tubular (WCOT) and support-coated open tubular (SCOT) columns, which are reported in earlier literature, for most routine applications. SCOT columns are sometimes encountered in very high temperature work. The wall-bonded phases are stable to at least 325°C and some types of coating will withstand temperatures of 370°C. The non-silicone based polymers, e.g. carbowax, cannot be bonded onto the wall of the column in the same way and columns with these coatings are less temperature stable. For instance, the temperature limit for a carbowax capillary column is *ca* 240°C. The most commonly used carrier gas in capillary GC is helium and the flow rates used are between 0.5 and 2 ml/min. Since the flow rate from the end of the capillary column is low compared to the internal space of some detectors, 'make up' gas often has to be added to the gas flow post column in order to sweep the sample through the internal volume of the detector at a reasonable rate. Typically *ca* 100 ng per component is loaded onto a capillary column.

Selectivity of liquid stationary phases

Kovats indices and column polarity

Kovats indices (*I*-values) are based on the retention time of an analyte compared to retention times of the series of *n*-alkanes. For a particular GC phase, *I*-values are very reproducible from one column or from one GC to another. However, they are slightly affected by GC programming conditions. *n*-Alkanes have most affinity for non-polar phases and tend to elute more quickly from polar phases. In contrast, a polar analyte will elute more slowly from a polar phase and thus relative to the *n*-alkanes, its retention time and thus its *I*-value will increase as the polarity of the

GC phase increases. A measure of the polarity of a stationary phase is given by its McReynold's constant (Table 11.1), which is based on the retention times of benzene, *n*-butanol, pentan-2-one, nitropropane and pyridine on a particular phase. The higher the McReynold's constant the more polar the phase. Many stationary phases are described by an OV-number. The higher the number after the OV the more polar the phase.

Table 11.1 McReynold's constants

Phase	Chemical type	McReynold's constant
Squalane	Hydrocarbon	0
Silicone OV-1	Methylsilicone	222
Silicone SE-54	94% methyl, 5% phenyl, 1% vinyl	337
Silicone OV-17	50% methyl, 50% phenyl	886
Silicone OV-225	50% methyl, 25% cyanopropyl, 25% phenyl	1813
Carbowax	Polyethylene glycol	2318

I-values provide a useful method for characterising unknown compounds and tables of *I*-values have been compiled for a large number of compounds.[3] Under temperature programming conditions, where the GC temperature rises at a uniform rate, e.g. 10°C/min, a plot of the carbon numbers of *n*-alkanes (where 1 carbon = 100) against their retention times is a straight line. Under isothermal conditions, where the column is maintained at the same temperature throughout the analysis, a plot of carbon number against the logarithm of the retention times of the *n*-alkanes is a straight line. Such calibration curves can be used to convert the retention time of a compound into an *I*-value.

Examples of the separation of mixtures by GC

Analysis of peppermint oil on two GC phases

Figure 11.5 shows the structures of some of the major components in peppermint oil. The use of the retention index system is illustrated in Figures 11.6 and 11.7 for peppermint oil run in comparison with *n*-alkane standards on both a weakly polar OV-5-type column and a polar carbowax column.

Figure 11.6 indicates approximate *I*-values for some of the components in peppermint oil on a BPX-5 column; this column selects mainly on the basis of molecular weight and shape. For example β-pinene has the same molecular weight as limonene but has a more compact shape and thus a lower *I*-value. Menthyl acetate has a higher *I*-value than menthol because of its higher molecular weight.

A carbowax column is highly selective for polar compounds. As can be seen in Figure 11.7 the group of polar compounds including menthol and menthone is

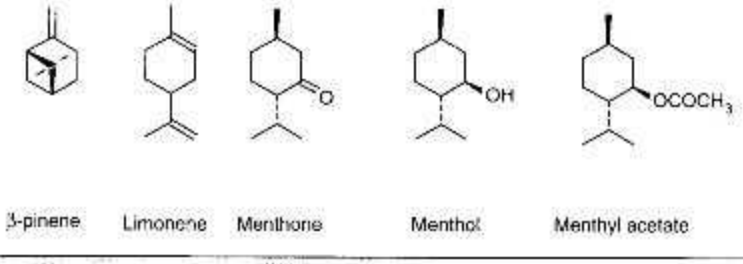


Fig. 11.5
The structures of some components in peppermint oil.

resolved more extensively on a carbowax column with the alcohol menthol and a number of other minor alcohols eluting at around 12 min. In addition, the less polar ketone menthone and a number of minor ketones elute at around 10 min. Menthyl acetate, which on the non-polar BPX-5 column ran later than menthol, runs earlier than menthol on the carbowax column because its polar alcohol group is masked by the acetate and it thus has a lower polarity than menthol.

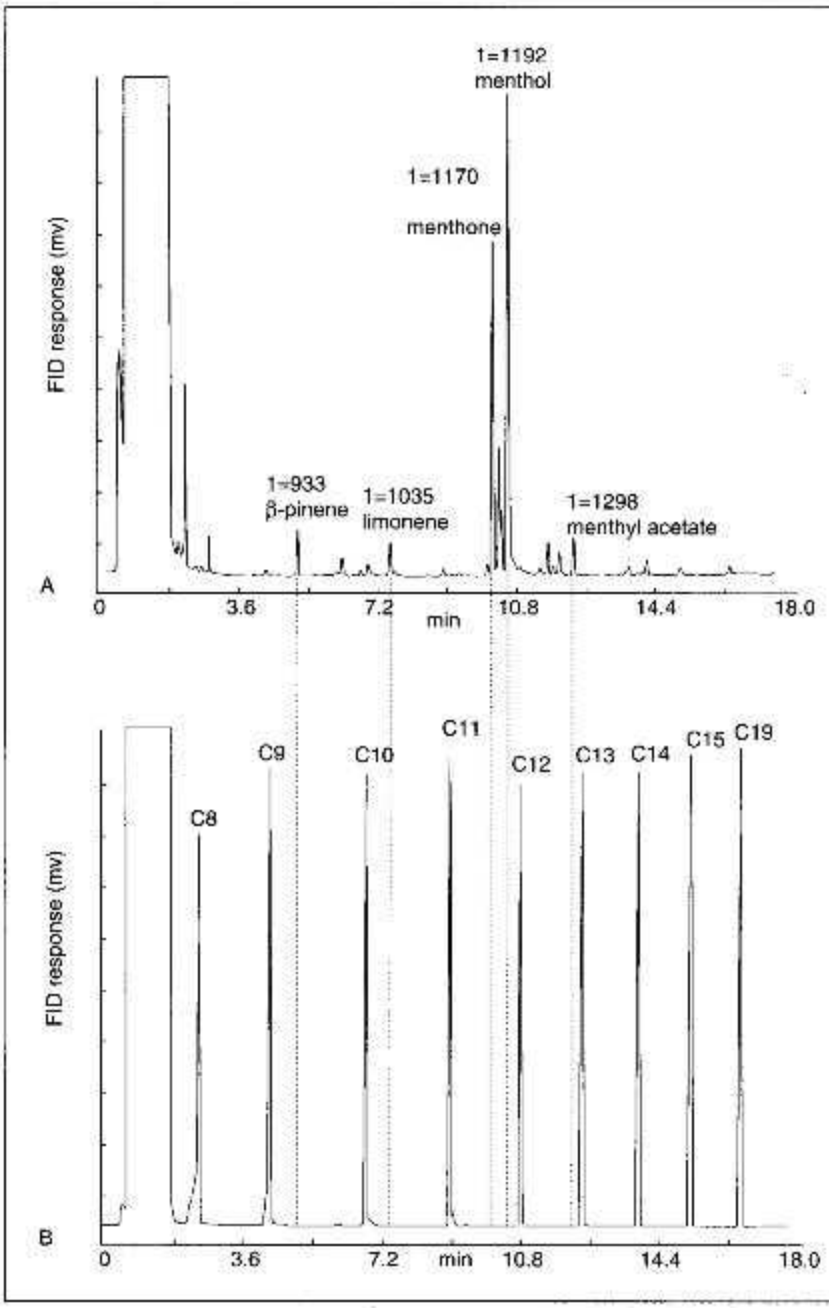


Fig. 11.6
(A) Peppermint oil analysed on a BPX-5 column (12 m × 0.25 mm i.d. × 0.25 μm film). Programmed 50°C (1 min), then 5°C/min to 70°C, then 10°C/min to 200°C. (B) n-alkanes C8–C16 chromatographed under the same conditions.

