Gas chromatography (GC) is applicable to a wide range of compounds of interest to toxicologists, pharmaceutical and industrial chemists, environmentalists and clinicians. If a compound has sufficient volatility for its molecules to be in the gas or vapour phase at or below 400°, and does not decompose at these temperatures, then the compound can probably be analysed by GC.

The separation is performed in a column (containing either a solid or liquid stationary phase) that has a continuous flow of mobile phase passing through it [usually an inert carrier gas, but more recently supercritical fluids (SCFs) have been used for some applications], maintained in a temperature–regulated oven. When a mixture of substances is injected at the inlet, each component partitions between the stationary phase and the gas phase as it is swept towards the detector. Molecules that have greater affinity for the stationary phase spend more time in that phase and consequently take longer to reach the detector. The detector produces a signal proportional to the amount of substance that passes through it, and this signal is processed and fed to an integrator or some other recording device. Each substance that elutes from the column has a characteristic retention time, defined as the time interval from injection to peak detector response. Fig.1 shows a schematic of a GC system.

Figure 1. A modern GC system

Identification of components was traditionally based primarily on peak retention time, but it is becoming increasingly more reliant on the nature of the response obtained from the detector. The analyst has two main goals: firstly, to make each different compound appear in a discrete band or peak with no overlap (or co–elution) with other components in the mixture, and, secondly, to make these bands uniform in shape and as narrow as possible. This is achieved partly by judicious choice of the column stationary phase and its loading, and partly by optimising the operating conditions of the column. In addition, the method of introducing the sample into the chromatograph, the detector choice and chemical modification to improve the volatility of the compounds also contribute to converting a mediocre analysis into a first–class one.

There has been a continuous synergism between enhanced detector performance and column performance; each advance being mutually dependent on the other. Initially, high–sensitivity detectors permitted the development of a precise column theory that, in turn, enabled the design of columns that had much higher efficiencies. This improved efficiency, however, produced peaks of small volume compared to the sensing volume and dispersion that occurred in the collecting tubes of the contemporary detectors. Consequently, efficiency became limited by the geometry of the detector, and not by its intrinsic sensitivity. Detector design was modified to incorporate smaller tube dimensions, and the volume of the sensing cell was thus reduced greatly. The introduction of capillary columns suitable for routine use in GC in the mid–1980s provided much higher resolution efficiencies and smaller peak volumes, which provoked further modifications in detector design. The latest column developments mean that peaks of only a few milliseconds are a real possibility in both GC and liquid chromatography (LC). Since this

matches the response time of the current sensor electronics, the ingenuity of the detector design engineers is again being tested.

Sommaire

# Gas chromatography columns

Packed columns contain a stationary phase (see below), either loaded directly into the column if it is a solid at its operating temperature, or coated onto the surface of a solid support if it is liquid at its operating temperature. Thus, the operating principle of GC can be distinguished as either gas–solid chromatography (mainly an absorptive process by the stationary phase) or gas–liquid chromatography (GLC; mainly partition of the analytes between the mobile and stationary phases), based on the physical characteristics of the stationary phase. Capillary columns, introduced in the early 1980s, have now replaced packed columns for most applications. The original glass columns were fragile and have been superseded by fused silica capillary columns. Fused silica is high–purity synthetic quartz, with a protective coating of polyimide applied to the outer surface. Since these columns retain their flexibility only as long as the coating remains undamaged, their operating temperature must be maintained below 360° for standard columns (400° for high–temperature polyimide coatings). The first capillary columns were 0.2, 0.25 or 0.32 mm internal diameter (i.d.) and between 10 and 50 m long. Subsequently ‘megabore’ (0.53 mm i.d.), ‘minibore’ (0.18 mm i.d.) and ‘microbore’ (0.1 or 0.05 mm i.d.) columns have evolved. When coated with a heat–resistant polymer, these have the advantages of flexibility and strength, and can be threaded with ease through intricate pipe work. A single column can be fitted into almost any manufacturer’s GC. Capillary columns provide improved resolution, sensitivity, durability, less bleed with increasing temperatures, ease of maintenance and repair and yield reliable and highly reproducible separations, typically over many hundreds or thousands of injections. This has reduced the number of columns required to achieve a satisfactory separation of quite complex mixtures.

The internal surface of the silica is deactivated by a variety of processes that can react silanol groups (Si–OH) on the silica surface with a silane reagent (usually a methyl or phenylmethyl surface is created). For gas–solid capillary chromatography, a fine layer (usually less than 10 µm) of stationary phase particles is adhered to the tubing (porous layer open tubular, or PLOT, columns).For gas–liquid capillary chromatography, the stationary phase may be coated or bonded directly on to the walls of the column (wall–coated open tubular, or WCOT), or on to a support (e.g. microcrystals of sodium or barium chloride) bonded to the column wall (support–coated open tubular, or SCOT).Stainless–steel capillaries are usually reserved for applications that require extremes of temperature, or where the possibility of column breakage cannot be tolerated. Nowadays, the internal surface is specially deactivated chemically or by lining with fused silica, which allows for more flexibility and greater durability.

# Solid stationary phases

In gas–solid chromatography, the stationary phase is an active solid at its operating temperature. A conventional packed column is filled completely by stationary phase particles, but in a capillary column a fine layer (usually less than 10 µm) of particles is adhered by proprietary processes to the inner surface of the tubing, to create a PLOT column. These solid phases may be inorganic materials (e.g. aluminium oxides, molecular sieves, silica gel, or graphitised carbon), or they may be organic polymers such as styrene. Both packed and capillary columns use similar solid–phase materials. Sample compounds undergo a dynamic gas–solid adsorption–desorption process with the stationary phase, and since the particles are porous, size exclusion and shape selectivity processes also occur. The carrier gas (mobile phase) merely serves to sweep towards the detector those solute molecules that are not currently adsorbed. The resultant columns are highly retentive, and separations impossible with liquid phases can be accomplished easily on PLOT columns above ambient temperature. These columns are generally reserved for the separation of low molecular weight materials, such as hydrocarbon and sulfur gases, noble and permanent gases, and low boiling solvents. Since PLOT columns occasionally shed particles, their use is not advised with detectors that are affected adversely by the intrusion of particulate matter (the mass spectrometer is particularly vulnerable, as the column interface operates under vacuum).

## Graphitised carbon black

Carbopaks are graphitised carbon black, having adsorptive surfaces of up to 100 $m^2/g$. They are usually modified with a light coating of a polar stationary phase. Difficult separations of the $C_1$ to $C_{10}$ hydrocarbons can be achieved rapidly. Carbopak C with 0.2% Carbowax 20M has been used to resolve substances abused by ‘glue–sniffers’. Carbopak C modified with 0.2% Carbowax 1500 and Carbopak C with 0.8% tetrahydroxyethylene diamine (THEED) are useful for the analysis of ethanol and ethylene glycol, respectively, in blood. Resolution is superior to that obtained with the Porapak and Chromosorb polymers, although the elution order is similar.

## Molecular sieves

Activated alumina is unique for its extremely wide pore–diameter range, and is very useful for separating most $C_1$ to $C_4$ molecules, light hydrocarbon saturates from unsaturates in the $C_1$ to $C_5$ range, and for separating benzene, toluene and xylenes. Deactivation of alumina with potassium hydroxide reverses the elution of some molecules (acetylene and *n*–butane).Carbosieves are granular carbon molecular sieves that give good separation of $C_1$ to $C_3$ hydrocarbons. Carboxen 1006 is useful in resolving formaldehyde, water and methanol, and impurities in ethylene. Zeolites (5A, 13X) give a good general separation of inorganic gases. Carbon dioxide is irreversibly adsorbed below

160°. Oxygen, nitrogen, carbon monoxide and methane are well separated. These columns have a tendency to adsorb water and carbon dioxide, which results in changes in retention over time.

## Polymers

Chromosorb 101–108 and Porapak are divinylbenzene cross–linked polystyrene copolymers. Incorporation of other functional groups, such as acrylonitrile and acrylic esters, into the polymer matrix provides moderately polar to polar surfaces with different pore sizes and surface areas (polarity increases with ascending number or letter). HayeSep phases are polymers of divinylbenzene and ethylene glycol dimethylacrylate. Separations range from free fatty acids to free amines, and small alcohols from methanol to pentanol. Tenax-TA is a porous polymer of 2,6–diphenyl–*p*–phenylene oxide, used both as a chromatographic phase and as a trap for volatile substances prior to analysis. It is also used for high–boiling alcohols, polyethylene glycols (PEGs), phenols, aldehydes, ketones, ethanolamines and chlorinated aromatics.

# Liquid stationary phases

In GLC, the stationary phase is a liquid or gum at the normal operating temperature. Components injected into the column are partitioned between the moving (mobile) gas phase and a stationary phase. Molecules that have greater affinity for the stationary phase spend more time immobilised in the column and consequently take longer to reach the detector. The process of immobilisation and subsequent release back into the mobile phase occurs thousands of times during the course of the analysis. The separation of components is dependent, to a large extent, on the chemical nature of the stationary phase. Stationary phases are essentially two types of high–boiling polymers, siloxanes (often incorrectly called silicones) and PEGs. Chiral stationary phases based on cyclodextrins (cyclic glucose chains) have specific applications in the separation of enantiomers and are discussed separately.

## Polysiloxanes

Standard polysiloxanes (PSXs) are characterised by their repeating siloxane backbone in which each silicone atom has the potential to attach two functional groups, the type and amount of which distinguish the stationary phase and its properties (Fig. 2). The basic PSX is 100% methyl–substituted, so when other groups are present, the amount is indicated as a percentage of the total substituent groups. For example, if 5% of Si atoms contain two phenyl groups and the remaining 95% of Si atoms are methyl–substituted, this may be written as (5% diphenyl/95% dimethyl)-PSX, (5% phenyl/95% methyl)-PSX or simply (5% phenyl)-PSX. In some instances, two

different groups are present on the same silicone atom, so a (10% cyanopropylphenyl/dimethyl)-PSX contains a total of 5% cyanopropyl, 5% phenyl and 90% methyl residues (see Fig.2).

While PSXs are generally less polar in nature than PEGs, the substitution of polar residues for a proportion of the methyl groups confers added polarity to the column. Polar phases retain polar compounds more effectively than do non–polar compounds, and vice versa. The 100% methyl–substituted-PSX is often considered the 'standard' non–polar phase, and has been used extensively in compilations of retention indices. This column is an ideal choice for starting a new application. However, substitution by *n*–octyl groups (up to 50%) renders the column extremely non–polar, and similar to squalene. Substitution with up to 5% phenyl groups still furnishes an essentially non–polar column with improved thermal stability. This phase has also been used for retention index (RI) work and is another good column with which to start a new application. Increasing the phenyl substitution to 20, 35 or 50% yields columns classed as intermediate in polarity, which predictably retain aromatic compounds relative to aliphatic solutes. All these are available as bonded phases that can be solvent rinsed, are not damaged by organic acids or bases, and can tolerate small injections of water if sufficiently highly loaded, but are sensitive to strong inorganic acids and bases.

Substitution of cyanopropylphenyl groups (typically 6% or 14%) creates an intermediate polarity mixed phase with unique elution relative to simple phenyl substitution, but renders the column more susceptible to damage from oxygen, moisture and mineral acids. 50% substitution is specifically designed to separate *cis*– and *trans*– fatty acid methyl esters (FAMEs). However, even low–level bleeding of the stationary phase produces a high background signal with certain types of detector (nitrogen–phosphorus detector). More polar columns are produced by substitution of bis–cyanopropyl and cyanopropylphenyl groups (80:20 or 90:10). 100% biscyanopropyl substitution gives the highest polarity of the PSXs. This phase can be operated at both high and low temperatures. To date, these are non–bonded columns, and should not therefore be rinsed with solvent. Trifluoropropyl/methyl-PSX is a mid–to–high polarity phase especially suited for otherwise difficult–to–separate positional isomers. Its unique interactions with nitro, halogen, carbonyl and other electronegative groups give it application in the analysis of herbicides and pesticides.

Increasing polarity in general is associated with some negative effects. Polar phases tend to have a narrower operating temperature range (higher minimum, lower maximum), are more prone to bleed at higher temperatures, more sensitive to moisture and oxygen, and consequently have a shorter life expectancy than non–polar phases. More recently, low–bleed arylene stationary phases (sometimes designated mass spectrometry or 'ms') have been introduced that have phenyl groups incorporated into the siloxane backbone (see Fig. 2). The incorporated phenyl groups confer additional strength to the backbone, which prevents the formation of cyclic fragments and associated 'bleed' at higher temperatures.

