the case of a magnetic analyser where the *m/z* ratio varies exponentially with the value of the magnetic field, there should be as many points as possible.

SIGNAL DETECTION AND DATA PROCESSING

Ions separated by an analyser are converted into electric signals by a detection system such as a photomultiplier or an electron multiplier. These signals are amplified before being re-converted into digital signals for data processing, allowing various functions such as calibration, reconstruction of spectra, automatic quantification, archiving, creation or use of libraries of mass spectra. The various physical parameters required for the functioning of the apparatus as a whole are controlled by computer.

01/2008:20244

2.2.44. TOTAL ORGANIC CARBON IN WATER FOR PHARMACEUTICAL USE

Total organic carbon (TOC) determination is an indirect measure of organic substances present in water for pharmaceutical use. TOC determination can also be used to monitor the performance of various operations in the preparation of medicines.

A variety of acceptable methods is available for determining TOC. Rather than prescribing a given method to be used, this general chapter describes the procedures used to qualify the chosen method and the interpretation of results in limit tests. A standard solution is analysed at suitable intervals, depending on the frequency of measurements; the solution is prepared with a substance that is expected to be easily oxidisable (for example, sucrose) at a concentration adjusted to give an instrument response corresponding to the TOC limit to be measured. The suitability of the system is determined by analysis of a solution prepared with a substance expected to be oxidisable with difficulty (for example, 1,4-benzoquinone).

The various types of apparatus used to measure TOC in water for pharmaceutical use have in common the objective of completely oxidising the organic molecules in the sample water to produce carbon dioxide followed by measurement of the amount of carbon dioxide produced, the result being used to calculate the carbon concentration in the water.

The apparatus used must discriminate between organic and inorganic carbon, the latter being present as carbonate. The discrimination may be effected either by measuring the inorganic carbon and subtracting it from the total carbon, or by purging inorganic carbon from the sample before oxidisation. Purging may also entrain organic molecules, but such purgeable organic carbon is present in negligible quantities in water for pharmaceutical use.

Apparatus. Use a calibrated instrument installed either on-line or off-line. Verify the system suitability at suitable intervals as described below. The apparatus must have a limit of detection specified by the manufacturer of 0.05 mg or less of carbon per litre.

TOC water. Use highly purified water complying with the following specifications:

– conductivity: not greater than 1.0 $\mu S \cdot cm^{-1}$ at 25 °C,

– total organic carbon: not greater than 0.1 mg/l.

Depending on the type of apparatus used, the content of heavy metals and copper may be critical. The manufacturer's instructions should be followed.

Glassware preparation. Use glassware that has been scrupulously cleaned by a method that will remove organic matter. Use *TOC water* for the final rinse of glassware.

Standard solution. Dissolve *sucrose R*, dried at 105 °C for 3 h in *TOC water* to obtain a solution containing 1.19 mg of sucrose per litre (0.50 mg of carbon per litre).

Test solution. Using all due care to avoid contamination, collect water to be tested in an airtight container leaving minimal head-space. Examine the water with minimum delay to reduce contamination from the container and its closure.

System suitability solution. Dissolve *1,4-benzoquinone R* in *TOC water* to obtain a solution having a concentration of 0.75 mg of 1,4-benzoquinone per litre (0.50 mg of carbon per litre).

TOC water control. Use *TOC water* obtained at the same time as that used to prepare the standard solution and the system suitability solution.

Control solutions. In addition to the *TOC water control*, prepare suitable blank solutions or other solutions needed for establishing the baseline or for calibration adjustments following the manufacturer's instructions; run the appropriate blanks to zero the instrument.

System suitability. Run the following solutions and record the responses: *TOC water* (r_w); *standard solution* (r_s); *system suitability solution* (r_{ss}). Calculate the percentage response efficiency using the expression:

$$\frac{r_{ss} - r_w}{r_s - r_w} \times 100$$

The system is suitable if the response efficiency is not less than 85 per cent and not more than 115 per cent of the theoretical response.

Procedure. Run the test solution and record the response (r_u). The test solution complies with the test if r_u is not greater than $r_s - r_w$.