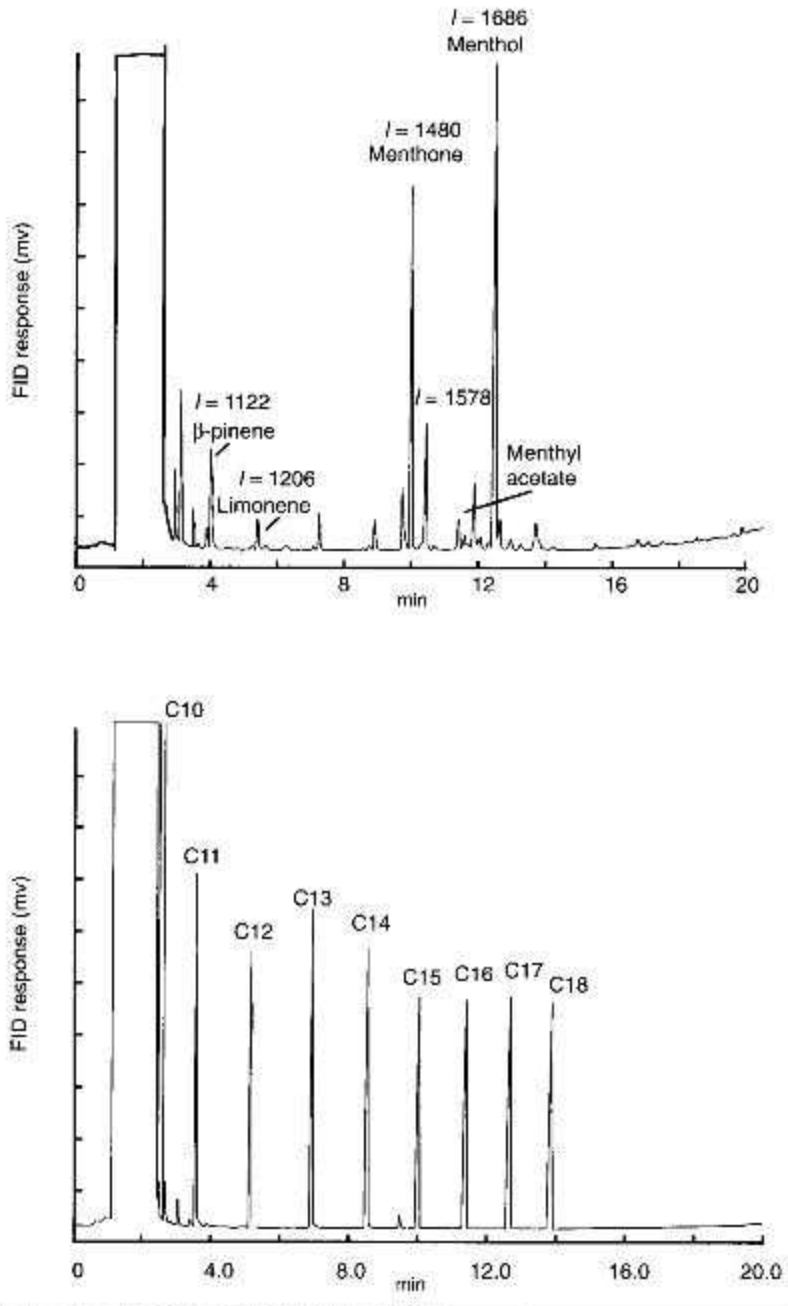
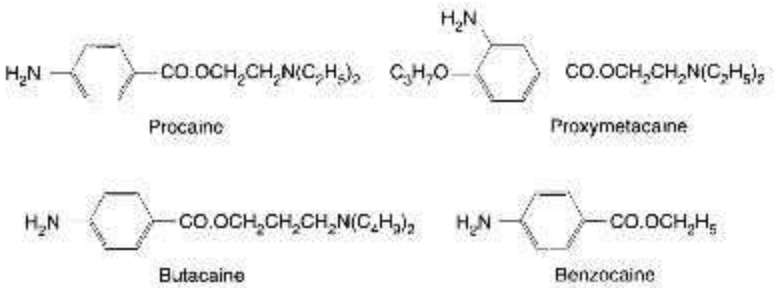


Fig. 11.7
(A) Peppermint oil analysed on a carbowax column (15 m × 0.25 mm × 0.5 μm film). Programmed 50°C (1 min) then 5°C/min to 70°C then 10°C/min to 200°C. (B) *n* alkanes C10–C18 chromatographed under the same conditions.

Self-test 11.1

Associate the following *I*-values obtained on an OV-1-type column with structures of the local anaesthetics shown below. *I*-values: 1555, 2018, 2323 and 2457. Note: oxygen in an ether linkage is equivalent to *ca* 1 CH_2 unit.



Answer: Procaine 2018; Proxymetacaine 2323; Butacaine 2457; Benzocaine 1555

Analysis of the fatty acid composition of a fixed oil by GC

A very polar phase such as carbowax is generally only used for samples requiring a high degree of polar discrimination for adequate separation or retention. An example of this is in the analysis of fatty acids with differing degrees of unsaturation. On a non-polar column such as BPX-5, a series of C-18 acids such as stearic, oleic, linoleic and linolenic acids, which contain respectively 0, 1, 2 and 3 double bonds, overlaps extensively. However, on polar columns such as carbowax they are separated.

The BP monographs for many of the fixed oils contain a GC analysis to confirm the content of the fatty acids composing the triglycerides (fatty acid triesters of glycerol) present in the oil. The monograph for almond oil states the composition of the fatty acids making up the triglyceride should be:

- palmitic acid (16:0) 4.0–9.0%
- palmitoleic acid (16:1) < 0.6%
- margaric acid (17:0) < 0.2%
- stearic acid (18:0) 0.9–2.0%
- oleic acid (18:1) 62.0–86.0%
- linoleic acid (18:2) (7.0–30.0%)
- linolenic acid (18:3) < 0.2%
- arachidic acid (20:0) < 0.1%
- behenic acid (22:0) < 0.1%.

The first number in brackets, e.g. 16, refers to the number of carbon atoms in the fatty acid and the second number, e.g. 0, refers to the number of double bonds in the fatty acid. The percentage of each component is determined in relation to the sum of the areas of the chromatographic peaks of all the components listed above.

In order to determine the fatty acid composition of the triglycerides, they have to be first hydrolysed and the liberated fatty acids converted to their methyl esters, which have a good chromatographic peak shape compared to the free acids. A convenient method for achieving hydrolysis and methylation in one step is shown in Figure 11.8.

A GC trace of methanolysed almond oil is shown in Figure 11.9. It can be seen that the methyl esters stearic, oleic and linoleic acid are incompletely resolved on a BPX-5 column. The esters of the minor C-20 and C-22 acids are also incompletely

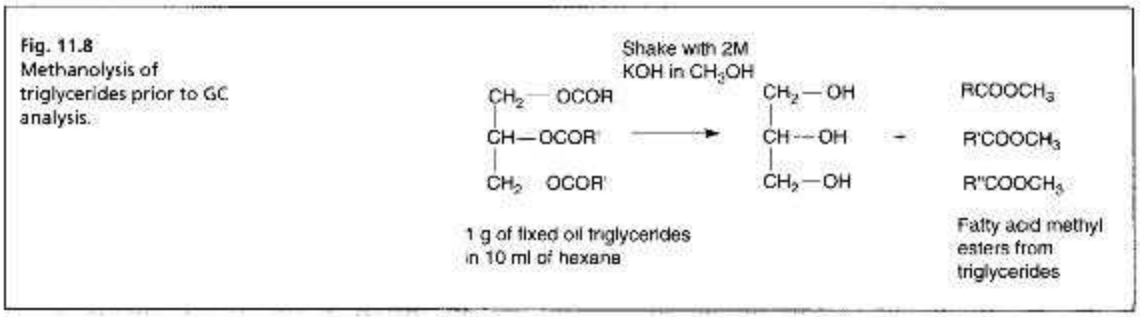


Fig. 11.8
Methanolysis of triglycerides prior to GC analysis.