## Polyethylene glycols

PEGs are widely used polar stationary phases and their general structure is in Fig. 2. They are less stable, less robust (especially to oxidative damage), have lower temperature limits and a shorter life expectancy than polar PSX phases, but they have unique resolving qualities. Acid–modified PEG (FFAP) substituted with terephthalic acid is especially useful to separate acidic polar compounds, such as acids, alcohols, acrylates, ketones and nitriles. Nitroterephthalic acid–substituted PEG (e.g. Nukol) is designed for volatile fatty acids and phenols. Both are highly resistant to damage from water–based samples. Base–modified PEG (CAM) is suited to analysis of strongly basic compounds, such as primary amines, that do not chromatograph well on polar PSXs. Since this phase is usually cross–linked, it cannot be used with water or alcohol, but it can be solvent rinsed. New bonded and cross–linked PEG phases are now available to separate free fatty acids and other organic acids. These show superior inertness and can tolerate repeated injections of water, alcohols, aldehydes and acids without the need for acidification treatment.

## Chiral phases

Second–generation chiral phases are based on cyclodextrins (toroidal shaped structures formed by $\alpha_{1\text{–}4}$ linkages of multiple glucose units). The enzyme cyclodextrin glucosyl transferase is used to cleave partially digested starch, and link the glucose units into three forms, referred to as $\alpha$, $\beta$, and $\gamma$, that have six, seven and eight glucose units, respectively. The mouth of the cyclodextrin molecule has a larger circumference than the base and is linked to secondary hydroxyl groups of the C2 and C3 atoms of each glucose unit. The primary hydroxyl groups are located at the base of the torus, on the C6 atoms. The number of glucose units thus determines the cavity size and electrophilic orientation (see Fig. 2), and affects the order of the enantiomeric forms. The hydroxyl groups can be functionalised selectively to provide various physical properties and inclusion selectivities. Six different cyclodextrin derivatives are manufactured: permethylated hydroxypropyl (PH), dialkylated (DA), trifluoroacetylated (TA), propionylated (PN), butyrylated (BP) and permethylated (PM). Changes in elution order can be seen between the different derivatives, and also between cyclodextrin cavity sizes. Unlike the cyclodextrins used in high performance liquid chromatography (HPLC), these phases separate both aromatic and non–aromatic enantiomers of a wide range of chemicals, including saturated alcohols, amines, carboxylic acids, epoxides, diols, lactones, amino alcohols, amino acids, esters, pyrans and furans. Each phase has an area of specificity, as summarised in Table .1. Derivatised cyclodextrins are thermally stable, highly crystalline and virtually insoluble in most organic solvents. Chiral phases are fragile, however, and unless chemically bonded or cross–linked, they cannot be washed with solvent or taken to temperatures outside the 0 to 225° range.

A subsequent development has been the embedding of PM cyclodextrins (usually 10 or 20% by volume) into columns that contain standard liquid stationary phases of intermediate polarity, such as 35% phenyl-PSX. Silyl–substituted cyclodextrins, such as 2,3–di-*O*-methyl–6-*O*–*t*–butyldimethylsilyl are also available embedded

(usually 25 to 35% by weight) in 20% phenyl-PSX, another intermediate polarity stationary phase. These columns are useful to separate positional isomers (phenols, xylenes, etc.), as well as enantiomers.

# Solute–stationary phase interactions

For liquid stationary phases, three major types of interaction determine chromatographic elution: dispersion, dipole and hydrogen bonding. Table.2 shows the contribution of each of these interactions for the common types of liquid stationary phases.

*Dispersion* is the dominant interaction for all PSX and PEG stationary phases; it can be simplified into the concept that the more volatile the compound (the lower its boiling point), the more likely it is to be in the mobile phase and so the faster it elutes from the column. Although this holds true for groups of compounds with similar functional groups or within homologous series, it cannot be applied universally. In general, a difference of 30° in boiling point is sufficient to predict and maintain elution order, but differences of less than 10° can be overturned by the influence of other interactions.

*Dipole* interactions of PEG phases and the cyanopropyl- and trifluoropropyl–substituted PSXs enable these phases to separate solute molecules that have different dipole moments. Such solutes are those with positional isomers of electronegative groups, such as pesticides, halocarbons and many pharmaceuticals.

Moderate *hydrogen bonding* is exhibited by PEGs and cyanopropyl–substituted PSXs, with less marked effects shown by phenyl- and trifluropropyl–substituted PSXs. Functional groups that exhibit strong hydrogen bonding include alcohols, carboxylic acids and amines; aldehydes, esters and ketones generally have less effect; hydrocarbons, halocarbons and ethers produce negligible hydrogen bonding. Although the amount of separation obtained through dipole interactions or through hydrogen bonding can be difficult to predict, resolution of compounds with smaller differences in dipoles or in hydrogen bonding strengths requires larger percentages of siloxane substitution.

# McReynolds constants

The retention behaviour of five probe compounds (benzene, butanol, pentan–2–one, nitropropane and pyridine) has been used traditionally to classify stationary phases in terms of their polarity (McReynolds 1970). The retention indices of each of these five reference compounds are measured on the stationary phase being tested, and then compared to those obtained under the same conditions on squalene (a standard non–polar phase). The differences

in the retention indices between the two phases (Δ*I*) for the five probe compounds are added together to give a constant, known as the *McReynolds constant*, which is used to compare the ability of stationary phases to separate different classes of compounds. Phases that provide McReynolds values of ±4 can be substituted freely for each other; those differing by ±10 units generally yield similar separations. Phases with McReynolds values below 100 are considered non–polar, those above 400 indicate a highly polar phase and values between 100 and 400 an intermediate polarity. Table .3 shows the McReynolds constants, operating temperature range, the relationship between capillary and packed column nomenclature and example applications for the most popular stationary phases. ΔIs for individual probes indicate the deviation from boiling point order and consequently represent the contribution of forces other than dispersion to elution for that probe. The probes are chosen to represent different functional groups as follows:

- Benzene for aromatics and olefins (pi–type interactions).
- Butan–1–ol for alcohols, nitriles, carboxylic acids and diols (electron–attracting effect).
- Pentan–2–one for ketones, ethers, aldehydes, esters, epoxides and dimethylamino derivatives (dipole–dipole effect).
- Nitropropane for nitro and nitrile derivatives (electron–donating effect).
- Pyridine for bases (non–bonding electron attraction and hydrogen–bonding effects).

| **Capillary phase**[a] | **Packed equivalent** | **Temp range (min/max)** | **McReynolds values**[b] | | | | | **ΣΔI** | **Applications** |
|---|---|---|---|---|---|---|---|---|---|
| | | | **x´** | **y´** | **z´** | **u´** | **s´** | | |
| SPB-Octyl | Squalene, Apiezon L | –60/300 | 3 | 14 | 11 | 12 | 11 | 51 | Separates by boiling point, polychlorinated biphenyls (PCBs) |
| *-1 | SE30, OV-1, OV-101, SP2100 | –60/320 | 4 | 58 | 43 | 56 | 38 | 199 | Amines, hydrocarbons, pesticides, PCBs, phenols, sulfur compounds, flavours, fragrances |
| *-5 | SE-52, OV-73 | –60/320 | 19 | 74 | 64 | 93 | 62 | 312 | Alkaloids, drugs, FAMEs, halogenated compounds, aromatic compounds |
| *-1301 | SE-54, CP-624 | –20/280 | 69 | 113 | 111 | 171 | 128 | 592 | Aroclors, alcohols, phenols, volatile organic acids |
| *-35 | OV-11 | 0/300 | 101 | 146 | 151 | 219 | 202 | 728 | Aroclors, amines, pesticides, drugs |

| Capillary phase[a] | Packed equivalent | Temp range (min/max) | McReynolds values[b] | | | | | ΣΔI | Applications |
|---|---|---|---|---|---|---|---|---|---|
| | | | x′ | y′ | z′ | u′ | s′ | | |
| *-1701 | OV-1701 | 10/280 | 67 | 170 | 153 | 228 | 171 | 789 | Aroclors, herbicides, pesticides, trimethylsilyl (TMS) sugars |
| *-50, *-17 | OV-17, SP-2250 | 30/310 | 125 | 175 | 183 | 268 | 220 | 971 | Drugs, glycols, pesticides, steroids |
| *-210 | OV-25 | –45/250 | 178 | 204 | 208 | 305 | 280 | 1175 | Aldehydes, ketones, organochlorines, organophosphates |
| *-225 | XE-60, OV-210 | 40/230 | 146 | 238 | 358 | 468 | 310 | 1520 | FAMEs, alditol acetates, neutral sterols |
| *-23 | OV-225 | 40/250 | 228 | 369 | 338 | 492 | 386 | 1813 | *cis–trans*FAMEs, stereoisomers |
| *-wax, *-20M | Carbowax 20M TPA | 35/280 | 305 | 551 | 360 | 562 | 484 | 2262 | Alcohols, free acids, essential oils, ethers, glycols, solvents, primary amines |
| *-FFAP | Carbowax–1500 PEG | 50/250 | 340 | 580 | 397 | 602 | 627 | 2546 | Acids, alcohols, aldehydes, acrylates, nitriles |
| Nukol | SP-1000, OV-351 | 60/200 | 314 | 569 | 372 | 578 | 504 | 2337 | Alcohols, free acids, essential oils, ethers, glycols, solvents |
| *-2330 | SP-2330 | 10/250 | 382 | 610 | 506 | 710 | 591 | 2799 | *cis–trans*FAMEs, positional isomers |
| *-2380 | – | 10/275 | 402 | 629 | 520 | 744 | 623 | 2918 | *cis–trans*FAMEs, positional isomers, alditol acetates |
| *-2340 | SP-2340, OV-275 | 25/250 | 419 | 654 | 541 | 758 | 637 | 3009 | *cis–trans*FAMEs, positional isomers |
| – | DEGS | 20/200 | 496 | 746 | 590 | 837 | 835 | 3504 | Acids, esters, phenols, terpenoids |
| – | EGS | 100/200 | 537 | 787 | 643 | 903 | 889 | 3759 | TMS or methyl sugars, acidic drugs |

| Capillary phase[a] | Packed equivalent | Temp range (min/max) | McReynolds values[b] | | | | | ΣΔI | Applications |
|---|---|---|---|---|---|---|---|---|---|
| | | | x′ | y′ | z′ | u′ | s′ | | |
| TCEP | TCEP | 10/145 | 594 | 857 | 759 | 1031 | 917 | 4158 | Flavours, fragrances, essential oils |
| α-Cyclodextrin in 35% phenyl-PSX | 30/240 | 102 | 243 | 142 | 221 | 170 | 878 | Enantiomers and isomers (see Table .1) | |
| β-Cyclodextrin in 35% phenyl-PSX | 30/240 | 119 | 264 | 154 | 134 | 187 | 858 | Enantiomers and isomers (see Table .1) | |

Table .3. Polarity (McReynolds values) of some common stationary phases, and example applications

[a] * is the proprietary prefix for the phase, for example; * = HP supplied by Hewlett Packard/Agilent; * = DB supplied by J&W; * = CPSil supplied by Chrompack; * = RT supplied by Resteck; * = SP supplied by Supelco; * = OV supplied by Ohio Valley. This list is not intended to be exhaustive. [b] x′ = benzene; y′ = butan–1–ol; z′ = pentan–2–one; u′ = 1–nitropropane; s′ = pyridine.

Moffat et al. (1974a) devised a system to assess the effectiveness of liquid stationary phases by calculating the discriminating power, and examined a number of phases commonly used in toxicology (Moffat et al. 1974b). Contrary to popular belief, it was shown that one column could be used to elute all the drugs studied, and that for screening purposes a single column, either SE-30 or OV-17 (100% dimethyl-PSX or 5% phenyl-PSX capillary equivalents), was sufficient for the reliable identification of drugs.

