and 120 per cent). Treat with the prescribed reagent, if necessary, and record the reflectance, the transmittance or fluorescence in the chromatograms obtained with the test and reference solutions. Use the measured results for the calculation of the amount of substance in the test solution.

Substances containing radionuclides. Prepare and apply a test solution containing about 100 per cent of the expected value. Determine the radioactivity as a function of the path length and report the radioactivity in each resulting peak as a percentage of the total amount of radioactivity.

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.

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2.2.28. GAS CHROMATOGRAPHY

Gas chromatography (GC) is a chromatographic separation technique based on the difference in the distribution of species between two non-miscible phases in which the mobile phase is a carrier gas moving through or passing the stationary phase contained in a column. It is applicable to substances or their derivatives which are volatilised under the temperatures employed.

GC is based on mechanisms of adsorption, mass distribution or size exclusion.

APPARATUS

The apparatus consists of an injector, a chromatographic column contained in an oven, a detector and a data acquisition system (or an integrator or a chart recorder). The carrier gas flows through the column at a controlled rate or pressure and then through the detector.

The chromatography is carried out either at a constant temperature or according to a given temperature programme.

INJECTORS

Direct injections of solutions are the usual mode of injection, unless otherwise prescribed in the monograph. Injection may be carried out either directly at the head of the column using a syringe or an injection valve, or into a vaporisation chamber which may be equipped with a stream splitter.

Injections of vapour phase may be effected by static or dynamic head-space injection systems.

Dynamic head-space (purge and trap) injection systems include a sparging device by which volatile substances in solution are swept into an absorbent column maintained at a low temperature. Retained substances are then desorbed into the mobile phase by rapid heating of the absorbent column.

Static head-space injection systems include a thermostatically controlled sample heating chamber in which closed vials containing solid or liquid samples are placed for a fixed period of time to allow the volatile components of the sample to reach equilibrium between the non-gaseous phase and the vapour phase. After equilibrium has been established, a predetermined amount of the head-space of the vial is flushed into the gas chromatograph.

STATIONARY PHASES

Stationary phases are contained in columns which may be:

- a capillary column of fused-silica whose wall is coated with the stationary phase,
- a column packed with inert particles impregnated with the stationary phase,
- a column packed with solid stationary phase.

Capillary columns are 0.1 mm to 0.53 mm in internal diameter (Ø) and 5 m to 60 m in length. The liquid or stationary phase, which may be chemically bonded to the inner surface, is a film 0.1 µm to 5.0 µm thick.

Packed columns, made of glass or metal, are usually 1 m to 3 m in length with an internal diameter (Ø) of 2 mm to 4 mm. Stationary phases usually consist of porous polymers or solid supports impregnated with liquid phase.

Supports for analysis of polar compounds on columns packed with low-capacity, low-polarity stationary phase must be inert to avoid peak tailing. The reactivity of support materials can be reduced by silanising prior to coating with liquid phase. Acid-washed, flux-calcinated diatomaceous earth is often used. Materials are available in various particle sizes, the most commonly used particles are in the ranges of 150 µm to 180 µm and 125 µm to 150 µm.

MOBILE PHASES

Retention time and peak efficiency depend on the carrier gas flow rate; retention time is directly proportional to column length and resolution is proportional to the square root of the column length. For packed columns, the carrier gas flow rate is usually expressed in millilitres per minute at atmospheric pressure and room temperature. Flow rate is measured at the detector outlet, either with a calibrated mechanical device or with a bubble tube, while the column is at operating temperature. The linear velocity of the carrier gas through a packed column is inversely proportional to the square root of the internal diameter of the column for a given flow volume. Flow rates of 60 ml/min in a 4 mm internal diameter column and 15 ml/min in a 2 mm internal diameter column, give identical linear velocities and thus similar retention times.

Helium or nitrogen are usually employed as the carrier gas for packed columns, whereas commonly used carrier gases for capillary columns are nitrogen, helium and hydrogen.

DETECTORS

Flame-ionisation detectors are usually employed but additional detectors which may be used include: electron-capture, nitrogen-phosphorus, mass spectrometric, thermal conductivity, Fourier transform infrared spectrophotometric, and others, depending on the purpose of the analysis.

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,