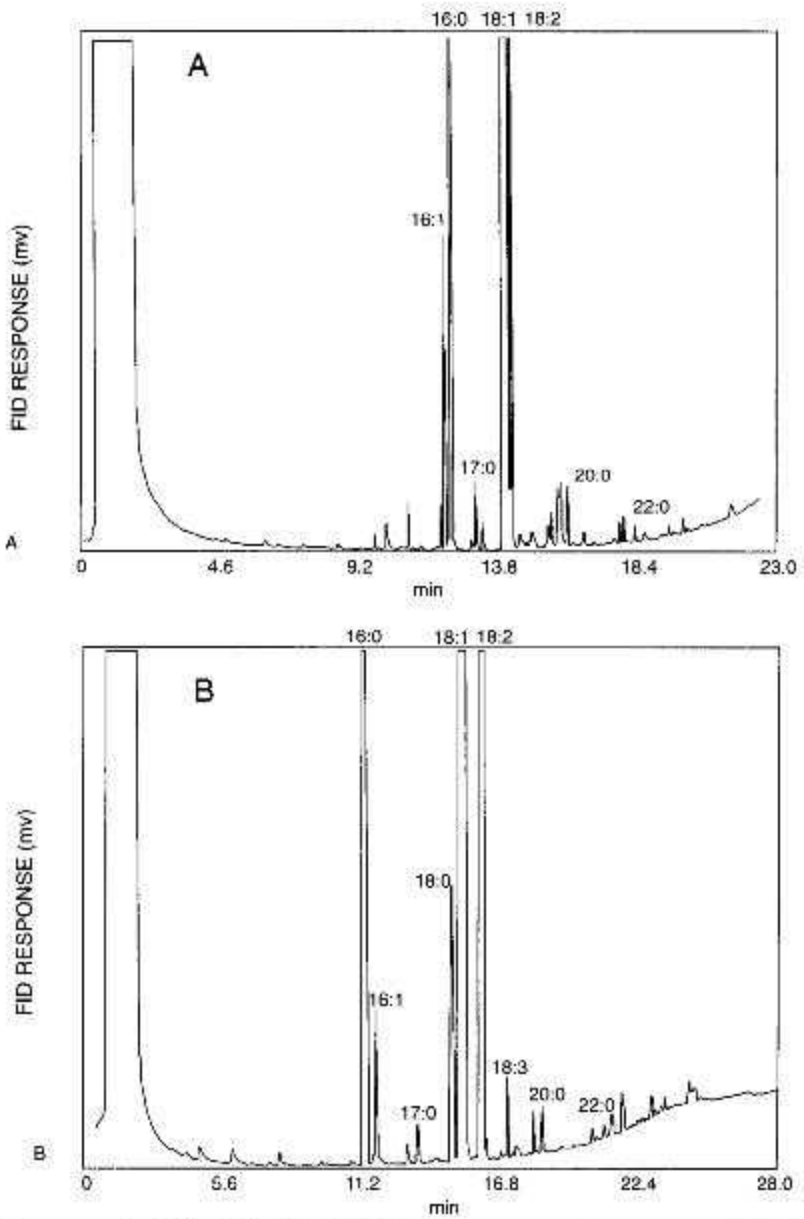


Fig. 11.9
(A) Separation of fatty acid methyl esters derived from almond oil on a BPX-5 column (12 m × 0.25 mm i.d. × 0.25 μm film). Programmed 100° (1 min) then 10°/min to 320°. The major peaks are shown as offscale so that the minor peaks can be seen clearly. (B) The same sample separated on a HP Stabiliwax column (15 m × 0.25 mm × 0.5 μm film). Programmed 140° (1 min) then 5°/min to 250° (5 min).

separated. When a carbowax column is used, complete separation of oleic (18:1), linoleic (18:2), stearic acid (18:0) and a small amount of linolenic acid (18:3) in the sample is achieved.

The chromatogram obtained on the carbowax column gave the percentage of areas of the peaks in this particular sample of almond oil as follows: 16:0 (7.0%), 16:1 (0.4%), 17:0 (0.12%), 18:0 (1.5%), 18:1 (62.8%), 18:2 (28.4%), 18:3 (0.16%), 20:0 (0.09%), 22:0 (0.09%). Thus the almond oil is within the BP specification given above (Fig. 11.9B).

Chiral selectivity

An advanced type of column selectivity is chiral discrimination. Since enantiomers have identical physical properties they are not separable on conventional GC columns. However, if chiral analytes are allowed to interact with a chiral environment they will form transitory diastereomeric complexes which result in their being retained by the column to a different extent. As increasing numbers of enantiomerically pure drugs are synthesised in order to reduce side-effects, this type of separation will become increasingly important.

Chirasil Val was one of the first chiral GC phases; it has one chiral centre as can be seen in its structure as shown in Figure 11.10.

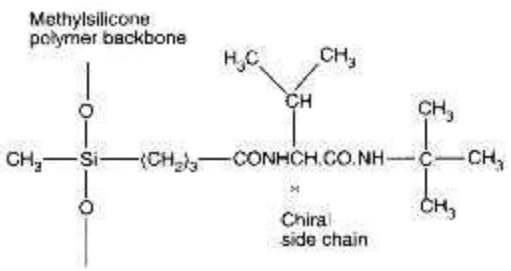
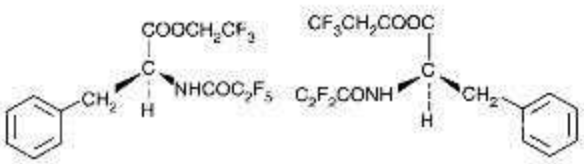


Fig. 11.10
The structure of Chirasil Val.

A number of variations on this type of coating have been prepared and offer some improvement over the original phase. Figure 11.11 shows the volatile pentafluoropropionamide–trifluoroethyl ester (PFP–TFE) derivatives of *L* and *D* phenylalanine. Figure 11.12 shows the separation of PFP–TFE derivatives of the *D* and *L* enantiomers of the amino acids phenylalanine and *p*-tyrosine on a Chirasil Val column, the *D*(*R*)-enantiomers elute first. Chirasil Val generally performs best for the separation of enantiomers of amino acids, for many other compounds it is not as effective.



PFP–TFE derivative of *L* phenylalanine PFP–TFE derivative of *D* phenylalanine

Fig. 11.11

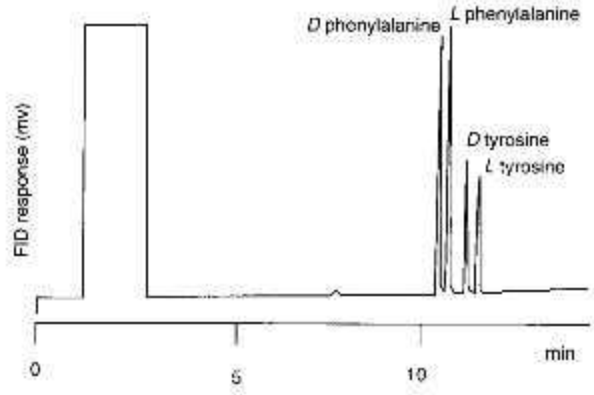


Fig. 11.12
Separation of the PFP-TFE derivatives of the D and L isomers of phenylalanine and *p*-tyrosine on a Chirasil Val column 25 m × 0.25 mm i.d. × 0.16 μm film. Programmed 120° (1 min) then 3°/min to 180°.

More recently alkylated cyclodextrins have been developed as chiral phases. These phases are based on cyclodextrins, which are cyclic structures formed from 6, 7 or 8 glucose units. Alkylation of the hydroxyl groups in the structure of the cyclodextrins lowers their melting points and makes them suitable as GC phases. The cyclodextrins contain many chiral centres and separate enantiomers of drugs according to how well they fit into the chiral cavities of the cyclodextrin units (see Ch. 12 p. 273).

An alternative to buying expensive chiral columns in order to separate enantiomers is to use a chiral derivatisation agent. These reagents can be based on natural products which usually occur in enantiomerically pure form. Chiral derivatising agents can often produce better separations than chiral columns, but if reaction conditions are too strong, there is a risk of small amounts of racemisation occurring in the analyte, i.e. chemical conversion of an enantiomer into its opposite. Reaction of an enantiomeric mixture with a chiral derivatising agent produces a pair of diastereoisomers which are separable by GC on non-chiral columns, e.g. the esters of menthol with (+) chrysanthemic acid.[4]

It can be seen in Figure 11.13 that, although the menthol portions of the esters are mirror images, addition of the chiral acylating reagent generates esters which are not mirror images but are diastereoisomers and thus have different physical properties.

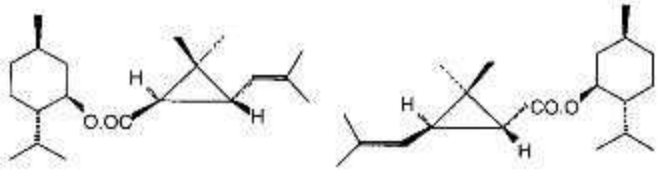


Fig. 11.13
(+) chrysanthemyl esters of menthol.

Use of derivatisation in GC

Derivatisation has been mentioned above without fully indicating why it is necessary for conducting GC analysis. Derivatisation is generally required prior to GC if a compound is highly polar so that good chromatographic peak shape can be achieved. A large number of derivatisation strategies are available.[2] In the following example, derivatisation is used to improve the peak shape of pseudoephedrine (Fig. 11.14).

