METHOD

Equilibrate the column, the injector and the detector at the temperatures and the gas flow rates specified in the monograph until a stable baseline is achieved. Prepare the test solution(s) and the reference solution(s) as prescribed. The solutions must be free from solid particles.

Criteria for assessing the suitability of the system are described in the chapter on *Chromatographic separation techniques* (*2.2.46*). The extent to which adjustments of parameters of the chromatographic system can be made to satisfy the criteria of system suitability are also given in this chapter.

Static head-space gas chromatography

Static head-space gas chromatography is a technique particularly suitable for separating and determining volatile compounds present in solid or liquid samples. The method is based on the analysis of the vapour phase in equilibrium with the solid or liquid phase.

APPARATUS

The apparatus consists of a gas chromatograph provided with a device for introducing the sample that may be connected to a module that automatically controls the pressure and the temperature. If necessary, a device for eliminating solvents can be added.

The sample to be analysed is introduced into a container fitted with a suitable stopper and a valve-system which permits the passage of the carrier gas. The container is placed in a thermostatically controlled chamber at a temperature set according to the substance to be examined.

The sample is held at this temperature long enough to allow equilibrium to be established between the solid or liquid phase and the vapour phase.

The carrier gas is introduced into the container and, after the prescribed time, a suitable valve is opened so that the gas expands towards the chromatographic column taking the volatilised compounds with it.

Instead of using a chromatograph specifically equipped for the introduction of samples, it is also possible to use airtight syringes and a conventional chromatograph. Equilibration is then carried out in a separate chamber and the vapour phase is carried onto the column, taking the precautions necessary to avoid any changes in the equilibrium.

METHOD

Using the reference preparations, determine suitable instrument settings to produce an adequate response.

DIRECT CALIBRATION

Separately introduce into identical containers the preparation to be examined and each of the reference preparations, as prescribed in the monograph, avoiding contact between the sampling device and the samples.

Close the containers hermetically and place in the thermostatically controlled chamber set to the temperature and pressure prescribed in the monograph; after equilibration, carry out the chromatography under the prescribed conditions.

STANDARD ADDITIONS

Add to a set of identical suitable containers equal volumes of the preparation to be examined. Add to all but one of the containers, suitable quantities of a reference preparation containing a known concentration of the substance to be determined so as to produce a series of preparations containing steadily increasing concentrations of the substance.

Close the containers hermetically and place in the thermostatically controlled chamber set to the temperature and pressure prescribed in the monograph; after equilibration, carry out the chromatography under the prescribed conditions.

Calculate the linear equation of the graph using a least-squares fit, and derive from it the concentration of the substance to be determined in the preparation to be examined.

Alternatively, plot on a graph the mean of readings against the added quantity of the substance to be determined. Extrapolate the line joining the points on the graph until it meets the concentration axis. The distance between this point and the intersection of the axes represents the concentration of the substance to be determined in the preparation to be examined.

SUCCESSIVE WITHDRAWALS (MULTIPLE HEAD-SPACE EXTRACTION)

If prescribed, the successive withdrawal method is fully described in the monograph.

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2.2.29. LIQUID CHROMATOGRAPHY

Liquid chromatography (LC) is a method of chromatographic separation based on the difference in the distribution of species between two non-miscible phases, in which the mobile phase is a liquid which percolates through a stationary phase contained in a column.

LC is mainly based on mechanisms of adsorption, mass distribution, ion exchange, size exclusion or stereochemical interaction.

APPARATUS

The apparatus consists of a pumping system, an injector, a chromatographic column (a column temperature controller may be used), a detector and a data acquisition system (or an integrator or a chart recorder). The mobile phase is supplied from one or several reservoirs and flows through the column, usually at a constant rate, and then through the detector.

PUMPING SYSTEMS

LC pumping systems are required to deliver the mobile phase at a constant flow rate. Pressure fluctuations are to be minimised, e.g. by passing the pressurised solvent through a pulse-dampening device. Tubing and connections are capable of withstanding the pressures developed by the pumping system. LC pumps may be fitted with a facility for “bleeding” the system of entrapped air bubbles.

Microprocessor controlled systems are capable of accurately delivering a mobile phase of either constant (isocratic elution) or varying composition (gradient elution), according to a defined programme. In the case of gradient elution, pumping systems which deliver solvent(s) from several reservoirs are available and solvent mixing can be achieved on either the low or high-pressure side of the pump(s).

INJECTORS

The sample solution is introduced into the flowing mobile phase at or near the head of the column using an injection system which can operate at high pressure. Fixed-loop and variable volume devices operated manually or by an auto-sampler are used. Manual partial filling of loops may lead to poorer injection volume precision.