Packed and capillary columns utilise similar stationary phases, although the method of introducing the phase into the column differs. In a capillary column, the internal surface of the silica is deactivated by a variety of processes that can react silanol groups (Si–OH) on the silica surface with a silane reagent (usually a methyl or phenylmethyl surface is created for most types of phases). The stationary phase is then bonded covalently, usually onto the internal wall of the column (WCOT), or less frequently onto a support (e.g. microcrystals of sodium or barium chloride) that is itself bonded to the internal surface of the column wall (SCOT). Cross–linked stationary phases have the individual polymer chains linked via covalent bonds. Both bonding and cross–linking impart enhanced thermal and solvent stability to the stationary phase (often designated ‘DB’), and should be used if they are available. For conventional packed column chromatography, however, the stationary phase must first be coated onto the surface of a solid support.

# Solid supports for packed columns

The raw material for the most commonly used supports is diatomaceous earth, calcined, usually with a flux, then crushed and graded into a number of particle sizes (60 to 80, 80 to 100 and 100 to 120 mesh). Chromosorb G has a surface area of about 0.5 $m^2/g$, and is suitable for low–loaded columns – the amount of stationary phase should not exceed 5% (w/w). Chromosorb W has a larger surface area (1 $m^2/g$) and accepts higher loadings, but is more fragile. Chromosorb P, obtained from crushed firebrick, accepts loadings up to 35% (w/w) for some phases. Supelcoport is the most inert of the diatomite supports, and can accept 20% (w/w) loadings. Carbopack, Porapak, HayeSep and Tenax-TA (see above) can also be used as solid supports. Table.4 shows the maximum suggested coating percentages for the most common solid supports.

Deactivated support materials are nearly always preferred. Deactivation procedures include acid or base washing to remove impurities and fines, and treatment with a silanising agent that reacts with surface hydroxyl groups to reduce adsorptive effects. Support materials that have the liquid phase chemically bonded to them are available. These offer decreased bleed rates of stationary phase, an advantage when operating a temperature programme or when using a mass spectrometer as the detector.

# Installing, conditioning and maintaining columns

# Column installation

Glass columns must have straight smooth ends to allow them to fit into unions at the injector and detector using either graphite or Vespel (polyimide) ferrules inside the nuts. Fused silica capillaries should also have their ends freshly cut after insertion through ferrules, to eliminate blockages. The injector end of the column should be fitted first, adjusting the height of the protrusion above the ferrule according to the type of inlet being used, then tightening the fittings just enough to prevent leakage when tested with a proprietary leak–testing fluid (not soap solution. which leaves a residue). The detector end of a packed column can be attached to a bubble–flow meter to ensure adequate flow through the column before connecting the detector end. The detector end of the capillary column may be immersed into a small tube of methanol to ensure adequate flow, the capillary end re–cut, then attached to the detector and checked for leaks. The detector is activated and the column tested at room temperature with an injection of 1 or 2 µL of methane, when a needle–sharp peak should be obtained. When the carrier-gas pressure has been adjusted to give a flow of approximately 20 mL/min of carrier gas for a packed column or 1 to 2 mL/min for a capillary, the column may be heated and a test mixture injected. Commercial

columns are invariably supplied with a chromatogram obtained from a test mixture, and it should be possible to obtain a performance equal to or better than the supplied chromatogram. Various test mixtures are used, including a mixture of dimethylphenol and dimethylaniline with straight–chain paraffins. Any acidity or alkalinity of the column is apparent by loss of the peak shape of the amine or phenol. The efficiency obtained is a function of the entire chromatographic system. Poor efficiency or peak shape often results from a non–swept volume somewhere in the system.

It may be necessary to add an additional gas supply to the column outlet to ensure that the detector is purged effectively, because most detectors are designed to operate with packed columns and a flow rate of about 30 mL/min, as opposed to the 1 or 2 mL/min delivered by a capillary column (see Dectectors below).

## Column conditioning

A new column requires conditioning before use to remove volatile impurities that remain after deactivation of the support, and/or the coating and packing processes. The column should be installed in the injector port only, with the detector end disconnected. With the column at room temperature, a low carrier gas pressure (14 to 35 kPa) should be maintained for half an hour to purge oxygen from the system. The temperature may then be raised by about 1°/min, until about 10° above the desired maximum operating temperature has been reached, and the column is maintained (2 h for a manufactured capillary, 12 h for a packed column or self–packed capillary). Care must be taken not to exceed the maximum operating temperature. After conditioning, the column is connected to the detector, and a period of further conditioning instigated if the background signal is excessive. Some phases (e.g. OV-17) are particularly oxygen–sensitive and can be ruined by careless conditioning. A constrictor fitted to the detector end of the column helps prevent back diffusion of oxygen if air or oxygen is supplied to the detector.

## Guard columns and retention gaps

A guard column and a retention gap are essentially the same thing, but are installed to serve different purposes. These 1 to 10 m lengths of fused silica tubing are attached to the front of the chromatography column via a press–snap connector or zero dead–volume union, and then installed into the injector port. The surface of the silica is deactivated to minimise solute interactions, but no stationary phase is added. The diameter tubing should be the same as the column, but if different it should ideally be of a wider bore. The function of a guard column is to trap deposits of non–volatile residues preventing their contamination of the analytical column. Solutes are not retained by the guard column (since there is no stationary phase) and pass directly onto the column. Portions can be cut periodically from the top of the guard column as deterioration in chromatography requires, without any appreciable

loss of resolution from the analytical column. A retention gap is used to improve peak shape when poor chromatography is the result of either a large injection volume (>2 µL), or there are solvent–stationary phase polarity mismatches. Greatest improvement is seen in early eluting peaks, or for solutes with similar polarity to that of the solvent.

## Maximum operating temperatures

Maximum operating temperatures for stationary phases are usually quoted assuming isothermal operation with a flame ionisation detector (see Table .3). Other detectors may impose different limits, the mass spectrometer being much more susceptible to bleeding of the stationary phase than the thermal conductivity detector. All phases bleed very slightly at high temperatures because of the loss of smaller sized (and hence lower boiling) polymer chains, although normally this is not noticeable. Operating temperature has a profound effect on column life, particularly for capillary columns. Loss of stationary phase, or breakdown of the thin film into pools to expose part of the tubing surface, results in serious loss of performance. Additionally, in columns that contain PSX phases with two different functional groups, one group (usually that which confers additional polarity) is preferentially lost. This results in a change of relative separation (or in RI– see discussion below) as well as a loss of resolution. The temperature limit of a column may be determined by the deactivation procedure used in production, rather than by the stationary phase itself. Newer silica columns have a very low metal oxide content, thought to act as a catalyst for the degradation of both sample and stationary phase, and thus enable phases to be run at higher temperatures. Fused silica capillary columns have a protective external coating of polyimide that is slowly degraded at elevated temperatures (maximum temperature 360°, but now to 400°), which can also limit column life. However, separations are usually achieved at much lower temperatures.

## Temperature programming

For complex mixtures with components of widely varying retention characteristics, it is often impractical to choose a column temperature that allows all the components to be resolved. Increasing the column temperature throughout the analysis dramatically reduces the time taken for higher boiling compounds to elute, and simultaneously improves the sensitivity of the assay, as the peaks are remarkably sharper. If the early eluting compounds are resolved inadequately, a lower starting temperature or slower initial ramp should be used, taking care to observe the temperature requirements of the type of injector used. All instruments currently manufactured are available with a temperature programme option, and a multi–ramp programmer is particularly useful for capillary chromatography. The first ramp can be used during splitless injection (see later) to bring the column rapidly up to the initial chromatography temperature, followed by a slower analytical ramp to perform the

separation. One problem with temperature programming is that the backpressure increases with temperature and reduces the carrier gas flow if a mass–flow controller is not used. For polar stationary phases, the polarity increases with temperature, which causes distortion of RI data (see discussion on the use of RIs below). Column bleed also increases, which results in an increasing baseline. For this reason the column should be well conditioned before use.

# Evaluating column performance

Column performance, whether capillary or packed, is made on the basis of its efficiency (the narrowness of a peak), the peak shape (whether it tails or fronts) and its ability to resolve compounds. This section deals with separation theory, and the reader may find it useful to refer at intervals to Fig.3.

# Retention time

Retention time ($t_R$) is the time taken for a given solute to travel through the column, and is the time assigned to the corresponding peak on the chromatograph. It is a measure of the amount of time the solute spends in the column, and is therefore the sum of time spent in both the stationary and the mobile phases.

## Retention time of a non–retained compound or hold–up time ($t_M$ or t)

The retention time $t_M$ is the time taken for a non–retained solute to travel along the column; it represents the transit time for the mobile phase (carrier gas) in the column and is a column–specific parameter, applicable only under the prevailing conditions of gas flow and oven temperature. It is the same for all solutes on the column, and no other peak can be expected to elute earlier than this time. $t_M$ is obtained by injecting a non–retained compound suitable for the detector system being used [butane or methane for flame ionisation detection (FID) or thermal conductivity detection (TCD), acetonitrile for nitrogen–phosphorus detection (NPD), methylene chloride for electron–capture detection (ECD), vinylchloride for photoionisation detection (PID) or electrolytic conductivity detection (ELCD)].

# Average linear velocity

The average linear velocity ($\bar{\mu}$) represents the average speed of carrier gas through the column, usually expressed in cm/s, and is considered more meaningful than measuring the flow (usually expressed in mL/min) at the column effluent, since flow is dependent on column diameter. This term directly influences solute retention times and column efficiency. Velocity is controlled by altering the column head pressure, and is calculated from the equation

where $L$ is the column length (cm), and $t_M$ is the retention time (s) of a non–retained solute.

# Retention factor

Retention factor ($k$) is the ratio of the amount of time a solute spends in the stationary and mobile phases and is calculated from $t_R$ and $t_M$ using the equation:

where $t_M$ is the retention time of a non–retained solute, $t_R$ is the retention time of the solute and $t'_R$ is the adjusted retention time of the solute.

Since all compounds spend an identical time in the mobile phase, k is a measure of retention by the stationary phase. A compound with a retention factor of 4 spends twice as much time in the stationary phase (but not twice as much time on the column) as a compound with a retention factor of 2. Thus, k provides relative rather than absolute information, and is to a large degree independent of the operating conditions.

# Separation factor

The separation factor ($\alpha$) is a measure of the time interval between two peaks. If $\alpha$ equals 1, then the peaks have the same retention time and co–elute. Separation factor is calculated using the equation:

where $k_1$ is the retention factor of the first peak, and $k_2$ the retention factor for the second peak.

The value of $\alpha$, however, does not indicate whether the peaks are resolved completely from one another. Two peaks may have only 0.01 min between them on one column, but still be resolved completely, while on another column they may have 0.1 min between them, but not be resolved adequately (refer to> Fig. 3).

# Number of theoretical plates or column efficiency

The theoretical plate is an indirect measure of peak width at a specific retention time. Higher plate numbers indicate greater column efficiency and narrower peaks. The number of plates per metre of column ($N$) is calculated from either form of Equation (28.4):

$N = 16(t_R/w_b)^2$(28.4a)

$N = 5.54(t_R/w_h)^2$(28.4b)

where $t_R$ is the time from injection to peak maximum for the solute, $w_b$ is the peak width at base in units of time and $w_h$ is the peak width at half height in units of time.

Efficiency is a function of the column dimensions (diameter, length, film thickness or loading), the type of carrier gas and its flow, as well as the chemical nature of the solute and the stationary phase.

# Separation (Trennzahl) number

Trennzahl formulated this measure of how many resolved peaks can be accommodated between adjacent members of a homologous series. The separation number for A and B, two members of a homologous series that differ by one methylene group, is given by:

where $\Delta t_R$ is the difference in retention time between A and B, and $w_{h(A)}$ and $w_{h(B)}$ are their peak widths at half height, respectively.

If $TZ = 0$, no other peak will fit between the two homologues; if $TZ = 1$, one peak will fit. For most applications in drug analysis the chromatogram only contains two or three compounds, but an efficient column maximises the probability that a peak consists of only one compound and that it is the compound of interest.

# Peak shape or asymmetry

A well–designed system should give symmetrical peaks, as tailing or fronting adversely affects resolution. Tailing may result from non–swept volume in the system or from component–stationary phase or component–support interactions. Tailing of polar compounds can often be remedied by the use of a more polar stationary phase. Fronting (shark's fin peaks) is usually caused by overloading, particularly with capillary columns, and can be resolved either by making a smaller injection or by using a column with a higher stationary phase ratio. Column capacity is the maximum amount of a solute that can be chromatographed successfully without loss of peak shape. Table .5 shows the relationship between column capacity, film thickness and column diameter. Peak fronting caused by thermal decomposition can be reduced by either lowering the injection temperature or using a cold on–column injector system.

