Solids. Ensure close and uniform contact between the substance to be examined and the whole crystal surface, either by applying pressure or by dissolving the substance in an appropriate solvent, then covering the crystal with the resulting solution and evaporating to dryness.

METHODS

Infrared spectroscopy is mostly used to identify substances, but it may also be carried out for quantitative applications. Quantitative analysis (based on the Beer-Lambert law, which relates the absorbance of a sample to its concentration) will not be described in this chapter.

The measurement is performed on an appropriately prepared sample. The data is then processed and evaluated, either to identify substances or quantify them (e.g. based on integration of IR-absorption bands).

Spectral quality may be enhanced by mathematical pretreatments. In practice, these are limited to spectral normalisation and subtraction of bands caused by carbon-dioxide and water vapour. The same pretreatments are performed on both the sample and the reference spectra.

Identification

Prepare the substance to be examined appropriately and record the spectra between 4000 and 650 cm^{-1}, unless otherwise prescribed.

Identification testing is performed by comparing the spectrum of the substance to be examined with the spectrum obtained from a Ph. Eur. chemical reference substance (CRS) or with a Ph. Eur. reference spectrum.

The spectrum of the current batch of the Ph. Eur. CRS may be recorded for immediate use or stored, for example, in a spectral library for future consultation. A stored spectrum may be used, provided traceability to the current batch of CRS is ensured.

In the case of substances that are not covered by individual monographs, a suitable reference standard may be used.

In all cases, spectra must be recorded using the same operating conditions and procedure, and especially the same measurement mode.

When comparison of the spectra recorded in the solid state show differences (see below), treat the substance to be examined and the reference substance in the same manner so that they recrystallise or are produced in the same crystalline form, or proceed as prescribed in the monograph, then record the spectra again. However, this procedure must only be done for substances where the monograph does not cover a particular form of a substance that exhibits polymorphism.

Several comparison procedures may be used, and the analyst must document and justify the method used and the specific acceptance criteria that allow a conclusion for identification. The spectra can be compared either by overlaying the spectra (in the whole spectral range or in the region of interest specified in the monograph) or by using mathematical calculations from the software. It is possible for example to perform:

- visual comparison based on band positions and relative intensities unless otherwise specified - the transmission minima (or absorption maxima) in the spectrum obtained with the substance to be examined correspond in position and relative size to those of the reference;
- calculation of the correlation coefficient between the 2 spectra - this value is calculated by the software and the identification threshold is defined by the user;
- evaluation by chemometric methods (e.g. Euclidean distance, Mahalanobis distance, classification methods); these methods involve the set-up, assessment and validation of the chemometric model by the analyst (see *5.21. Chemometric methods applied to analytical data*).

Impurities in gases

For the analysis of impurities, use a cell transparent to infrared radiation and of suitable optical pathlength (e.g. 1-20 m). Fill the cell as prescribed under Gases. For detection and quantification of the impurities, proceed as prescribed in the monograph.

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2.2.25. ABSORPTION SPECTROPHOTOMETRY, ULTRAVIOLET AND VISIBLE

PRINCIPLE

Ultraviolet and visible (UV-Vis) spectroscopy (or spectrophotometry) is based on the ability of atoms, molecules and ions to absorb light at specific wavelengths in the ultraviolet (approximately 180-400 nm) and visible (approximately 400-800 nm) range. This absorption is associated with changes in electronic energy in the form of temporary transitions of electrons to an excited state at a higher energy orbital. As each energy level of a molecule or molecular ion also has associated vibrational and rotational sub-levels, this results in many permitted transitions, which are generally impossible to separate, thereby producing absorption bands rather than sharp lines. These bands are characteristic of the functional groups and bonds in a molecule.

UV-Vis spectroscopy measurements involve exposing a sample to light and measuring the attenuation and/or scattering of the emerging (transmitted or reflected) light either at a single wavelength or over a specified wavelength range.

APPLICATIONS

UV-Vis spectroscopy is traditionally used for the quantitative and qualitative analysis of liquid samples, but is also suitable for solid and gaseous analytes and has other applications such as the determination of physical or chemical properties.

UV-Vis spectroscopy as described in this chapter can be applied in various ways:

- when a monograph or general chapter refers to this chapter, the requirements described in the relevant paragraphs of this chapter are mandatory;
- when used as the detection method in chromatographic systems as described in general chapter *2.2.46*, the requirements listed in the relevant paragraphs of this chapter are mandatory;
- when used as a process analytical technology (PAT) tool for PAT applications similar to the applications described in this chapter, the provisions herein apply; for other PAT applications, the principles are the same, however the criteria are established bearing in mind the intended purpose of the analysis, using a risk-based approach.

EQUIPMENT

Spectrophotometers used for carrying out measurements in the UV-Vis region typically consist of:

- a suitable light source (such as a deuterium lamp for the UV region, a tungsten-halogen lamp for the visible region or a xenon lamp to cover the entire UV-Vis range); UV-Vis spectrophotometers often have 2 sources;
- a monochromator such as a grating system;
- other optical components, such as lenses or mirrors, that relay light through the instrument and that may also be used to generate more than one beam of light, i.e. in double-beam spectrophotometers, as opposed to single-beam spectrophotometers;

- a sample container, holder or sampling device; examples include conventional cuvettes, fibre-optic probes and immersed transmission cells (e.g. high-purity quartz or sapphire transparent to UV-Vis radiation); the choice depends on the intended application, paying particular attention to its suitability for the type of sample to be analysed;
- a single-channel (e.g. photomultiplier, photodiode) or multi-channel detector (e.g. photodiode array (PDA) or charge-coupled device (CCD));
- suitable computerised data processing and evaluation systems.