STATIONARY PHASES

There are many types of stationary phases employed in LC, including:

- silica, alumina or porous graphite, used in normal-phase chromatography, where the separation is based on differences in adsorption and/or mass distribution,
- resins or polymers with acid or basic groups, used in ion-exchange chromatography, where separation is based on competition between the ions to be separated and those in the mobile phase,
- porous silica or polymers, used in size-exclusion chromatography, where separation is based on differences between the volumes of the molecules, corresponding to steric exclusion,
- a variety of chemically modified supports prepared from polymers, silica or porous graphite, used in reversed-phase LC, where the separation is based principally on partition of the molecules between the mobile phase and the stationary phase,
- special chemically modified stationary phases, e.g. cellulose or amylose derivatives, proteins or peptides, cyclodextrins etc., for the separation of enantiomers (chiral chromatography).

Most separations are based upon partition mechanisms utilising chemically modified silica as the stationary phase and polar solvents as the mobile phase. The surface of the support, e.g. the silanol groups of silica, is reacted with various silane reagents to produce covalently bound silyl derivatives covering a varying number of active sites on the surface of the support. The nature of the bonded phase is an important parameter for determining the separation properties of the chromatographic system.

Commonly used bonded phases are shown below:

octyl	= Si-$[CH_2]_7$-CH_3	C_8
octadecyl	= Si-$[CH_2]_{17}$-CH_3	C_{18}
phenyl	= Si-$[CH_2]_n$-C_6H_5	C_6H_5
cyanopropyl	= Si-$[CH_2]_3$-CN	CN
aminopropyl	= Si-$[CH_2]_3$-NH_2	NH_2
diol	= Si-$[CH_2]_3$-O-CH(OH)-CH_2-OH	

Unless otherwise stated by the manufacturer, silica based reversed-phase columns are considered to be stable in mobile phases having an apparent pH in the range 2.0 to 8.0. Columns containing porous graphite or particles of polymeric materials such as styrene-divinylbenzene copolymer are stable over a wider pH range.

Analysis using normal-phase chromatography with unmodified silica, porous graphite or polar chemically modified silica, e.g. cyanopropyl or diol, as the stationary phase with a non-polar mobile phase is applicable in certain cases.

For analytical separations, the particle size of the most commonly used stationary phases varies between 3 µm and 10 µm. The particles may be spherical or irregular, of varying porosity and specific surface area. These parameters contribute to the chromatographic behaviour of a particular stationary phase. In the case of reversed phases, the nature of the stationary phase, the extent of bonding, e.g. expressed as the carbon loading, and whether the stationary phase is end-capped (i.e. residual silanol groups are silylated) are additional determining factors. Tailing of peaks, particularly of basic substances, can occur when residual silanol groups are present.

Columns, made of stainless steel unless otherwise prescribed in the monograph, of varying length and internal diameter (Ø) are used for analytical chromatography. Columns with internal diameters of less than 2 mm are often referred to as microbore columns. The temperature of the mobile phase and the column must be kept constant during an analysis. Most separations are performed at room temperature, but columns may be heated to give higher efficiency. It is recommended that columns not be heated above 60 °C because of the potential for stationary phase degradation or changes occurring to the composition of the mobile phase.

MOBILE PHASES

For normal-phase chromatography, less polar solvents are employed. The presence of water in the mobile phase is to be strictly controlled to obtain reproducible results. In reversed-phase LC, aqueous mobile phases, with or without organic modifiers, are employed.

Components of the mobile phase are usually filtered to remove particles greater than 0.45 µm. Multicomponent mobile phases are prepared by measuring the required volumes (unless masses are specified) of the individual components, followed by mixing. Alternatively, the solvents may be delivered by individual pumps controlled by proportioning valves by which mixing is performed according to the desired proportion. Solvents are normally degassed before pumping by sparging with helium, sonication or using on-line membrane/vacuum modules to avoid the creation of gas bubbles in the detector cell.

Solvents for the preparation of the mobile phase are normally free of stabilisers and are transparent at the wavelength of detection, if an ultraviolet detector is employed. Solvents and other components employed are to be of appropriate quality. Adjustment of the pH, if necessary, is effected using only the aqueous component of the mobile phase and not the mixture. If buffer solutions are used, adequate rinsing of the system is carried out with a mixture of water and the organic modifier of the mobile phase (5 per cent *V/V*) to prevent crystallisation of salts after completion of the chromatography.

Mobile phases may contain other components, e.g. a counter-ion for ion-pair chromatography or a chiral selector for chromatography using an achiral stationary phase.

DETECTORS

Ultraviolet/visible (UV/Vis) spectrophotometers, including diode array detectors, are the most commonly employed detectors. Fluorescence spectrophotometers, differential refractometers, electrochemical detectors, mass spectrometers, light scattering detectors, radioactivity detectors or other special detectors may also be used.

METHOD

Equilibrate the column with the prescribed mobile phase and flow rate, at room temperature or at the temperature specified in the monograph, until a stable baseline is achieved. Prepare the solution(s) of the substance to be examined and the reference solution(s) required. The solutions must be free from solid particles.

Criteria for assessing the suitability of the system are described in the chapter on *Chromatographic separation techniques* (*2.2.46*). The extent to which adjustments of parameters of the chromatographic system can be made to satisfy the criteria of system suitability are also given in this chapter.