Peak shape is usually expressed by the peak asymmetry ($A_s$). In Fig. 3, the peak asymmetry factor for substance B is given by:

where a vertical line is drawn through the peak maximum and XYZ is drawn at 10% of the peak height. A symmetrical peak has $A_s = 1$.

# Use of retention indices

If gas chromatographic retention data are to be exchanged between laboratories, they must be independent of the instrument used. The concept of RI has been shown to be more reliable than that of relative retention time (i.e. the retention time of the solute relative to that of a reference compound). The RI system uses a homologous series of compounds (i.e. a series of compounds that increase in size by an additional methylene unit) to provide the reference points on the scale. The most commonly used is the system described by Kovats (1961) using straight–chain saturated hydrocarbons (n–paraffins or *n*–alkanes). For any column temperature and stationary phase, the elution times of members of a series of *n*–alkane homologues are assumed to increase by an index of 100 for each additional methylene unit. On this scale, $H_2$ has an index of zero, methane has an index of 100,

ethane 200, and so on up the scale of alkanes. The RIs of unknown substances are measured against this scale, obviating the need to correct data between laboratories because of variations in retention time. The method is illustrated in Fig.4, in which phenobarbital has a retention time of 4.5 min and a RI of 1957.

where $t_R$ is the retention time, $P_z$ is the carbon number of the smaller *n*–alkane, $P_{z+n}$ is the carbon number of the larger *n*–alkane and *x* is the unknown solute.

Retention indices collected from many sources show remarkable agreement, even when measurements were made on different (though equivalent) phases and at different temperatures (Ardrey and Moffatt 1981).

Most capillary GC is performed in temperature–programmed mode and the relationship between the retention time and chain length is almost linear, provided the ramp rate is constant throughout the run and $n > 7$. The simplified Equation (28.7b) is used to calculate RI (see Fig. 4 for an illustration).

Other homologous series have been proposed and good results were found with the alkan–2–ones and *n*–aldehydes. Use of other series (*n*–alkylesters, *n*–alkalylbenzenes, *n*–alkyl iodides) has been less successful. When using a specific detector to identify compounds, the accuracy is increased by splitting a small percentage of the column effluent to an FID to give a 'real time' calculation of RI, rather than relying on either historical retention times for *n*–alkanes or to use an alternative homologous series. Alternatively, Franke et al. (1993) have proposed the use of a RI reference mix (a selected group of compounds that structurally resemble those under investigation) rather than an homologous series. Retention indices for unknown compounds can then be normalised relative to the known RI values of the reference compounds. The main advantage of this approach is that both temperature–dependent and column–ageing effects on RI, which arise from polarity mismatch between the homologous series and the investigated solutes, are somewhat reduced and the data are therefore more reproducible over time.

As would be expected, there is better agreement for non–polar (most hydrocarbon–like) compounds than for polar compounds, and also for non–polar versus polar stationary phases where there is less difference in retention time between the *n*–alkane index markers. Using capillary columns, reproducibility can be as good as to within one RI for a non–polar phase, and to within a few units for a more polar one.

Good temperature, pressure and flow control, and precise measurement of the injection time and peak elution, are essential for accurate measurement of RIs. The carrier-gas flow rate and the polarity of the stationary phase are temperature dependent. Thus, the partitioning of polar compounds into the stationary phase is affected by temperature to a greater extent than that for the *n*–alkanes. Differences between constant flow and constant

pressure modes of operation are exaggerated when the column is ramped in several stages. It is common practice to have an initial fast rate of increase in temperature followed by one or more slower ramp rates, since the number of low–boiling compounds of interest is usually lower than the number of higher boiling compounds. Inaccuracies in RI calculation can also arise with high solute concentrations because of problems identifying the crest of the peak. This can also result in a trace component that elutes on the back of the concentrated one, as the major component begins to take part in the separation process by acting as a 'dynamic stationary phase'. This delays the elution of the trace component.

Column deterioration with use can lead to a preferential destruction or loss of the more labile component of a mixed phase. For example, in columns that contain mixed cyanopropyl- and methyl-PSX phases, the cyanopropyl group is preferentially lost so that the column polarity is reduced. The elution of the index markers (*n*–alkanes) remains unchanged, but the progressive loss of cyanopropyl substituents results in a poorer interaction with polar compounds and an apparent *decrease* in their RI. With single–component stationary phases, the effect is still present, though less noticeable, as there is loss of retention of both RI markers and polar compounds.

# Inlet systems

The inlet system provides the means of introducing the specimen into the GC. Obtaining a narrow sample band at the start of the chromatographic process is critical to achieve good resolution, since broad sample bands usually produce broad peaks, especially for analytes that elute early. The choice of injector depends on the characteristics of the specimen or residue, the quantity and characteristics of the analytes to be separated, the temperature and nature of the stationary phase and the column. Solids may be dissolved in a suitable solvent and injected with a micro–syringe. It is best to keep the solution as concentrated as possible to reduce the size of the solvent peak. Liquids can be injected using a micro–syringe, but with sensitive detection systems the sample should be dissolved in a suitable solvent to reduce the sample size and avoid overloading the detector. Gases and vapours may be introduced by injection through the inlet port septum using a gas–tight syringe.

The four main types of GC injectors are Megabore direct (or packed column), split, splitless and cold on–column. In reality, splitless is an extreme example of split injection and both are carried out using the same hardware. Conventional glass syringes of 1 to 10 µL volume with stainless steel needles can be used on the packed column, or vaporisation capillary injectors, and the injection is made by piercing a silicone rubber septum. Care must be taken to select septa that have low bleed characteristics at the operating temperature, and those with Teflon backs are most reliable in this respect. Unstable materials can be decomposed by the high temperature of the injection system, particularly if the system is constructed of metal. For labile substances cold on–column injection is preferred, but clean extracts must be used to minimise column contamination.

# Megabore direct (or packed column) injection

The packed column is usually inserted such that the top butts directly onto the septum or septum plate, and is housed in a heated port. The sample is injected directly on to the top of the column, and the carrier gas (typically 10 to 40 mL/min) sweeps the compounds directly along the column. A deactivated glass–wool plug at the top of the column serves as a filter to retain non–volatile co–injected material, and must be replaced regularly to prevent turbulence or blockage of the carrier gas flow. Packed column inlets (6.3 mm diameter) can usually be modified by insertion of a reducing fitting and glass liner to take Megabore (0.53 or 0.45 mm i.d.) columns, but the high flow requirements to sweep the compounds onto the column in a narrow band (4 mL/min minimum) preclude the use of narrower capillaries. One type of liner usually has a restriction at the top to prevent backflush and septum contamination, to which the top of the column is abutted to reduce interaction of the sample with the steel surface of the injection port. The injection is made directly onto a capillary column (sometimes referred to as hot on–column injection), and it is essential to maintain clean specimens to avoid poor chromatographic performance. The installation of a retention gap (see below) may be useful in this situation. This type of injection is ideally suited to high–boiling compounds, and minimises degradation of thermally labile compounds. An alternative type of liner (flash vaporisation) avoids direct injection onto the capillary by having a second restriction about half way down, to which the top of the column is abutted. This creates a vaporisation chamber above the column into which the injection is made. Chromatography is generally more efficient, since the second taper acts as a concentrating zone for the solutes, and produces a narrow solvent front, which enables analysis of highly volatile compounds. Glass wool should not be inserted into these liners. The injector temperature is usually held about 50° above the boiling point of the solvent, with the initial column temperature (if a temperature programme is available) some 10° below the boiling point. Usually, experimentation is needed to balance the injector temperature with the column temperature and carrier gas flow to obtain the most efficient chromatography. This is the simplest capillary injector available and is compatible with most samples. The highest concentration is usually limited by the column capacity, and the smallest amount by the sensitivity of the detector.

# Split and splitless injectors

Split injectors are used for more concentrated samples, since only a fraction of the sample actually enters the column. An inlet splitter allows a high flow of carrier gas through the injector while maintaining a low flow (1 to 4 mL/min) through the column: the excess gas and associated sample components are vented to the atmosphere through the split line. The ratio of these two flows (the split ratio) controls the proportion of the injected sample that reaches the column. The total flow through the injector may be from 10 to 100 mL/min, which gives split ratios of 10:1 to 100:1. A good splitter should be linear, that is it should split high- and low–boiling point compounds equally. The function of the splitter is not primarily to reduce

sample volume, but rather to ensure that the sample enters the column as a compact plug. Split injections, therefore, produce some of the most efficient chromatographic separations, and allow the use of very narrow capillary columns. A lower split ratio channels a larger fraction of the injected sample down the column and may result in column overload. High split ratios waste large amounts of carrier gas and insufficient analyte may reach the column.
In splitless injection, all the carrier gas passes to the column. This is useful for very volatile compounds, for low sample concentrations or for trace analysis. The flow rate in the injector is the same as that in the column (1 to 4 mL/min), and the only path for the injection to take is into the column, since the split vent is closed. At a fixed time after injection (usually 15 to 60 s), the injector is purged by opening the split vent to introduce a much larger flow of carrier gas through the injector (typically 20 to 60 mL/min) and any remaining sample in the injector is discarded through the split vent. Since the rate of sample transfer onto the column is so slow (because of the low gas flow), peaks are usually somewhat broader than for split injections. Temperature conditions can be adjusted to narrow or focus the sample band at the top of the column. Splitless injections should therefore be made with the initial column temperature at least 10° below the boiling point of the solvent (see Table 28.6), and the initial temperature should be held at least until after the purge activation time. Solvent condenses on the front of the column and traps the solute molecules, which focusses the sample into a narrow band (known as the solvent effect).Individual solutes with a boiling point 150° above the initial column temperature condense and focus at the top of the column in a process known as cold trapping. Either the solvent effect or cold trapping must occur before efficient chromatography can be obtained. Some newer chromatographs have the option of a pulsed splitless injection. In this mode, the column head pressure is increased immediately upon injection (typically to 174 kPa) and held there for 30 to 60 s, before returning to the normal operating pressure. This facilitates band sharpening and, while the process is not guaranteed to increase the fraction of the injection delivered onto the column, sensitivity is often improved because of improved chromatography.

Glass liners for split and splitless injectors come in a variety of shapes and volumes and it is prudent to start with a straight liner, and to investigate some of those that cause turbulence (e.g. the inverted cup style) later if this is unsatisfactory. A plug of deactivated glass wool in the liner helps prevent the deposition of non–volatile or particulate material on the column, but may cause some peak discrimination, and for the best results needs to be placed at a consistent position in the liner. Packing splitless injection liners with deactivated glass wool may decrease the chromatographic performance, but this must be weighed against the potential for damage to the stationary phase from the repeated injection of non–volatile or particulate material.

The analysis of trace amounts of components or contaminants in complex matrices such as foods, beverages and environmental samples is difficult. Adequate sensitivity to detect trace components is provided by specific detectors such as the NPD or ECD, but regulatory standards require positive identification of these compounds by mass spectroscopy (MS). To overcome the inferior sensitivity of MS, large–volume injectors have been developed. Examples include the Apex pre–column separating inlet (PSI), the temperature–programmed sample inlet (PTV) from Gerstel, and time–coupled time–resolved chromatography (TCRC). The inlet typically consists of a length (10 to 50 cm) of standard (2 mm i.d.) glass chromatography column that can be deactivated or packed with traditional

materials. The first two injectors are mounted directly in the GC injector port; the latter is a free–standing column coupled by a four–way valve into the GC inlet. Injection volumes range from 125 µL for the PSI, 1 mL for the PTV and up to 20 mL for the TCRC. Injection of larger volumes (up to 60 mL) are possible for some applications, but result in discrimination in favour of high–boiling components and loss of volatiles.

Large–volume injectors remove the solvent from the sample prior to its introduction onto the capillary column, typically by low–temperature evaporation through the split vent. As the sample is concentrated towards the bottom of the injector, the injector is heated, the split vent closed and the analytes introduced onto the GC column in splitless mode. Those injectors that can be heated selectively and cooled allow the precise introduction of selected components only from the sample, and thus reduce the quantity of non–volatile components (e.g. sugars) that might overload or destroy the analytical column. The TCRC has a small mobile oven (2 to 8 mm width) that can be scanned along the length of the column to produce band compression. Prior to the next injection, the injector columns are usually baked to vent high–boiling compounds to waste. Sensitivity can often be improved 50- to 100-fold and time is saved in sample preparation, since extensive clean–up or extraction procedures are no longer required.