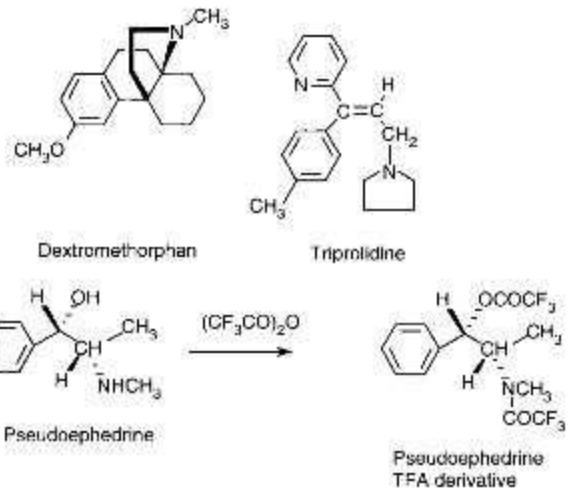


Fig. 11.14
The components in a decongestant + derivatisation of pseudoephedrine with trifluoroacetic anhydride (TFA).

A decongestant syrup was basified with ammonia and extracted into ethyl acetate, thus ensuring that the components extracted were in their free base forms rather than their salts, which is important for obtaining good chromatographic peak shape. Salts of bases will thermally dissociate in the GC injector port but this process can cause a loss of peak shape and decomposition.

If the extract is run directly, the trace shown in Figure 11.15A is obtained. The free bases of triprolidine and dextromethorphan give good peak shape but pseudoephedrine which is stronger base and which has in addition a hydroxyl group in its structure gives a poor peak shape. This can be remedied by masking the polar alcohol and amine groups of pseudoephedrine by reaction with trifluoroacetic anhydride TFA. Treatment with TFA does not produce derivatives of the tertiary bases in the extract. This reagent is very useful because it is very reactive and boils at 40°C thus excess reagent can be evaporated very easily prior to GC analysis and thus unlike many reagents it does not leave any residue.

Silylating reagents are another popular class of derivatisation reagents. These reagents introduce residues into the sample, although this is not a great problem if the analyte is relatively involatile. An example of a silylation reaction is shown on page 226.

Summary of parameters governing capillary GC performance

Carrier gas type/flow

According to the Van Deemter equation hydrogen and helium give higher efficiencies at high flow rates compared with nitrogen. For practical analysis times hydrogen or helium are used in capillary GC and typical flow rates for hydrogen and helium are in the range of 30–50 cm/s; nitrogen has its optimum flow rate at 10–20 cm/s. Table 11.2 shows typical pressure settings to achieve optimal flow rate for three columns. The gas flow rate decreases with increasing temperature and this may have an influence on column efficiency. Modern instruments have flow programming so that the flow can be set to remain constant as the temperature rises.

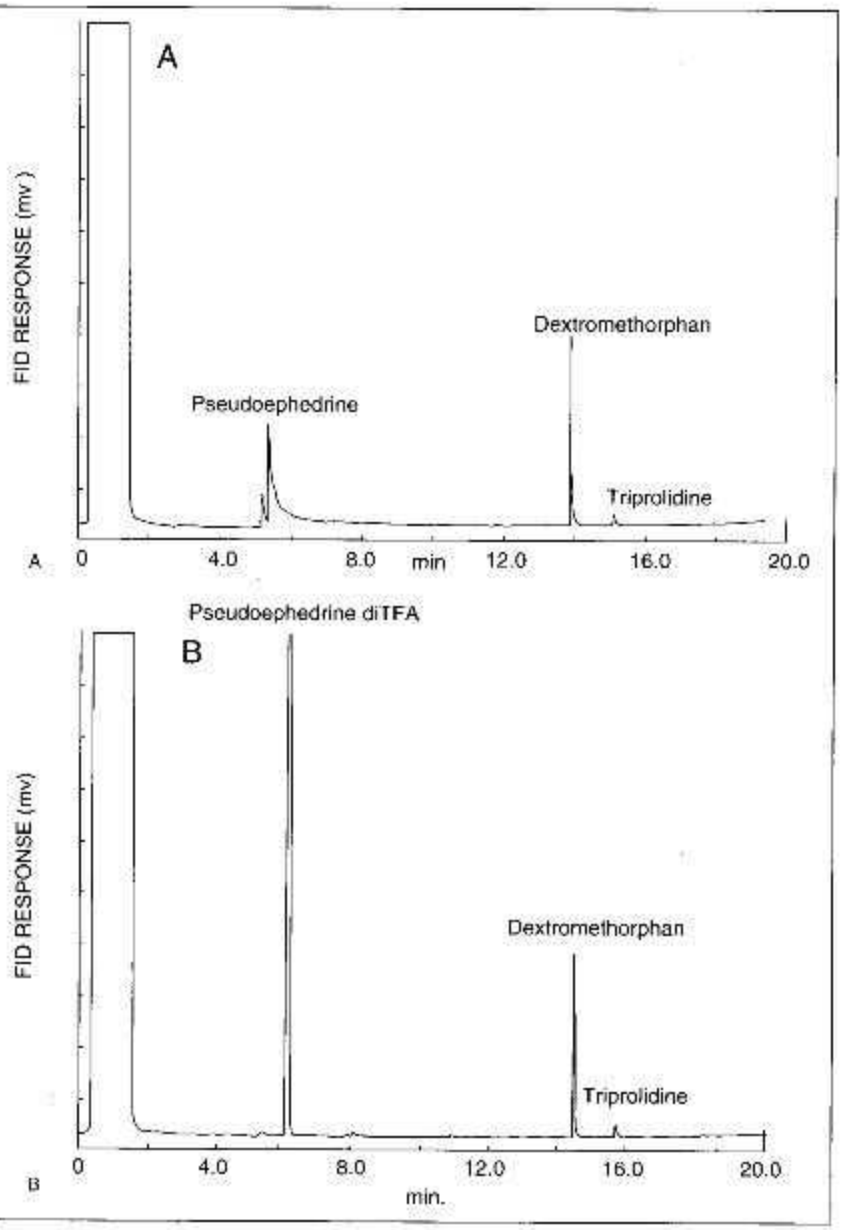


Fig. 11.15
GC traces of an extract from a decongestant syrup. (A) Underivatised and (B) after treatment with trifluoroacetic anhydride.

Table 11.2 Effect of temperature on flow rate at constant pressure

Column	Pressure	Temperature (C)	T_o (s)	Flow rate	Temp	Flow rate (C)	T_o (s)
25 m × 0.5 mm i.d.	22.2 KPa	100°	83	30 cm/s	250°	23.7 cm/s	106
25 m × 0.25 mm i.d.	91.1 KPa	100°	83	30 cm/s	250°	23.7 cm/s	106
12 m × 0.25 mm i.d.	42.8 KPa	100°	40	30 cm/s	250°	23.7 cm/s	51

Column temperature

As column temperature increases the degree of resolution between two components decreases because the degree of interaction with the stationary phase is reduced as the vapour pressure of the analytes increases. Lower temperatures produce better resolution.

Column length

The separating power of a column varies as the square root of its length. Thus if a two-fold increase in resolution is required and a four-fold increase in column length would be required, this would result in a four-fold increase in analysis time. The increased resolution afforded by length can often be replaced with a decrease in temperature ensuring that more interaction with the stationary phase occurs, especially if the stationary phase has characteristics that enable it to select one analyte more than another.

Film thickness phase loading

The greater the volume of stationary phase the more a solute will partition into it. If the film thickness or loading of stationary phase doubles then in theory the retention of an analyte should double. Thus thicker films are used for very volatile materials to increase their retention time and to increase resolution between analytes without increasing the column length.

Internal diameter

The smaller the internal diameter of a capillary column the more efficient the column is for a given stationary phase film thickness on the capillary wall. This is because the mass transfer characteristics of the column are improved with the analyte being able to diffuse in and out of the mobile phase more frequently because of the shorter distance for transverse diffusion (Ch. 10 p. 201).

Self-test 11.2

A fixed temperature is used and the head pressure is adjusted so that the linear velocity of a helium carrier gas through the following capillary columns is 20 cm/s: (i) 30 m × 0.25 mm i.d. × 0.25 μm OV–1 film; (ii) 15 m × 0.15 mm × 0.2 μm OV–1 film; (iii) 12 m × 0.5 mm i.d. × 1.0 μm OV–1 film.

a. List the columns in the order in which they would increasingly retain a *n*-hexadecane standard.
b. List the columns in order of increasing efficiency.