The method can also be applied using on-line instrumentation that has been adequately calibrated and shown to have acceptable system suitability. The location of instrumentation must be chosen to ensure that the responses are representative of the water used.

01/2008:20245

2.2.45. SUPERCRITICAL FLUID CHROMATOGRAPHY

Supercritical fluid chromatography (SFC) is a method of chromatographic separation in which the mobile phase is a fluid in a supercritical or a subcritical state. The stationary phase, contained in a column, consists of either finely divided solid particles, such as a silica or porous graphite, a chemically modified stationary phase, as used in liquid chromatography, or, for capillary columns, a cross-linked liquid film evenly coated on the walls of the column.

SFC is based on mechanisms of adsorption or mass distribution.

APPARATUS

The apparatus usually consists of a cooled pumping system, an injector, a chromatographic column, contained in an oven, a detector, a pressure regulator and a data acquisition device (or an integrator or a chart recorder).

Pumping system

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-damping device. Tubing and connections are capable of withstanding the pressures developed by the pumping system.

Microprocessor controlled systems are capable of accurately delivering a mobile phase in either constant or varying conditions, 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

Injection may be carried out directly at the head of the column using a valve.

Stationary phases

Stationary phases are contained in columns which have been described in the chapters on *Liquid chromatography* (*2.2.29*) (packed columns) and *Gas chromatography* (*2.2.28*) (capillary columns). A capillary column has a maximum internal diameter (Ø) of 100 µm.

Mobile phases

Usually the mobile phase is carbon-dioxide which may contain a polar modifier such as methanol, 2-propanol or acetonitrile. The composition, pressure (density), temperature and flow rate of the prescribed mobile phase may either be constant throughout the whole chromatographic procedure (isocratic, isodense, isothermic elution) or may vary according to a defined programme (gradient elution of the modifier, pressure (density), temperature or flow rate).

Detectors

Ultraviolet/visible (UV/Vis) spectrophotometers and flame ionisation detectors are the most commonly employed detectors. Light scattering detectors, infrared absorption spectrophotometers, thermal conductivity detectors or other special detectors may be used.

METHOD

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.

01/2008:20246

2.2.46. CHROMATOGRAPHIC SEPARATION TECHNIQUES

Chromatographic separation techniques are multi-stage separation methods in which the components of a sample are distributed between 2 phases, one of which is stationary, while the other is mobile. The stationary phase may be a solid or a liquid supported on a solid or a gel. The stationary phase may be packed in a column, spread as a layer, or distributed as a film, etc. The mobile phase may be gaseous or liquid or supercritical fluid. The separation may be based on adsorption, mass distribution (partition), ion exchange, etc., or may be based on differences in the physico-chemical properties of the molecules such as size, mass, volume, etc.

This chapter contains definitions and calculations of common parameters and generally applicable requirements for system suitability. Principles of separation, apparatus and methods are given in the following general methods:

- paper chromatography (*2.2.26*),
- thin-layer chromatography (*2.2.27*),
- gas chromatography (*2.2.28*),
- liquid chromatography (*2.2.29*),
- size-exclusion chromatography (*2.2.30*),
- supercritical fluid chromatography (*2.2.45*).

DEFINITIONS

The following definitions have been used to calculate the limits in monographs.

With some equipment, certain parameters, such as the signal-to-noise ratio, can be calculated using software provided by the manufacturer. It is the responsibility of the user to ensure that the calculation methods used in the software are compatible with the requirements of the European Pharmacopoeia. If not, the necessary corrections must be made.

Chromatogram

A chromatogram is a graphical or other representation of detector response, effluent concentration or other quantity used as a measure of effluent concentration, versus time, volume or distance. Idealised chromatograms are represented as a sequence of gaussian peaks on a baseline.

RETENTION DATA

Retention time and retention volume

Retention measurements in elution chromatography may be given as the retention time (t_R) directly defined by the position of the maximum of the peak in the chromatogram. From the retention time, the retention volume (V_R) may be calculated.

$$V_R = v \times t_R$$

t_R = retention time or distance along the baseline from the point of injection to the perpendicular dropped from the maximum of the peak corresponding to the component,

v = flow rate of the mobile phase.