## Cold on–column injection

Cold on–column injection is most suited to compounds that are thermally labile. The injection needle must be fine enough to enter the column bore, usually fused silica or stainless steel with a fused silica insert. The top of the column is held at a temperature low enough for the solvent that contains the sample to condense, usually by an air- or carrier gas–cooled sleeve. The solvent temporarily swamps the stationary phase and ensures that the sample components concentrate in a narrow band. Any solvent or sample that remains in the injector is backflushed with carrier gas, often by automatic valves. The proximal end of the column is then brought rapidly to the operating temperature, when the solvent vaporises and chromatography begins. The potential for rapid column contamination or deterioration means that cold on–column injection is usually restricted to those applications where its use is essential.

## Volatiles interface

The volatiles interface allows automated analysis of gaseous samples. The interface is a low–volume highly inert switching block, and is ideally suited to trace–level detection. A portion of the carrier gas supply is diverted through the specimen sampler and released under controlled conditions onto the column. The remainder of the carrier gas goes to a flow sensor, which prevents fluctuations in column gas flow that would otherwise occur when the switching valves are opened and closed. The interface can be run in split, splitless or cold–on column modes as described in the sections above. Samples may be introduced from external devices, such as air samplers or purge and trap devices (see section below), or from headspace analysis, which permits analysis of volatile substances in a liquid sample while minimising contamination of the column. This technique is used in the assay of ethanol and

other solvents in blood and for complex household preparations, such as polishes, which contain volatile substances.

## Thermal desorption and purge–and–trap injection

The analysis of samples that have been pre–concentrated onto solid adsorbents is common in the fields of industrial air monitoring, analysis of residues in food, soil and water, petrochemical analysis, and environmental monitoring. The methods of preparing samples for analysis are described in the section on specimen preparation. These samples require special interfaces with GCs to ensure good chromatography. In some instances the sample preparation device and injector are manufactured as stand–alone pieces of equipment that require very little modification of conventional injectors, while others must be dedicated pieces of equipment. Once collected, the concentrated sample must be desorbed into the chromatograph using the heated injector port. The major problem here is the possible introduction of water into the chromatograph from moisture adsorbed during collection from high–humidity samples. Release of solutes from the adsorbent should be as rapid and complete as possible to allow for rapid and sensitive analysis and for a narrow sample band to be introduced into the chromatograph. This is achieved either by cooling the column oven cryogenically to refocus the sample in the injector prior to injection or by using a dry purge system coupled to the GC via a volatile interface (see above) designed to operate above ambient temperature. Here, the specimen is thermally desorbed from the collection tube onto a narrower (1 mm i.d.) tube of the same adsorbent material. The concentrated solute is then released into the chromatograph, ensuring rapid and complete sample introduction. Adsorbents must be thermally stable to reduce interference from background contaminants.

With solid–phase micro–extraction (SPME) the adsorbed sample is introduced into the heated injector port via a special sleeved needle (see under specimen preparation). This technique requires the injector liner to be narrow (usually 0.75 mm as opposed to 2 or 4 mm) to increase the linear velocity of carrier gas through the liner and ensure a narrow band of sample is introduced onto the column.

## Solid injection

When solvent interference is serious the sample may be injected as a solid. The ‘moving needle’ injector has found application in steroid analysis and for the determination of anticonvulsant drugs. A solution of the material to be injected is placed on the tip of the glass needle with a syringe. A small flow of carrier gas sweeps the solvent out of the top of the device to waste. The dry residue is then introduced by moving the needle into the heated injection zone of the chromatograph with a magnet. This form of injection can only be used with compounds that do not volatilise with the solvent.

## Backflush

Upon vaporisation, the injected sample undergoes considerable expansion, sometimes up to 100 to 1000 times its original volume, which creates a pulse of pressure that often exceeds the column carrier-gas pressure. If the volume of the liner is smaller than the expanded solvent volume (see Table.6), some of the sample is propelled out of the injector in a process known as backflush. This can appear as a broad tailing solvent front, since it now takes longer to flush the expanded solvent out of the injector and carrier-gas line. Backflush can also cause injector contamination, since the analytes condense in the cooler carrier-gas line, from where they may bleed continuously into the injector and cause high background or spurious peaks. Carryover or peak ghosting can occur when the next injection backflushes and carries previously condensed compounds back into the vapour phase and onto the column. Backflush can usually be solved by using a smaller injection volume, a less expansive solvent, a lower injector temperature, a liner with an upper restrictor or a faster carrier gas flow. The use of an adjustable septum purge gas (0.5 to 1 mL/min usually) also decreases the potential for backflush, as components that would normally condense on the cooler septum and travel into the carrier-gas lines are swept away by the septum purge. Too high a purge flow results in loss of highly volatile components.

| Solvent | Boiling point (°) | Expansion volume (µL) per µL of solvent[a] | Suggested GC oven starting temperature (°) |
|---|---|---|---|
| Methylene chloride | 40 | 330 | 15–30 |
| Carbon disulfide | 46 | 355 | 15–30 |
| Acetone | 56 | 290 | 30–45 |
| Methanol | 65 | 525 | 40–55 |
| *n*-Hexane | 69 | 165 | 40–60 |
| Ethyl acetate | 77 | 215 | 45–65 |
| Acetonitrile | 85 | 405 | 55–75 |
| iso-Octane | 99 | 130 | 70–90 |
| Water | 100 | 1180 | 70–90 |
| Toluene | 111 | 200 | 80–100 |

[a]Values are given at 250° and 105 kPa head pressure.

Table 6. Boiling points and expansion volumes for commonly used injection solvents

## Injector discrimination

Injector discrimination occurs because not all the compounds in the sample vaporise at the same rate. Since the sample remains in the liner for a limited time, this usually results in some loss of higher–boiling solutes. This can be alleviated by increasing the residence time of the sample within the injector, or by using a higher injector temperature or smaller injection volume. However, there is usually a compensatory loss in lower–boiling compounds. Discriminating behaviour can usually be managed by making reproducible injections.

# Gas pressure and flow control

For accurate and reproducible GC, either a constant carrier-gas flow or a constant carrier-gas pressure must be maintained. Under isothermal conditions, simple pressure control is adequate for packed or capillary columns and back pressure can be monitored by a pressure gauge between the flow controller and the injector. A decrease indicates a leaking septum and an increase suggests contamination of the injector liner or the top of the column. This also ensures that the flow controller is performing correctly. Since the back pressure rises to equal the supply pressure, flow becomes pressure–controlled. Flow control is highly desirable, if not essential, during temperature programming with packed columns and can be used to advantage with capillary columns. The added convenience of a digital (electronic) flow controller may be worthwhile.

Since the carrier gas becomes less viscous as the column oven temperature rises, the gas pressure must be increased as the run progresses to maintain constant velocity (or constant flow) throughout the analysis. Fig.5 shows the effects of increasing the column temperature on the carrier gas flow and velocity if the head pressure is held constant during the run. As flow and velocity do not respond identically to increasing temperature (see Fig. 5d), late eluting analytes are recovered more quickly using constant flow than under constant pressure conditions. Furthermore, since column efficiency is a function of the carrier gas velocity (Fig.6), resolution at the end of the chromatogram is improved under constant flow conditions. Switching between conditions of either constant flow or constant pressure can sometimes resolve otherwise co–eluting compounds. Table .7 shows the relationship between flow and pressure for various lengths and diameters of capillary columns. It shows the calculated head pressure (kPa) required to achieve the stated gas velocity or flow through a 25 m column operating at 150°. Note that head pressure values above 280 kPa are not usually practicable using standard pressure regulators. Increasing the column length has a direct and proportional increase on head pressure for both velocity and flow calculations.

The way in which carrier-gas velocity affects column efficiency is best demonstrated by reference to the van Deemter curves in Fig.6. These demonstrate that the optimum column efficiency (minimum plate height, H) occurs at intermediate velocity, and that column efficiency is compromised at both low and very high velocity. A small loss in efficiency for a shorter analysis time is usually tolerated. Curves are shown for the three most common

carrier gases (helium, nitrogen and hydrogen), and it can be seen that the chromatography is much less tolerant to changes in nitrogen velocity than to helium. Helium is favoured by most users, as analysis times are half that with nitrogen, with only a slight loss in efficiency. While hydrogen gives the best dynamic range and shortest analysis times, there are safety issues relating to its use. While the gas used for the carrier gas should always be of the highest purity available, a lower quality gas can sometimes be used for the makeup or detector, since these do not contribute to column deterioration by oxidation. Regardless of quality, it is advisable always to use a scrubber (to remove oxygen and hydrocarbons) followed by a dryer (to remove water vapour) between the supply and the instrument. Metal trap bodies are recommended, as plastics are permeable to impurities in laboratory air, especially when large amounts of organic solvents are used. Most traps have an indicator to show when they are saturated, and they can be changed without interruption to the gas flow. Stainless steel or copper tubing is recommended for plumbing of all gases, as plastics are permeable to moisture and oxygen, and Teflon, nylon, polyethylene, polypropylene and PVC contain contaminants that degrade gas purity.

# Detector systems

The choice of chromatography detector for an application depends on factors such as cost, ease of operation, consumables supply, sensitivity, selectivity and the linear working range.

Some detectors respond to almost all solutes, while others (selective detectors) respond only to solutes with specific functional groups, atoms or structural configurations. Additional functional groups can often be added to solutes, generally after extraction (see below under derivatisation), to achieve a response from a selective detector and gain additional sensitivity and selectivity. The use of detectors such as the ECD to identify amenable compounds, and the NPD to detect compounds that contain phosphorus and nitrogen, removes many of the extraneous peaks frequently observed when using non–selective detectors, such as the FID. However, these selective detectors have also led to the detection of substances such as plasticisers from blood–collection tubes or transfusion lines, which interfere in many toxicological analyses. Detectors that detect the presence of a solute and also give information about its structure are increasingly popular and MS, Fourier transform infra-red spectroscopy and atomic emission spectrometry have been invoked to achieve this goal. Detector sensitivity is measured as signal–to–noise ratio, in which the signal corresponds to the height of the peak, and the noise to the height of the baseline variability. A signal–to–noise ratio of 8 to 10 is considered sufficient to confirm the presence of a peak. Each type of detector has a linear operating range in which the response obtained is directly proportional to the amount of solute that passes through, although this can be modified slightly by the nature of the solute and the chromatographic conditions (mobile phase type and flow, detector temperature). The linear operating range is considered to be exceeded when the incremental response obtained from the detector varies by more than 5% from that expected.

Most detectors (except MS) rely on gas other than the mobile phase (combustion, reagent or purge gas) for their operation. Usually, a total flow of at least 30 mL/min is necessary to sweep the solute molecules physically through the body of the detector at sufficient speed to prevent refluxing and produce narrow peaks. Thus, the addition of a 'makeup' gas is invariably required with capillary columns. Recommended gases and their flows for each detector are included in the manufacturer's instruction manuals, and it is important to follow these guidelines (and those on maintenance) to achieve the stated performance.

Here, only the most widely used detectors are considered in detail. Several other types of detectors are available; for a more detailed discussion, the reader is referred to the text by Scott (1996).

## Flame ionisation detector

This is the most widely used of all detectors, since it responds to nearly all classes of compound. The effluent from the column is mixed with hydrogen and the mixture burnt at a small jet in a flow of air. A polarising current is applied between the jet and an electrode situated above it. When a component elutes from the column it burns in the flame to create ions that carry a current between the electrodes and provide the signal. The background current and noise are both low. Any of the usual carrier gases can be used and minor changes in gas flow are without effect. Sensitivity is moderate (0.1 to 10 ng), with linearity extending sometimes as high as six orders of magnitude. The response of the FID is dependent on the number of carbon atoms in the molecule, but the response is lowered if oxygen or nitrogen is also present in the molecule. It responds to all organic compounds that contain carbon–hydrogen bonds with the exception of formic acid. Both the sensor design and electronics are simple, and manufacturing cost is therefore low. The FID is easy to clean, and when operating with capillary columns it is virtually maintenance free. With packed columns, however, there is a tendency for a build up of stationary phase bleeding from the column, which must be periodically removed. The insensitivity of the detector to water is a useful feature that allows aqueous solutions to be used.