Answers: a. (ii) (i) (iii) b. (iii) (i) (ii)

GC detectors

There are many GC detectors available although the flame ionisation detector remains the most widely used and the most widely applicable to quality control of pharmaceutical products. However, newer detectors such as the plasma emission detector for analysis of trace impurities or the GC–FTIR detector for the structural characterisation of components in mixtures are becoming increasingly important.

Selectivity in a detector is most often required for sensitive bioanalytical methods where trace amounts of compounds are being analysed in the presence of interferants which are also present in the sample matrix. The properties of some commonly used detectors are summarised in Table 11.3.

Table 11.3 Commonly used GC detectors

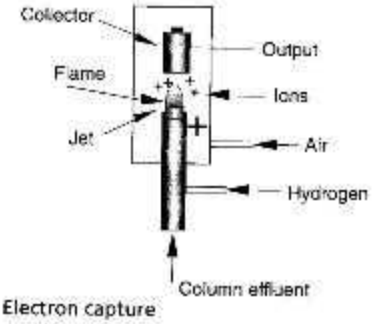
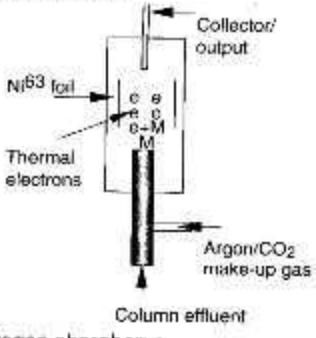
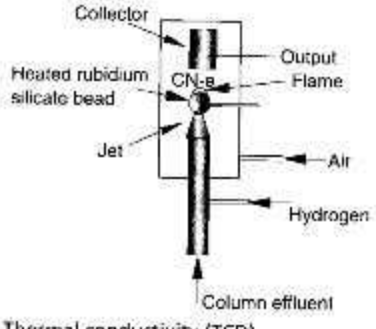
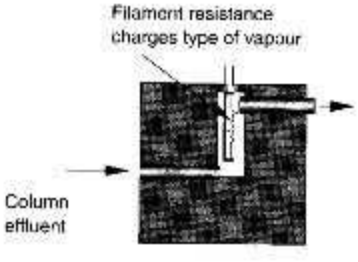
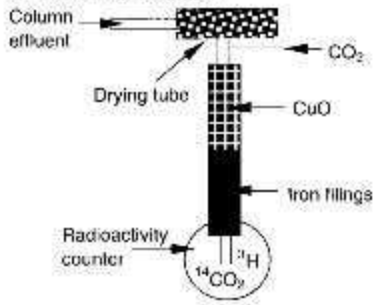
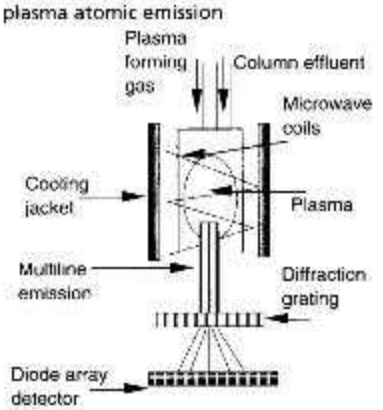
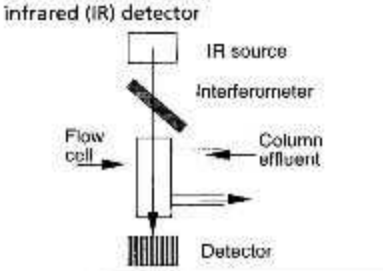
Detector	Applications
Flame ionisation 	Compounds are burnt in the flame producing ions and thus an increase in current between the jet and the collector. Detects carbon/hydrogen-containing compounds. Insensitive to carbon atoms attached to oxygen, nitrogen or chlorine. In combination with capillary GC it may detect as low as 100 pg–10 ng. Wide range of linear response *ca* 10^6
Electron capture 	Compounds with a high affinity for electrons enter the detector and capture the electrons produced by the radioactive source thus reducing the current to the collector. Highly halogenated compounds can be detected at the 50 fg–1 pg level. Has a large internal volume therefore some chromatographic resolution may be lost. Linearity of response is not as great as FID, e.g. 10^3. Mainly used for analysis of drugs in body fluids. Has wide application in environmental monitoring, e.g. chlorofluorocarbons in the atmosphere
Nitrogen phosphorus 	Nitrogen and phosphorus-containing compounds react with the alkali metal salt in the detector to produce species such as CN , various phosphorus anions or electrons all of which produce an increase in current which generates the signal. Detects phosphorus compounds at the pg level, nitrogen compounds at the low ng level. Highly selective for nitrogen and phosphorus-containing compounds. Used mainly in the analysis of drugs and their metabolites in tissues and bodily fluids
Thermal conductivity (TCD) 	Responds to cooling effect of the analyte passing over the filament. Relatively insensitive to organic compounds in comparison to FID. It is a universal detector which can be used to determine water vapour. It is also non-destructive so that analytes can be collected after detection, if required. Used to determine water in some BP assays, e.g. water in the peptides menotrophin, gonadorelin and salcatonin

Table 11.3 Commonly used GC detectors (Cont.)

Detector	Applications
Radiochemical detector 	^{14}C and ^3H present in the molecule are respectively converted to $^{14}CO_2$ and $^3H_2$. Its sensitivity depends on the degree of radioactivity of the analyte. Useful for metabolic labelling studies, making metabolites of drugs easy to detect. The detector tends to work better with packed columns since it has a large internal space
Microwave-induced plasma atomic emission 	A hot plasma of argon is produced by heating to > 6000°C which causes all the elements in the compound to produce emission spectra. The individual emission lines are passed through diffraction grating and detected by a diode array detector. Detects individual elements, e.g. chlorine in organochlorine pesticides and metals in organometallics. Sensitive to the pg level for some elements. Most widely used in environmental monitoring but also has useful potential for impurity profiling of drugs and metabolism studies
Fourier transform (FT) infrared (IR) detector 	Essentially just an FT–IR instrument coupled to a GC, thus allowing IR spectra of compounds eluting from the GC column to be obtained. More useful for structure elucidation rather than quantitative studies. The detector is sensitive to the 10 ng level. Used as a tool for qualitative identification. There are some examples of quantitative applications, e.g. determination of propandiol in acyclovir cream.[3]

Applications of GC in quantitative analysis

HPLC has more or less supplanted GC as a method for quantifying drugs in pharmaceutical preparations. Many of the literature references to quantitative GC assays are thus old and the precision which is reported in these papers is difficult to evaluate based on the measurement of peak heights or manual integration. It is more difficult to achieve good precision in GC analysis than in HPLC analysis and the main sources of imprecision are the mode of sample introduction, which is best controlled by an autosampler, and the small volume of sample injected. However, it is possible to achieve levels of precision similar to those achieved using HPLC methods. For certain compounds that lack chromophores, which are required for detection in commonly used HPLC methods, quantitative GC may be the method of choice, for analysis of many amino acids, fatty acids, and sugars. There are a number

of assays in the BP, US Pharmacopoeia and the European Pharmacopoeia which are based on GC, but the selection of compounds analysed in this way appears to be rather random and many of the assays described could also be carried out by HPLC. The BP format for assays (both for HPLC and GC assays) is, most often, to run three samples. These are: a calibration standard containing more or less equal amounts of a pure standard and an internal standard (Solution 1); an extract of the sample containing no internal standard to check for interference from the formulation matrix (Solution 2); and an extract from the sample containing the same amount of internal standard as Solution 1 (Solution 3). This is illustrated in Figure 11.16 for the analysis of methyltestosterone in a tablet formulation using testosterone as an internal standard p. 259.

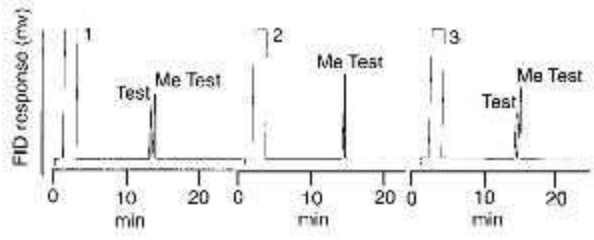


Fig. 11.16
Chromatograms of Solutions 1, 2 and 3 prepared for the analysis of methyltestosterone tablets. RTX-1 column 15 m × 0.25 mm i.d. × 0.25 μm film. Programmed 150°(1 min) then 10°/min to 320°C (5 min).