## Nitrogen–phosphorus detector or alkali flame ionisation detector

The introduction of alkali metal vapours (usually supplied by an electrically heated bead of rubidium or caesium chloride) into the flame or 'plasma' of an FID confers an enhanced response to compounds containing phosphorus

and nitrogen. By adjusting the plasma gases the detector can be made virtually specific for phosphorus compounds (e.g. a phosphorus:carbon response ratio of 50 000:1 and a phosphorus:nitrogen response ratio of 100:1). Even when optimised for nitrogen compounds, it retains its response to phosphorus (e.g. a nitrogen:carbon response ratio of 5000:1 and a nitrogen:phosphorus response ratio of 10:1). This detector is particularly useful for drug analysis, since most drugs contain nitrogen, while the solvent and the bulk of the co–extracted material from a biological sample do not. The NPD is ideal to detect pesticides that contain phosphorus, and therefore has wide application in environmental and regulatory analysis (air, soil, water and residues in food). The extreme sensitivity to compounds that contain phosphorus can be further exploited by the preparation of derivatives that contain this element. Sensitivity is excellent (1 to 10 pg), with a good linear range of up to four or six orders of magnitude. A disadvantage is the need for the supply of three gases and, unlike the FID, their control is absolutely critical to selectivity. The detecting element (bead) lasts between 1 and 3 months depending on usage. Stationary–phase bleeding from packed columns coats the bead and collector assembly and can be rinsed off using methanol or dilute (0.1 M) sulfuric acid. Most of the early problems that arose from poor reproducibility in bead coating have now been resolved, and the most stable detectors nowadays have a geometry that enables the bead to be located and fixed in its optimal position with relative ease.

# Electron–capture detector

The early form of this detector consists of a small chamber with a pair of electrodes and a radioactive source, usually $^{63}$Ni, placed close to the cathode to ionise the carrier gas. Potential applied to the electrodes produces a steady background current. Electron–capturing solutes arriving in the chamber remove some of the electrons and reduce the detector current. The response of the detector is therefore a loss of signal rather than an increase, as is given by most other detectors. Although the ECD can be polarised from a suitable low–voltage direct–current supply, it is more sensitive when a pulsed power supply is used, and in modern detectors the polarising pulses are modulated to maintain a constant current. A voltage dependent on the modulation frequency is generated as the output signal. Additional carrier gas is necessary, even with packed columns, to obtain a flow of at least 60 mL/min to purge the detector adequately and avoid peak broadening and distortion. Sensitivity can also be improved dramatically by raising the operating temperature of the detector, and decreasing the makeup gas flow.

The ECD is a selective detector with a very high sensitivity to compounds that have a high affinity for electrons; for many compounds, the sensitivity of the ECD often exceeds that of MS, and sometimes even that of the NPD. Compounds that contain a halogen, nitro group or carbonyl group are detected at concentrations of 0.1 to 10 pg, 1 to 100 pg and 0.1 to 1 ng, respectively. This makes it very useful for compounds such as the benzodiazepines or halogenated pesticides and herbicides. Alternatively, the great sensitivity of the detector may be utilised by preparing derivatives with halogenated reagents, such as trifluoroacetic, heptafluorobutyric or pentafluoropropionic (PFP) anhydrides. Linearity (at best only two or three orders of magnitude) is a limiting factor

for quantitative analysis. In older models, the addition of a small amount of quench gas, such as methane, improves stability and linearity, and is essential if argon or helium carrier gas is used. Newer models can be operated successfully with helium as both carrier and detector gas. The ECD, because of its high sensitivity, can be contaminated easily: an impure cylinder of gas can damage a detector beyond repair in a matter of only a few hours. Cleaning is difficult, although some material can be removed by heating the detector to its maximum operating temperature overnight, and the injection of water in 100 µL aliquots through an empty glass column can also help. However, if contamination is avoided, it is virtually maintenance free. The radioactive source requires special handling procedures that may be subject to federal regulations. More recently, it has been shown that this detector can work with greater sensitivity and operate over an increased linear range using a helium plasma in place of the radioactive source.

# Fourier–transform infra-red detector

In the Fourier–transform infra-red detector (FTIRD), the column effluent is conducted through a light pipe and swept by a scavenging gas into the path of an infrared light beam that has been processed by an interferometer. The interferometer directs the entire source light to a beam splitter, which sends the light in two directions at right angles. One beam takes a fixed pathlength to a stationary mirror, while the other takes a variable pathlength to a computerised moving mirror. The two beams are recombined, and the difference in path lengths creates constructive and deconstructive interference, or an interferogram. The recombined beam is then passed through the sample. Analyte molecules absorb light energy of specific wavelengths from the interferogram, and the sensor reports variation in energy versus time for all wavelengths simultaneously. For molecules to be infra-red active they must be able to undergo a change in dipole moment with the transition to their excited state. As a result, many compounds that are symmetrical do not respond.

Fourier transform refers to the mathematical computation that converts the data from an intensity versus time plot into an intensity (% transmission) versus frequency spectrum. Each dip in the spectrum corresponds to light absorbed, and can be interpreted as characteristic of specific functional groups in the molecule. Computer libraries allow for easy and rigorous comparison of spectra. FTIR can be fully quantitative, but it is relatively insensitive (10 ng range). Its advantages are that it is non–destructive, and it can distinguish between isomers (MS cannot). Because of the logistical difficulties of combining FTIR with GC, this combination of techniques has started to emerge only recently.

# Atomic emission detector

With the atomic emission detector (AED), carrier gas that elutes from the column delivers solutes into a high–temperature helium plasma, where heat energy is absorbed by the constituent elements. In returning to their ground state, energy is emitted as light, the wavelength of which is characteristic for each element. Emitted light is focussed by a quartz lens and spherical mirror onto a diffraction grating, and the dispersed light focussed onto a diode array that is continuously scanned (wavelength usually 170 to 800 nm). Typically, some 15 elements can be monitored simultaneously, and each is plotted against time. The composite chromatogram allows the percentage elemental composition of each peak to be determined. Sensitivity is very good, but the detector is complex and expensive to operate and is not widely used.

# Mass spectrometer

A GC is an almost ideal inlet device for quadruple MS. The detector is maintained under vacuum, and in the most common technique of electron impact (EI) the column effluent is bombarded with electrons. Compounds absorb energy, which causes them to ionise and fragment in a characteristic and reproducible fashion. The resultant ions are focused and accelerated into a mass filter that allows fragments of sequentially increasing mass to enter the detector stepwise. The mass filter scans through the designated range of masses (usually up to about 700 amu) several times per second. The abundance of each mass at a given scan time produces the mass spectrum, which can be summed and plotted versus time to obtain a total ion chromatogram. The MS detector can be operated either in full scan mode (collecting all the ions within a given mass range) or selected ion monitoring (SIM) mode, which collects only pre–selected masses characteristic for the compound under study. Sensitivity for the two modes of operation is quite different, 1 to 10 ng for full scan, increasing to 1 to 10 pg in SIM because of the dramatic decrease in background noise. The linear range is excellent and often spans five or six orders of magnitude. Recent advances in computer technology, coupled with improved detector design, have revolutionised the use of the MS detector from a research tool to one of routine application.

# Ion–trap mass spectrometer

As with other forms of mass spectrometers, EI or chemical ionisation (CI) is used to produce an ion source, but this is focussed into the ion–trap mass spectrometer in pulses rather than continuously. The fundamental difference is that all the solute ions generated over the entire pulse period are trapped in the detector and are then sequentially ejected in increasing mass number from the trap into the electron multiplier. The addition of helium into the trap

(133 mPa) contracts the ion trajectory to the centre of the trap, where it is further focussed by the ring electrode, to form dense ion packets that are expelled more efficiently than diffuse clouds, and thus greatly improve resolution. The spectral patterns can be quite different to those produced by mass filter spectrometers, and are often characteristic of the conditions under which the instrument is run, which makes comparison difficult between instruments. However, because the ion collection period is longer, the sensitivity of the ion trap in full scan mode is similar to that obtained in SIM on the average MS. Furthermore, an improved mass range (sometimes up to several thousand amu) gives this type of detector many applications, particularly for quantitative trace analysis, and for higher mass components.

# Dual detector systems

The simultaneous use of a combination of a universal detector (FID) with a specific detector to monitor the effluent of a column can provide useful information about the properties of functional groups and substituents in a molecule. The FID response is roughly dependent on the number of carbon atoms in a molecule and is quite predictable. However, the ECD response varies widely for different compounds, is dependent on the electron–deficient part of the compound, and is difficult to predict. The NPD response of a compound depends to some extent on the number of phosphorus or nitrogen atoms in a molecule, but it also depends on their environment. Thus, by using the FID as a reference, and measuring the ECD or NPD response relative to it, another characteristic for identification is obtained in addition to retention behaviour.

Dual detector systems can be used in several ways. The column can be split at the detector end and the effluent passed into two different detectors that operate in parallel. This approach allows the most flexibility, since the choice of detectors is wide, and the effluent can be split in proportion to the sensitivity required from each detector. For capillary columns this is accomplished easily with zero dead volume press–fit tee connectors, but it is a more complicated operation for packed columns. Additional makeup gas may be required to ensure a good flow through the detectors, and care should be taken to use tubing of a smaller or equal total area to the analytical column to avoid loss of peak shape through refluxing at the detector. Alternatively, the GC oven houses two completely separate, but identically matched columns, each connected to a single detector. This is not an ideal approach, as matching columns is difficult and has to be checked at frequent intervals. Another approach is to stack the detectors in series, and some manufacturers deliberately provide detectors in identical modules for this purpose. There are limitations to the choice of possible detector combinations, as the first detector must always be a non–destructive detector, such as the ECD, AED or FTIRD.

# Specimen preparation

Prior to chromatography, it is usually necessary to isolate the compound(s) of interest from either a biological matrix (plasma, urine, stomach contents, hair and tissue) or some other matrix, such as soil, air or water. Removal of extraneous material and concentration of the compounds of interest usually take place simultaneously. The high water solubility of some drug metabolites (e.g. glucuronide conjugates) requires chemical conversion to a less polar entity to permit isolation from water–based samples, and a hydrolysis procedure is often used for this purpose.

# Isolation and concentration

# Protein precipitation

If the analyte is present in blood in high concentration, a simple protein precipitation step often provides a suitable extract, although the possibility of losing significant amounts of analyte with the precipitate must be considered. Mixing with a solution of mercuric chloride or barium sulfate readily precipitates plasma proteins, and centrifugation provides a supernatant for direct injection onto the chromatography column. Use of perchloric or trichloroacetic acids (10%) is not advised, unless the resultant solution is neutralised prior to injection. Dimethylformamide is a good organic precipitation reagent that is well tolerated by most GC stationary phases. Other organic precipitating agents are methanol, acetone and acetonitrile, all of which should be added in the proportion of two volumes to each volume of blood. While the extract is still water based, most columns with a high stationary–phase loading (5 µm film thickness) can tolerate the injection of 1 µL of water. If the column is not water tolerant, it is possible to evaporate small volumes of the supernatant to dryness for reconstitution in a more suitable solvent.

# Liquid–liquid extraction

Liquid–liquid extraction is the most frequently used method to isolate and concentrate solutes for GC. The pH of the specimen is adjusted to ensure the compounds to be extracted are not ionised (basic for bases, acid for acidic compounds). Bearing in mind that some portion of the aqueous acid or base will dissolve in the solvent, the use of strong mineral acids or alkalis is not advised as this adversely affects column performance. Best results are obtained with acidic buffers (phosphate or acetate) and with ammonium hydroxide or basic buffers (borate), using

a 5:1 ratio of solvent to specimen. The solvent chosen should be sufficiently polar to partition the compound of interest without co–extracting excessive amounts of polar contaminants. For more water–soluble drugs, such as *β*-blockers, the addition of 2 to 10% of a polar solvent (e.g. isopropanol or butanol) is helpful, or solid sodium chloride can be added to 'salt out' the analyte. If a derivatisation step is to be carried out subsequently, the use of a solvent compatible with the derivatisation eliminates the need for an evaporation step. Use of solvents with a higher density than the sample (e.g. dichloromethane) can lead to difficulty in isolation of the organic phase. Purification of extracts by back extraction (re–extraction of the analytes from the organic solvent at the opposite pH followed by re–extraction into solvent at the original pH) may be helpful for trace analysis. The use of a small volume of solvent for the final extraction serves as a concentration step without the need for separation and evaporation of the organic phase.

## Solid–liquid or solid-phase extraction

Solid–liquid extraction uses a polypropylene cartridge with a small amount (200 mg to 3 g) of high capacity (1 to 20 mL) silica–based packing at the base of the reservoir. On introduction of the sample matrix, the compounds of interest are withheld by the packing. Impurities are then rinsed selectively from the column, and the final elution releases the compound of interest. Evaporation followed by reconstitution in a suitable solvent provides a clean concentrated sample ready for analysis by GC. Bonded phase packings that have been modified by the addition of various functional groups are available. The mechanisms of interaction for the matrix, analytes and packings are similar to those in LC. Polar stationary phases retain polar analytes (normal phase) and are eluted with organic solvents, while non–polar stationary phases retain non–polar analytes (reverse phase) and are eluted with aqueous solvents. Ion–pair extraction uses a non–polar stationary phase and polar analyte, with a counter ion added to the sample solution, and allows retention of the (now neutral) analyte by a reversed–phase mechanism. In ion–exchange extraction, the adsorbent surface is modified with ionisable functionalities. Analytes with ionic charges opposite to those on the packing are retained. Solvents that contain counter ions of greater strength are used to elute the analytes of interest from the tube.

## Solid–phase micro–extraction

SPME requires no solvents or complicated apparatus and can concentrate volatile and non–volatile compounds in both liquid and gas samples. The unit consists of a fused silica fibre attached to a stainless–steel plunger coated with a stationary phase (mixed with solid adsorbents as required). The plunger is inserted through a septum into a vial that contains the sample, and the fibre exposed by depressing the plunger either into the liquid or the headspace for 20 to 30 min. The retracted fibre is inserted into the injection port of the GC, and is desorbed when

the plunger is depressed. The unit may be reconditioned and used 50 to 100 times. For field analysis, adsorbed samples can be stored and transported in the needle sealed in a special container for subsequent analysis by GC (or LC). Pesticides recovered from water samples have been shown to be more stable when stored in this way than in water. The special small–volume injection liner fits any model of chromatograph, and produces sharper peaks because of the higher linear gas velocity, with little or no backflush. Suitable stationary phases are:

- 100 µm dimethyl-PSX film for low molecular weight compounds or volatiles, or a thinner film (7 µm) for larger weight semivolatile compounds
- 85 µm polyacrylate film for polar compounds
- 65 µm film of dimethyl-PSX–divinyl benzene for volatile alcohols and amines
- for surfactants, 50 µm Carbowax–templated resin
- for trace level volatiles, a 75 µm Carbowax–carboxen phase is suitable.

An alternative approach uses a small magnetic stir bar encapsulated in glass and coated with a layer of dimethyl-PSX. The bar is left to stir in the sample for 30 to 120 min and then removed and placed in a thermal desorption tube. From there, it is introduced onto the GC as described in the section on thermal desorption injectors. Both approaches give similar performance for higher–boiling compounds (>350°), but SPME is inferior for lower–boiling compounds such as naphthalene and fluorene (b.p. 218 and 298°, respectively).

# Supercritical fluid extraction

A SCF is a substance that is maintained above its critical temperature and pressure, where it exhibits physicochemical properties intermediate between those of a liquid and a gas. Properties of gas–like diffusivity, gas–like viscosity and liquid–like density combined with a pressure–dependent solvating power provided the impetus to apply SCFs to analytical separation. The initial applications most often involved isolation of flavours and contaminant residues from food and soil. These have now been extended to the isolation of drugs from blood and other aqueous–based media by using adsorbents added in–line (such as molecular sieves, diatomaceous earth, silica gel, etc.) to filter proteinaceous material and adsorb water. It is possible, by adding small volumes of co–solvent to the SCF, to extract highly polar solutes with excellent efficiency. In contrast to the conventional extracting solvent, the fluid most often used in supercritical fluid extraction (SFE), supercritical $CO_2$, is non–polluting, non–toxic and relatively inexpensive. Additionally, extractions are carried out quickly at temperatures that avoid degradation of temperature–sensitive analytes and provide clean extracts with extremely high efficiency. Several dedicated SFE analysers are available; each consists of a gas supply, pump and controller used to pressurise the gas, temperature–controlled oven, extraction vessel, internal diameter regulator and collection device. The $CO_2$ supply is compressed to a selected pressure (e.g. 28 000 kPa) and its temperature adjusted (e.g. 50°). As the supercritical $CO_2$ passes through the sample material, the solutes are extracted to an

equilibrium solubility level, typically about 10% (w/w). The gaseous solution that leaves the extractor is passed through the pressure reduction valve, where the pressure (and thus the dissolving power) of the $CO_2$ is reduced. The solutes precipitate in the separator, and the $CO_2$ is recycled through the system several times until the extraction is completed, when it is vented to waste.

# Headspace analysis

This method of isolation is used for analytes with volatility higher than that of the common extraction solvents.

# Purge and trap

Purge and trap is a powerful procedure for extracting and concentrating volatile organic compounds from soil, sediment, water, food, beverages, etc. It is especially useful for poorly water–soluble compounds and those with boiling points above 200°. The procedure involves bubbling an inert gas (nitrogen or helium) through an aqueous sample or suspension at ambient temperature, which causes volatile organic compounds to be transferred into the vapour phase. During the purge step, purge gas sweeps the vapour through a trap containing adsorbent materials that retain the volatilised compounds. Water vapour may be removed by dry purging. The trap is rapidly heated to 5 to 10° below the desorb temperature. The valve is then switched to join the trap flow to the carrier-gas flow, and the trap heated to its desorb temperature for a fixed time. Adsorbent tubes are usually packed with multiple beds of sorbent materials, each one more active than the preceding one, which allows compounds with a wide range of boiling points and polarities to be analysed simultaneously. During purge, the smaller and more non–polar solutes are readily carried down the beds, and since the carrier gas passes in the opposite direction during the desorb phase, the larger and more polar compounds do not come into contact with the innermost active beds, from which their release may be difficult to effect.

# Thermal desorption

This technique is used extensively for air monitoring in industrial hygiene, environmental air, indoor air or source–emission monitoring. The device may be portable or fixed and of varying size. Air is pumped continuously through the device at a fixed rate, during which time components are extracted gradually and concentrated onto the adsorbent beds; the arrangement of the beds is the same as described above for the purge and trap, and prevents potentially irreversible binding of large molecules. The direction of the flow is simply reversed during

desorption. Analysis requires a special interface to the GC, which is described above in the section on Thermal desorption. The adsorbents must have high capacity to remain active during the entire sampling period, and show an acceptable pressure drop during sampling. Ideally, a minimal amount of unwanted analytes should be absorbed, as these will contribute to the background noise.

## Tissues and hair

Tissues and hair require treatment prior to drug extraction to break down the biological matrix and enable a good recovery of the drug. For solid tissues, good results are obtained by incubation of a portion of the tissue with a mixture of a collagenase, a protease and a lipase in a buffer of suitable pH. For small amounts of tissues (100 mg), overnight treatment at room temperature suffices, although gentle agitation or occasional mixing speeds up the process. Larger amounts of tissue benefit from mechanical homogenisation prior to incubation. For the analysis of hair, an initial washing to remove residues from cosmetic products or environmental contaminants is recommended, followed by incubation with either caustic alkali (for basic drugs) or mineral acid (for acidic drugs). After adjustment of the pH, drug recovery can proceed by the usual procedures established for the specific compounds under investigation.

## Hydrolysis

Recovery of conjugated drug metabolites from biological fluids can be increased by hydrolytic cleavage of the conjugate bond prior to extraction. This offers a vast improvement in sensitivity for qualitative analysis, particularly from urine, and is essential to identify drugs (e.g. laxatives) that are excreted almost exclusively as conjugated metabolites. However, reliable quantitative analysis of conjugated metabolites requires that the unconjugated metabolite must first be removed or quantified, and then the total (conjugated plus unconjugated) metabolite be measured after hydrolysis in a subsequent separate procedure. For quantitative work, appropriate standards that contain conjugated metabolites must be carried through the procedure to monitor the efficiency of the hydrolysis step.

## Enzymatic hydrolysis

The use of a specific enzyme to cleave chemical bonds is the more specific of the two approaches, but incurs additional cost and time. It also provides cleaner extracts, and therefore prolongs the life of the chromatography

column. There are a number of commercial preparations of purified glucurase and sulfatase harvested from different species. It is important to pay attention to the pH and temperature optima of the specific enzyme preparation. Temperature–tolerant preparations allow heating up to 60°, which permits relatively short (2 h) incubation times.

## Chemical hydrolysis

This quicker and less expensive approach can provide suitable extracts for chromatography for some analytes, although they are generally more demanding in terms of clean–up procedures. Typically, strong mineral acids or alkalis are used, often with boiling or treatment in a microwave or pressure cooker. Extracts must be neutralised, otherwise the chromatography column deteriorates quickly. Care should be taken to ensure the stability of the analytes to the hydrolysis conditions. Vigorous hydrolysis conditions often yield undesirable by–products, or if several compounds can be hydrolysed to a single entity, preclude accurate identification of the original compound present. For example, both the acid and the enzymatic hydrolysis of benzodiazepines remove glucuronide conjugates, but acid hydrolysis also converts two or three drugs to the same benzophenone compound (diazepam, temazepam and ketazolam are all converted into 2–methylamino–5–chlorobenzophenone). While this compound has good chromatography characteristics, the approach is unsuitable for those applications (such as forensic analysis) that require absolute identification of the drug ingested.

## Derivative formation

Derivatisation enables the analysis of compounds that otherwise could not be monitored readily by GC. To some extent the availability of stable polar stationary phases in capillary columns and the use of temperature programming has negated the requirement for derivatisation, although it is still widely used. Choice of reagent is based on the functional group that requires derivatisation, the presence of other functional groups in the molecule and the reason for performing the reaction. Although the retention characteristics are changed, the order of elution of a series of derivatives will be the same as that for the parent compounds. The preparation of derivatives modifies the functionality of the solute molecule to increase (or sometimes decrease) volatility, and thereby shortens or lengthens the retention time of a substance, or to speed up the analysis.

Another common reason for derivatisation is to improve resolution and reduce tailing of polar compounds (hydroxyl, carboxylic acids, hydrazines, primary amines and sulfydryl groups). For instance, hydroxylated compounds often have long retention times and column adsorption causes tailing, which results in low sensitivity. However, they readily form silyl ethers and these derivatives show excellent chromatography, and sensitivity can

often be improved by a factor of 10 or more. Derivatisation can also help to remove the substance peak away from interfering material. For example, the reaction of amfetamine with acetone enables successful differentiation from methylethylketone on most stationary phases. Derivatives may also be used to make the molecule amenable to detection by selective detectors, or can be used to improve the fragmentation pattern of the compound in the mass spectrometer.

The reaction may be carried out during extraction (e.g. extractive alkylation), on the dry residue after solvent extraction (e.g. silylation) or during injection (e.g. methylation). In choosing a suitable reagent, certain criteria must be used. A good reagent produces stable derivatives without harmful by–products that interact with the analytical column, in a reaction that is almost 100% complete. Poor reagents cause rearrangements or structural alterations during formation, and contribute to loss of sample during reaction. Most manufacturers of derivatising reagents provide information on the potential uses of each product, along with standard operating instructions. Entire texts, such as that by Blau and Halket (1993), are devoted to this topic.

# Chiral separations

Chiral compounds can be derivatised to improve their chromatographic characteristics, and the enantiomers separated on a chiral stationary phase. Both enantiomers behave similarly, provided that steric hindrance does not preclude a reaction with one enantiomer. An alternative approach is to use a chiral derivatising reagent which, when reacted with enantiomers, produces diastereoisomers that can then be separated on a conventional stationary phase. As with enantiomers, diastereoisomers still produce similar mass spectra, but are resolved in time by the chromatography column. This approach is less expensive and also less restrictive, since a dedicated column is not required. Care should be taken to ensure the enantiomeric purity of the derivatising reagent, and to guard against racemisation during the reaction. *n*-Trifluoroacetyl–l–propyl chloride (TPC) in triethylamine and chloroform (or ethyl acetate) is a commonly used chiral reagent that couples with enantiomeric amines. Excess reagent is washed off with 6M HCl and the organic phase dried over magnesium sulfate. For chiral alcohols, (1*R*,2*S*,5*R*)-(–)-menthylchloroformate (MCF) reacts well if pyridine is used as a catalyst.