Analysis of methyltestosterone in tablets

A calibration solution containing *ca* 0.04% w/v of methyltestosterone and *ca* 0.04% w/v testosterone in ethanol is prepared (Solution 1). A weight of tablet powder containing *ca* 20 mg of methyltestosterone is extracted with 50 ml of ethanol to prepare Solution 2. Solution 3 is prepared by dissolving tablet powder containing *ca* 20 mg of methyltestosterone in *exactly* 50 ml of ethanol containing *exactly* the same concentration of testosterone as Solution 1. In this example 0.5 μl amounts of the solutions were injected into the GC in the splitless mode.

Solution 1 gives a response factor for the calibration solution as follows:

$$\frac{\text{area of methyltestosterone peak in calibration solution}}{\text{area of testosterone peak in calibration solution}}$$

Solution 3 gives a response factor for the sample as follows:

$$\frac{\text{area of methyltestosterone peak in sample solution}}{\text{area of testosterone peak in sample solution}}$$

The amount of methyltestosterone in the tablet powder can be calculated as follows:

$$\text{amount of methyltestosterone} = \frac{\text{response factor for sample}}{\text{response factor for calibration solution}} \times \%\text{w/v of methyltestosterone in calibration solution} \times \frac{\text{vol. sample solution}}{100}$$

Data from analysis of methyltestosterone tablets

- Weight of 5 tablets = 0.7496 g
- Stated content of methyltestosterone per tablet = 25 mg

- Weight of tablet powder taken for assay = 0.1713 g
- Solution 1 contains: 0.04% w/v methyltestosterone and 0.043% w/v testosterone
- Solution 3 contains: the methyltestosterone extracted from the powder taken for assay and 0.043% w/v testosterone
- Solution 1: Peak area testosterone = 216 268; Peak area methyltestosterone = 212 992
- Solution 3: Peak area testosterone = 191 146; Peak area methyltestosterone = 269 243.

Calculation example 11.1

$$\text{Response factor for Solution 1} = \frac{212\,992}{216\,268} = 0.9849$$

$$\text{Response factor for Solution 3} = \frac{269\,243}{191\,146} = 1.409$$

Amount of methyltestosterone in the tablet powder determined by analysis =

$$\frac{1.409}{0.9849} \times 0.04 \times \frac{50}{100} = 0.02861 \text{ g} = 28.61 \text{ mg}$$

Amount of methyltestosterone expected in tablet powder =

$$\frac{\text{weight of powder analysed}}{\text{weight of 5 tablets}} \times \text{stated content of 5 tablets} = \frac{0.1713}{0.7496} \times 5 \times 25 = 28.57 \text{ mg}$$

$$\text{Percentage of stated content} = \frac{28.61}{28.57} \times 100 = 100.1\%$$

A dilution factor may be incorporated into this calculation if the sample is first extracted and then diluted in order to bring it into the working range of the instrument. This approach to quantitation does not address the linearity of the method but since the variation in the composition of formulations should be within ± 10% of the stated amount there is some justification for using it. The precision of the method is readily addressed by carrying out repeat preparations of sample and calibration solutions.

Analysis of atropine in eyedrops

Another group which is used to mask polar groups in molecules in order to improve GC peak shape is the trimethylsilyl group. Atropine Eye Drops BP are used to dilate the pupil prior to cataract surgery. The 1993 BP method for the analysis of Atropine Eye Drops BP uses derivatisation with a trimethylsilyl group to mask an alchohol group as shown in Figure 11.17.

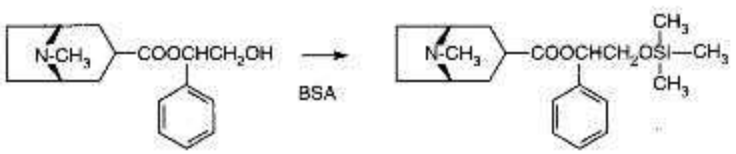


Fig. 11.17 Trimethylsilylation of atropine.

The method involves extraction of atropine and a homatropine internal standard from the aqueous phase which is rendered alkaline by the addition of ammonia followed by trimethylsilylation with *N,O*bistrimethylsilyl acetamide (BSA).

In the calculation using the results of this experiment it is better to use amount rather than concentration as a standard measure since after the initial accurate volume measurement used for the addition of the standard and internal standard to the calibration solution (Solution 1) and for the addition of the internal standard to a fixed volume of eyedrops the volumes need only be measured approximately; this is the advantage of using an internal standard (Fig. 11.18). The following formula is used:

$$\text{amount of atropine in the eyedrop sample} = \frac{\text{response factor for sample}}{\text{response factor for calibration solution}} \times \text{amount of atropine in calibration solution}$$

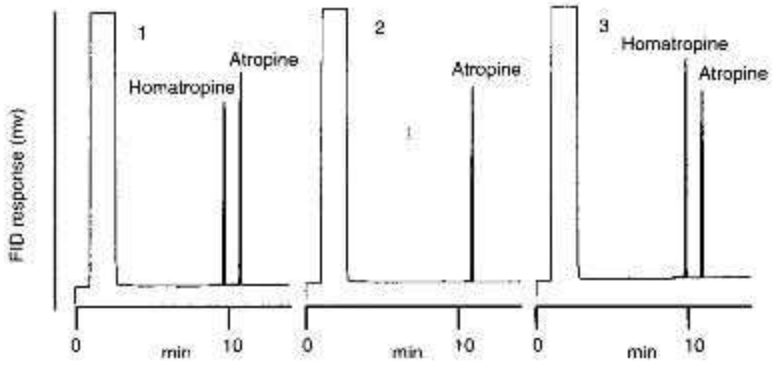


Fig. 11.18
Chromatograms of solutions 1, 2 and 3 prepared for the analysis of atropine in eyedrops. RTX-1 column 15 m × 0.25 mm i.d. × 0.25 μm film. Programmed 140°C (1 min) then 10°/min to 320°C (5 min).

Brief description of the assay

Solution 1 is prepared from *exactly* 5 ml of 0.4092% w/v atropine sulphate solution and *exactly* 1 ml of 2.134% w/v homatropine hydrobromide solution. The solution is basified and extracted, the solvent is removed and the residue is treated with 2 ml of BSA and then diluted to 50 ml with ethyl acetate. Solution 3 is prepared from *exactly* 2 ml of eyedrops and *exactly* 1 ml of 2.134% w/v homatropine hydrobromide solution. The solution is basified and extracted, the solvent is removed and the residue is treated with 2 ml of BSA and then diluted to 50 ml with ethyl acetate.

Data from analysis of eyedrop formulation

- Volume of eyedrops analysed = 2.0 ml
- Stated content of eyedrops = 1.0% w/v
- Solution 1: Peak area homatropine TMS = 118 510; Peak area atropine TMS = 146 363
- Solution 3: Peak area homatropine TMS = 145 271; Peak area atropine TMS = 117 964.

Calculation example 11.2

Amount of atropine sulphate in Solution 1 = $0.4092 \times \frac{5}{100} = 0.02046$ g.

Response factor for Solution 1 = $\frac{146\,363}{118\,510} = 1.2350$.

Response factor for Solution 3 = $\frac{117\,964}{145\,271} = 0.8120$.

Amount of atropine sulphate in Solution 3 = $\frac{0.8120}{1.2350} \times 0.02046$ g = 0.01345 g.

This was the amount originally present in 2 ml of eyedrops therefore percentage of w/v of atropine sulphate in eyedrops

$= 0.01345 \times \frac{100}{2} = 0.6725\%$ w/v.

The amount determined in the eyedrops is well below the stated amount of 1% w/v and this is because this sample of eyedrops was *ca* 10 years old and had probably suffered extensive degradation.

Self-test 11.3

Calculate the percentage of the stated content of hyoscine hydrobromide in travel sickness tablets from the following data. The assay is carried out in a manner similar to the eyedrop assay described above. The amount of atropine added as an internal standard does not enter into the calculation if we assume that the same amount is added to Solutions 1 and 3.

- Weight of 20 tablets = 2.1881 g
- Weight of tablet powder taken = 0.9563 g
- Stated content per tablet = 0.6 mg
- Concentration of hyoscine hydrobromide standard solution = 0.0341% w/v
- Volume of hyoscine hydrobromide solution added to Solution 1 = 15 ml
- Area of hyoscine peak in Solution 1 = 147 881
- Area of atropine peak in Solution 1 = 159 983
- Area of hyoscine peak in Solution 3 = 167 799
- Area of atropine peak in Solution 3 = 173 378.

Answer 102.1%

Quantification of ethanol in a formulation

Gas chromatography provides a useful method for quantifying very volatile materials. In this case columns are required, which strongly retain volatile compounds. Ethanol is used in the preparation or tinctures and in disinfectant solutions. Typically ethanol may be quantified against a related alcohol. In the 1993 BP assay of chloroxylenol solution ethanol is quantified against a propan-1-ol internal standard. The column used is packed with Porapak Q: Porapak is an example of a porous polymeric stationary phase which retains low molecular weight compounds strongly. These types of phases are also effective in separating gases such as CO_2, ammonia and acetylene. As an alternative to a Porapak column, a thick film (e.g. 5 μm film) GC capillary column may be used for this type of analysis.

Determination of manufacturing and degradation residues by GC

Determination of pivalic acid in dipivefrin eye drops

GC provides a useful technique for estimating volatile degradation products. For example the pivalic acid release from the hydrolysis of dipivefrin in an eyedrop preparation (Fig. 11.19) used for treating glaucoma may be estimated by GC.[6] Isovaleric acid, which is an isomer of pivalic acid, provides a suitable internal standard. Breakdown products of esters are more likely to occur in aqueous formulations such as eyedrops or injections.

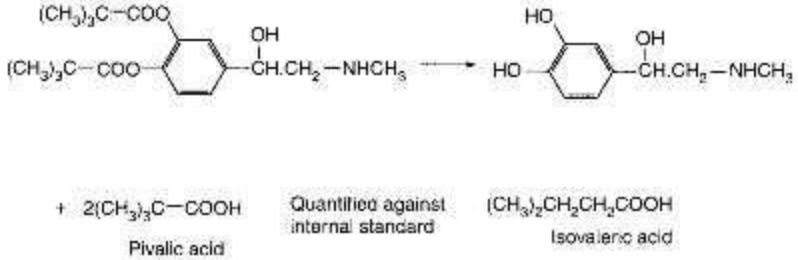


Fig. 11.19 Breakdown of dipivefrin resulting in formation of pivalic acid.

Dimethylaniline in bupivacaine injection (Fig. 11.20)

Dimethylaniline is both a manufacturing impurity in bupivacaine and since it is formulated in injections a possible breakdown product, although hydrolysis of amides is much slower than hydrolysis of esters. The BP uses a spectrophotometric method to assay for this impurity but GC provides a more sensitive and specific method for this determination.

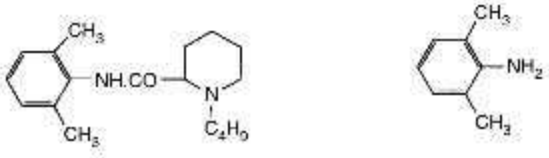


Fig. 11.20 Bupivacaine and its degradation product.

The GC trace obtained from injection of a 10% w/v solution of bupivacaine free base extracted from an injection gave the trace shown in Figure 11.21. It is apparent from comparison with a standard for dimethylaniline that there is ≤ 0.1% of the impurity present although a number of other peaks due to excipients or impurities can be seen in the GC trace.

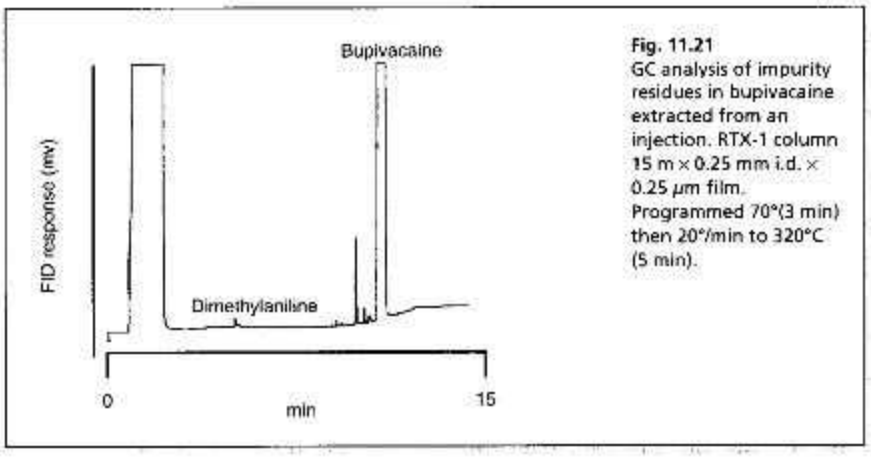


Fig. 11.21
GC analysis of impurity residues in bupivacaine extracted from an injection. RTX-1 column 15 m × 0.25 mm i.d. × 0.25 μm film. Programmed 70°(3 min) then 20°/min to 320°C (5 min).

Determination of a residual glutaraldehyde in a polymeric film

Sometimes derivatisation can provide a highly specific method of detecting impurities. In this example the low molecular weight impurity glutaraldehyde, which is not stable to direct analysis by GC, is reacted with a high molecular weight derivatising reagent pentafluorobenzyloxime; the reaction is shown in Figure 11.22. This reaction stabilises the analyte and increases its retention time into a region where it can be readily observed without interference from other components extracted from the sample matrix. The derivative is also highly electron capturing. In this example a GC method was found to be superior to a HPLC method using derivatisation with dinitrophenylhydrazine since the residues from the reagent produced less interference in the analysis.

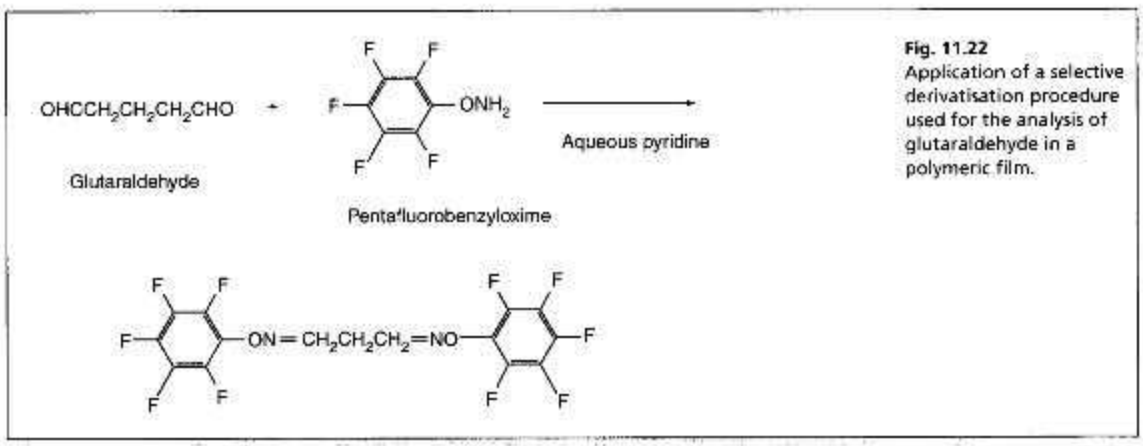


Fig. 11.22
Application of a selective derivatisation procedure used for the analysis of glutaraldehyde in a polymeric film.

The converse of this type of reaction has been used to determine hydrazine as a manufacturing impurity in the drug hydralazine by reaction of the hydrazine residue with benzaldehyde to form a volatile derivative for GC analysis.[7]

Determination of residual solvents

Typical BP procedures

The current BP methods for determination of solvent residues in pharmaceuticals remaining from the manufacturing process rely on direct injection of the sample dissolved in a suitable solvent (often water) and are based on packed column GC. Some examples are given in Table 11.4.

Table 11.4 Some BP procedures for the analysis of residual solvents

Drug	Residues	GC conditions
Ampicillin sodium	Dichloromethane	10% polyethylene glycol 60°
Ampicillin sodium	Dimethyl aniline	3% OV-17 80°
Colchicine	Ethyl acetate and chloroform	10% polyethylene glycol 75°
Gentamycin sulphate	Methanol	Porapak Q 120°
Menotrophin	water	Chromosorb 102, 114°, TCD
Warfarin sodium	Propan-2-ol	10% polyethylene glycol 70°

Determination of residual solvents and volatile impurities by head space analysis

A more refined method for determining residual solvents and volatile impurities is based on head space analysis.

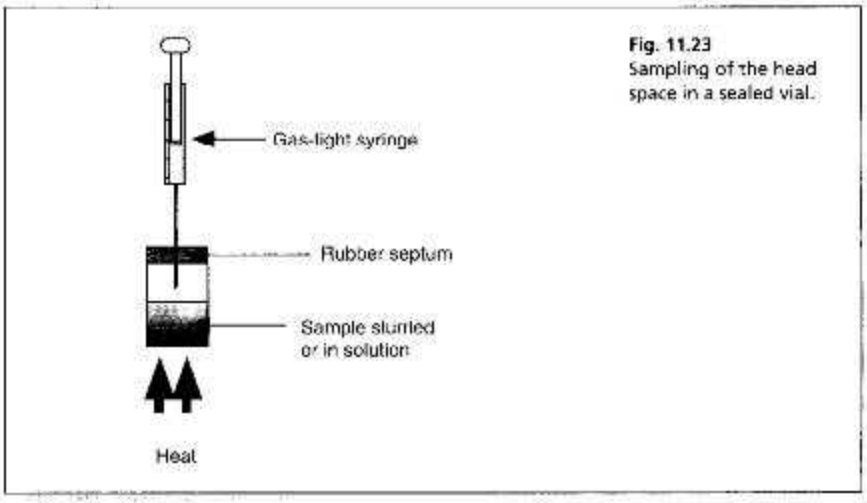


Fig. 11.23 Sampling of the head space in a sealed vial.