# Quantitative determinations

Quantitative work usually requires some form of sample preparation to isolate the drug from the bulk of the sample and some degree of concentration or, more rarely, dilution. These processes inevitably introduce a degree of analytical error. A further difficulty is caused by the non–reproducibility of injected volumes. To compensate for these errors, it is usual to compare the response of the unknown with the response of an added internal standard.

The internal standard should be added as early as possible in the assay process and should have chromatographic properties matching the drug's as closely as possible, preferably with a longer retention time. It is often possible to obtain unmarketed analogues of drugs, or compounds specially synthesised for use as internal standards (e.g. a methyl addition or a halogen substitution). However, the internal standard usually does not behave exactly as the drug and careful control of variables, such as pH, is necessary. If a derivative is to be prepared, the internal standard should also be amenable to derivatisation. Use of an inappropriate internal standard can seriously affect precision (Dudley 1980). If a mass spectrometer is being used as the detector, then the ideal internal standard is a $^{3}H$ or $^{13}C$ substituted analogue of the drug, a number of which are readily available at reasonable cost. Calibration should include points of higher and lower concentrations than the sample, and quality assurance samples should be included at appropriate concentrations in frequently run assays. Peak measurement may be by peak height or by the peak area obtained by integration. If the peaks show even a modest degree of tailing, use of peak area usually provides a more accurate quantitative result. A plot of the ratio of peak height (or area) of the drug to internal standard versus concentration is a straight line with most detectors. Care should be taken in the preparation of standards to match the matrix to that of the specimens, and to allow for any associated salt or water of crystallisation in the calculation of the concentration. The best results are obtained when the amount of internal standard used produces a peak response ratio of one at the mid–point of the calibration range.

# Optimising operation conditions to customise applications

Additional sensitivity can be achieved by increasing sample size, using a concentration step, derivatisation, injecting a larger sample volume, selecting a different stationary phase and using the detector at a higher sensitivity level.

When attempting a new analysis, it is advisable first to review published literature for a method that can be copied or for a method that involves a similar type of compound and can be adapted. Column manufacturers' catalogues are a useful source of information and invariably show examples of separations performed with their columns. Data on boiling points and RI (see monographs in Part Two) are also useful indicators. If the review is not helpful, a start can be made with a standard column, selecting either a 100% methyl-PSX capillary column (25 m with a 0.5 µm film) or a OV1–packed column (1.7 m with a 3% loading on 100 to 120 mesh support) and using standard flow conditions (1 to 2 mL/min helium for a capillary or 30 to 60 mL/min for packed). The oven temperature should be taken from 80 to 300° at 10°/min (or started at 200 or 250° if only an isothermal oven is available). A solution of the compounds of interest in ethanol or methanol should be injected with the injector temperature set at 250°. If a peak tails, derivatisation or using a more polar stationary phase should be considered. Fine–tuning is carried out once some peaks have been obtained. Having established the chromatography, the extraction and concentration steps can be determined. Manufacturers' catalogues are again a useful source for both derivatisation and solid–phase extraction procedures.

Good preventative maintenance is essential. The injector (or liner) should be cleaned periodically, and any glass wool changed regularly (approx. every 100 to 1000 injections, depending on the quality of the extracts). For capillary columns, the performance is improved by periodically removing the first 5 to 10 cm of capillary tubing (a retention gap could be considered for dirty samples), and for packed columns by replacing the glass wool and first few centimetres of packing. It is advisable to monitor performance by selecting certain performance criteria (e.g. a certain response size or amount of acceptable separation between two closely eluting components) to indicate when maintenance is required. The manufacturers' instructions for cleaning detectors should be followed.

The presence of traces of contaminants in the carrier gas supply shortens the column life drastically, and also causes detector deterioration. In–line filters (to remove oxygen, hydrocarbons, etc.) and molecular sieves (to remove water vapour) are strongly recommended, and the use of stainless–steel gas tubing minimises further contamination. Carrier–gas flow should be optimised for a particular column and a particular carrier gas. This is most important for capillary columns. Fig. 6 shows the relationship between efficiency expressed as the HETP versus carrier-gas velocity (van Deemter plot) for a 28 m by 0.25 mm i.d.WCOT OV-101 column. Modifying the mobile phase in GC has very little effect compared with that observed with HPLC or thin–layer chromatography (TLC) and, in general, affects efficiency rather than selectivity. Nitrogen gives higher efficiency, but at the expense of longer analysis time, while the less dense, but more hazardous, hydrogen gives lower efficiency, but faster analysis. In practice, nitrogen is usually used for packed columns and helium for capillary columns. Certain detectors impose restrictions on the choice of carrier gas, but an additional supply of gas can be added to the column effluent to purge the detector. Experimenting with higher flow and a lower operating temperature (or *vice–versa*) can give rewarding results for the separation of compounds that elute closely. This effect is particularly noticeable for two compounds that have different polarities, as the retention of the more polar compound is influenced to a greater extent the longer it resides in the column (non–polar compounds elute in boiling point sequence). Conditions of constant flow improve the efficiency of late–eluting peaks and produce faster chromatography than do constant pressure conditions.

For a particular separation, the lowest temperature compatible with a reasonable analysis time should be used. In general, retention times double with each 20° decrease in temperature. If the time is excessive, it is generally better to reduce the stationary phase loading or use a shorter column than to increase the column operating temperature. There is a maximum temperature at which a column can be operated and there is also a minimum temperature below which efficiency drops sharply. Manufacturers give the temperature operating ranges for each of their stationary phases (see Table .3). The stationary phase must be a liquid at the temperature of operation, and if a column is run at too low a temperature to obtain longer retention times the stationary phase may still be in the solid or semi–solid form. When using temperature programming, experimentation with a faster initial ramp followed by a slower subsequent ramp or an isothermal period can help resolve problematic separations.

Efficiency can also be improved by decreasing the column diameter or increasing the column length. The resultant increase in analysis time (particularly if the flow must be reduced to accommodate the increased pressure demand imposed by a narrower column), can usually be offset by using a slightly higher operating temperature

(temperature increases affect retention time much more than do increases in gas flow). As shown in Table .8, reducing the diameter of a capillary column markedly increases efficiency, but the retention time remains constant only as long as the same phase ratio is maintained. Therefore, unless there is a simultaneous reduction in film thickness, retention increases in direct proportion to the phase ratio.

| **Film thickness *d* (µm)** | **Column internal diameter (mm)** | | | | | | |
|---|---|---|---|---|---|---|---|
| **0.10** | **0.18** | **0.20** | **0.25** | **0.32** | **0.45** | **0.53** | |
| 0.10 | 250[a] | 450 | 500 | 625 | 800 | 1125 | 1325 |
| 0.18 | 139 | 250 | 278 | 347 | 444 | 625 | 736 |
| 0.25 | 100 | 180 | 200 | 313 | 400 | 450 | 663 |
| 0.40 | 63 | 113 | 125 | 156 | 200 | 282 | 331 |
| 0.42 | 107 | 119 | 149 | 190 | 265 | 315 | |
| 0.50 | 90 | 100 | 125 | 160 | 225 | 265 | |
| 0.83 | 60 | 75 | 96 | 136 | 160 | | |
| 0.85 | 59 | 74 | 94 | 133 | 156 | | |
| 1.00 | 50 | 63 | 80 | 113 | 133 | | |
| 1.27 | 49 | 63 | 88 | 104 | | | |
| 1.50 | 42 | 53 | 75 | 88 | | | |
| 2.55 | 25 | 31 | 44 | 52 | | | |
| 3.00 | 21 | 27 | 38 | 44 | | | |
| 5.00 | 13 | 16 | 23 | 27 | | | |
| Efficiency *N*[b] | 12 500 | 6600 | 5940 | 4750 | 3710 | 2640 | 2240 |

Table .8. Relationship between film thickness, phase ratio (β)[a], efficiency (*N*)[b] and column diameter

[a] Phase ratio β = *r*/2*d*, where *r* = column radius µm, *d* = film thickness µm.[b]*N*, theoretical plates per metre, maximum efficiency calculated for a solute with *k* = 5.

The solvent used for the sample can sometimes produce unexpected derivatives that give different retention times (traces of acetic anhydride that remain in butyl acetate avidly derivatise primary amines at room temperature). An inert non–polar solvent should be used if possible to minimise the co–extraction of unwanted contaminants. Acetone, other ketones, ethyl acetate and carbon disulfide readily form derivatives with primary amines and should be avoided.

The choice of injector type and injection solvent also play an important part in the chromatography. A solvent volume should be chosen that does not expand to exceed the capacity of the injector (see Table .6), otherwise backflush and irreproducible results are obtained. Split injection significantly reduces the amount of solvent and

associated contaminants that enter the column and, although the analyte response is reduced, the improvement in the signal–to–noise ratio often results in enhanced sensitivity.

The use of a selective detector, such as an ECD (with the preparation of a strongly responsive derivative if appropriate), can improve sensitivity typically up to 100–fold. Similarly, switching from full scan to selected ion monitoring in MS improves the sensitivity, usually by a factor of ten. However, selective detectors should not be used as a substitute for cleaning up of sample extracts, as loading contaminants onto the column affects the chromatography adversely, even if the selective detector does not respond to the compounds. Increasing the detector temperature may also improve sensitivity.

Fronting or splitting of peaks indicates column overload. If the detector sensitivity permits, the best option here is to inject a smaller sample volume (or a more dilute sample), rather than to increase the column loading or diameter, otherwise efficiency is also affected.

If trace impurities are sought in the presence of a preponderant component, a number of stationary phases of differing polarities should be tried. Trace impurities are seen easily if they emerge before the main component of a mixture, while they may be lost completely in the tail if they elute just after the large peak. Early peaks are also sharper and thus, for the same peak area, higher – an effect that can contribute enormously to the successful detection of trace substances.

# Specific applications

The systems given below are applicable to the routine screening, separation and identification of groups of drugs and chemicals. They are not exhaustive lists and references to specific systems for individual drugs and chemicals are given in the relevant monographs in Part 2. Some of these systems use columns that are identical or very similar in terms of discriminating power (see Table .3), but are operated with different temperature programmes for specific groups of compounds. Moreover, some groups of substances are chromatographed as derivatives rather than as the parent compounds.

The most commonly used general screening system is a 100% dimethyl-PSX (methyl-PSX or X-1) capillary column (for packed columns, SE-30, OV-1 or OV-101 are equivalent). This should always be used for screening purposes, since it has the best chance of eluting any compound of interest. Analysts have collaborated to compile comprehensive lists of retention indices using this system (de Zeeuw 2002), some of which are included in Part 3 (see Index of Gas Chromatographic Data).

Most of the data are for the drugs themselves, but thermal decomposition may occur and the peak observed may be for the decomposition product (referred to as ‘artifact’) rather than the original drug. Where the drug is known to chromatograph badly, or to decompose, data are given for suitable derivatives [e.g. methyl or ethyl esters for the sulfonamides, and trimethylsilyl (TMS) derivatives for hydroxides]. Wherever possible, the RI of the drug is given, since this is a more reproducible parameter than retention time or relative retention (see discussion above). However, if a laboratory prefers routinely to use the latter parameters, the RI data can be converted easily after chromatography of a few representative drugs and using a regression analysis of RI against either retention time or relative retention. RIs for some additional non–drug substances that might interfere with toxicological analyses, but are not included in the monographs, are located in Part 3. A nitrogen phosphorus (alkali flame ionisation) detector is the best detector for nitrogenous drugs and phosphorus–containing pesticides, but a FID should also be used, since some drugs do not contain nitrogen (e.g. some anti–inflammatory agents).ECDs are excellent for benzodiazepines and halogen–containing compounds, such as some phenothiazines and herbicides. Extra selectivity can always be obtained by using element–specific detectors (e.g. those for phosphorus and sulfur for compounds that contain these elements). Additional specificity or confirmation of identity can be obtained by using a mass–selective detector, such as MS or ion–trap detector. In any analysis for an unknown compound, the data obtained from complimentary techniques, such as TLC, HPLC or colour tests, should always be assessed for compatibility with the GC result. (In the tables of retention indices given here, a dash indicates that no value is available for the compound, not that it does not elute.)