The simplest method of sampling is to put the sample into a sealed vial and heat it as shown in Figure 11.23. The sample, either in solution or slurried with a relatively involatile solvent with little potential for interference, e.g. water, is put into a sealed vial fitted with a rubber septum and heated and agitated until equilibrium is achieved. Then a fixed volume of head space, e.g. 1 ml is withdrawn. The sample is then injected into a GC in the usual way. If capillary column GC is used a split injection has to be used to facilitate sample injection: a flow of 10:1 out of the split vent would ensure that a 1 ml sample could be injected in about 5 s with the flow through the column being 1 ml/min. Several points are important to note:

(i) Partition equilibrium must be established by heating for an appropriate length of time and at an appropriate temperature
(ii) A clean room is required away from all other sources of volatiles such as laboratory solvents
(iii) Potential interference from rubber septa must be checked
(iv) Reactive contaminants may react with the sample matrix at high temperatures
(v) If the sample is ground and mixed in preparation for the head space analysis care has to be taken to ensure that no volatiles are lost.

For best reproducibility the process should be automated and for quantitative accuracy it would be best to use the method of standard additions (Ch. 6 p. 123). Suitable columns include packed columns containing Porapak Q or long thick film (3–5 μm) capillary columns, which for best selectivity should be coated with a phase which is moderately polar to polar.

Figure 11.24 shows a chromatographic trace for residual solvents in a sample of cephalosporin obtained by automatic sampling of the head space in a sealed vial.[8] The cephalosporin sample (300 mg) was suspended in diethylene glycol (DEG) (the low vapour pressure of this material meant that it was not present in large amounts in the head space).

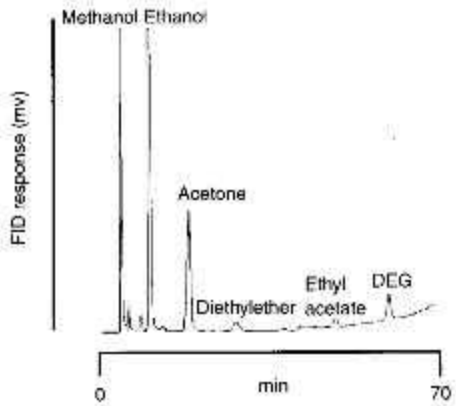


Fig. 11.24
Solvent impurities in cephalosporin analysed by sampling of the head space above a heated DEG solution of the drug. Separation was carried out on column (10 feet × 1/8 in i.d.) packed with Porapak Q. Reproduced with permission from J. Chromatology (see Reference 8).

Self-test 11.4

Which of the following capillary columns would be most suitable for use in the determination of residual solvents by head space analysis (consult Table 11.1):

(i) OV-1 column 12 m × 0.2 mm i.d. × 0.25 μm film
(ii) OV-17 column 15 m × 0.33 mm i.d. × 0.5 μm film
(iii) OV-225 column 30 m × 0.5 mm i.d. × 3 μm film
(iv) OV-1 column 25 m × 0.5 mm i.d. × 1 μm film.

Answer
(iii) OV-225

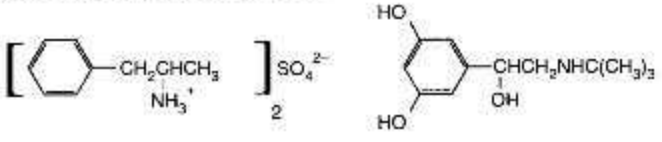
Purge trap analysis

Another form of head space analysis uses a purge trapping device to trap volatile impurities. In this technique a gas, e.g. helium, is bubbled through the sample which is dissolved in suitable solvent (usually water) and the volatile impurities are thus 'stripped' from the solution and passed in the stream of gas through a polymeric adsorbant where they become trapped and thus concentrated. The stream of gas is then switched so it passes in reverse direction through the polymeric trap, which is heated to desorb the trapped volatiles and the gas stream is then diverted into the GC. This type of procedure is used in environmental analysis to concentrate volatiles in water which are present at low levels.

Self-test 11.5

Suggest an analytical procedure based on GC for solving the following problems:

(i) Determination of residual solvents in a penicillin.
(ii) Determination of the stability, in a formulation, of a drug with a propyl ester group.
(iii) Determination of an amphetamine sulphate in tablet form.
(iv) Determination of terbutaline in tablet form.



Amphetamine Terbutaline

Answers: (i) see p. 232; (ii) see p. 239; (iii) see p. 226; (iv) see p. 226

Applications of GC in bioanalysis

In order to determine an optimum dosage regimen for a drug and to determine its mode of metabolism, methods for analysis of the drug and its metabolites in blood, urine and tissues have to be developed. Analysis of drugs in biological fluids and tissues by GC is quite common although GC–MS (see Ch. 9) has replaced many GC methods which are reliant on less selective types of detector.

A typical application of GC to the determination of a drug in plasma is in the determination of the anti-epileptic drug valproic acid[9] after solid phase extraction (see Ch. 15) by GC with flame ionisation detection. In this procedure, caprylic acid, which is isomeric with valproic acid, was used as an internal standard. The limit of detection for the drug was 1 μg/ml of plasma. The trace shown in Figure 11.25 indicates the more extensive interference from background peaks extracted from the biological matrix which occurs in bioanalysis compared to the quality control of bulk materials.

An example of the use of GC with nitrogen selective detection is in the quantification of bupivacaine in plasma.[10] Bupivacaine contains two nitrogen atoms in its structure which makes it a good candidate for this type of analysis. The limits of detection which can be achieved with a nitrogen selective detector for this compound are much better than methods based on flame ionisation detection, which are much less selective.

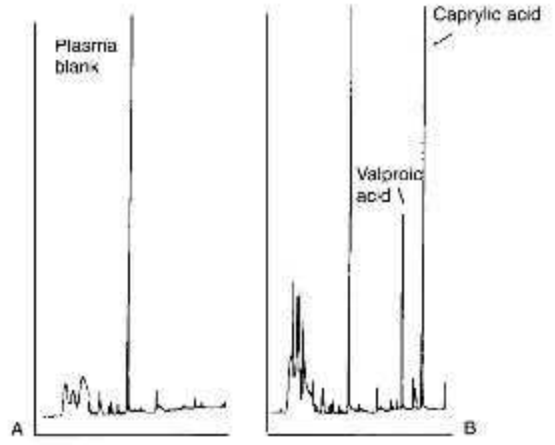
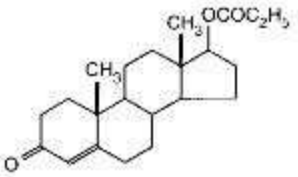


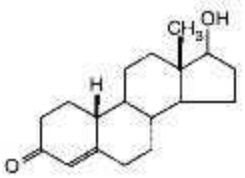
Fig. 11.25
(A) The GC trace of an extract of blank plasma obtained from a patient. (B) The GC trace of an extract of plasma obtained from the same patient after treatment with valproic acid (peak 1) to which caprylic acid (peak 2) has been added as an internal standard.

Additional problems

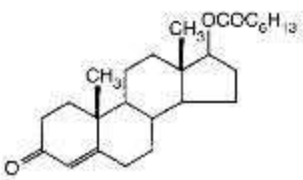
1. Indicate the order of elution of the following compounds from an OV-1 column.



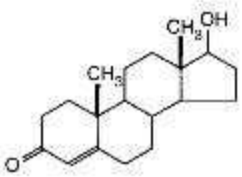
1 Testosterone propionate



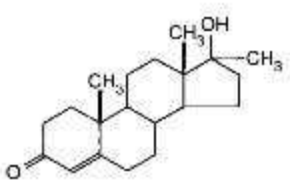
2 Nandrolone



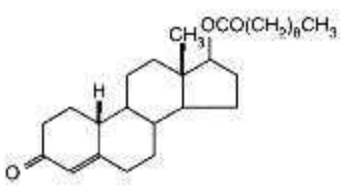
3 Testosterone heptanoate



4 Testosterone



5 Methyltestosterone



6 Nandrolone decanoate

Answers: 2,4,5,1,3,6

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Additional reading

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