Control of cuvettes. For benchtop instruments, cuvettes or cells with a defined path length are used. These can be made of different materials such as quartz or glass. The tolerance for the path length of quartz and glass cuvettes is ± 0.5 per cent (e.g. ± 0.005 cm for a 1 cm cuvette). Plastic cuvettes may also be used but the tolerance interval is wider; therefore, their use must be thoroughly justified and based on a risk assessment.

The following method may be applied to check the cleanliness of optical cuvette windows and any significant differences in their thickness or parallelism: fill the cuvette with *water R* and measure its apparent absorbance against air at 240 nm for quartz cuvettes and 650 nm for glass cuvettes; rotate the cuvette 180° in its holder and measure the apparent absorbance again at the same wavelength.

When using scanning instruments, it is recommended to scan over the optical region of interest.

When using double-beam spectrophotometers, measures should be taken (e.g. matching the cuvettes) to ensure that any difference between the absorbance of the cuvettes will not have a significant impact on the analysis to be performed.

Acceptance criteria:

- the apparent absorbance is not greater than 0.093 for 1 cm quartz cuvettes (UV region) and 0.035 for 1 cm glass cuvettes (visible region);
- the absorbance measured after rotation (180°) does not differ by more than 0.005 from the value previously obtained.

MEASUREMENT

Transmission mode. Transmission mode provides a measure of the transmittance (T), at a given UV-Vis wavelength, of a sample placed between the light source and the detector. Transmittance is the ratio of the intensity of the transmitted light to the intensity of the incident light and is given by the following equation:

$$T = \frac{I}{I_0}$$

I = intensity of transmitted radiation;

I_0 = intensity of incident radiation.

A spectrum may be obtained by plotting the variation in transmittance (T) or absorbance (A) as a function of wavelength.

The absorbance is defined as the logarithm to base 10 of the reciprocal of the transmittance for monochromatic radiation. It is a dimensionless quantity expressed in absorbance units (AU), given by the following equation:

$$A = \log_{10}\left(\frac{1}{T}\right) = \log_{10}\left(\frac{I_0}{I}\right)$$

According to the Beer-Lambert law, which applies to clear diluted solutions, in the absence of interfering physico-chemical factors, the absorbance (A) is proportional to the path length (l) of the radiation through the sample, and to the concentration (c) of the substance in solution in accordance with the following equation:

$$A = \varepsilon c l$$

ε = molar absorption coefficient, in litres per mole per centimetre;

c = molar concentration of the substance in solution, in moles per litre;

l = absorption path length, in centimetres.

The specific absorbance ($A_{1\text{ cm}}^{1\text{ per cent}}$) of the substance is generally used in monographs and is related to absorbance (A) as follows:

$$A = A_{1\text{ cm}}^{1\text{ per cent}} \times c_m \times l$$

c_m = mass concentration of the substance in solution, in grams per 100 millilitres.

$A_{1\text{ cm}}^{1\text{ per cent}}$ represents the specific absorbance of a dissolved substance and refers to the absorbance of a 1 g/100 mL (or 1 per cent *m/V*) solution in a 1 cm cuvette or cell and is measured at a defined wavelength. The relationship between $A_{1\text{ cm}}^{1\text{ per cent}}$ and ε is:

$$A_{1\text{ cm}}^{1\text{ per cent}} = \frac{10\varepsilon}{M_r}$$

M_r = relative molecular mass.

Transmittance or absorbance measurements are generally used for liquids (dispersions and solutions), but can also be used for solids (including tablets and capsules). For measurements of solids, a suitable sample accessory is used. Liquid samples are examined using a cell or cuvette with a suitable path length (typically 0.01-1 cm) and made of a material that is transparent to UV-Vis radiation, or by using a fibre-optic probe of a suitable configuration immersed in the liquid.

Diffuse reflection mode. Diffuse reflection mode provides a measure of reflectance (R), which is given by the following equation:

$$R = \frac{I}{I_0}$$

I = intensity of light reflected and/or scattered from the sample;

I_0 = intensity of light reflected and/or scattered from a blank or reference reflective surface.

Depending on the chemical composition and physical characteristics of the sample, the UV-Vis radiation may be absorbed as it passes through the sample. In diffuse reflection mode, it is the non-absorbed radiation which is partially reflected and/or scattered back from the sample that is measured by the detector. UV-Vis reflectance spectra are typically obtained by calculating and plotting $\log_{10}(1/R)$ as a function of the wavelength.

This measurement mode is generally selected for solids. The sample is examined either in a suitable device (e.g. a sample holder) or in direct contact with a probe. For process monitoring, the material can be analysed through a polished window interface (e.g. quartz or sapphire), or in-line using a

probe. Care must be taken to ensure that the measurement conditions are as reproducible as possible from one sample to another.

Operation of the equipment. The factors below affect the spectral response and must always be taken into account.

Choose a measurement mode that is appropriate for the intended application and the sample type.

Define the measuring conditions taking into account the sample size and sample probe in such a way as to obtain a satisfactory signal-to-noise ratio (e.g. beam size, measurement time and number of measurements). For scanning spectrophotometers, also select the scan range, scan rate and slit-width that provide the necessary optical resolution for the intended application without losing the required signal-to-noise ratio or the linearity of the analytical method. When using spectrophotometers with array sensors, there is no need to adjust the beam size, scan range, scan rate or slit-width since the optical resolution is typically fixed and the full spectrum is always recorded.

Before an absorbance measurement is carried out, the zero position of the absorption (baseline correction) should be set or determined for the wavelengths of interest or over the appropriate range of wavelengths.

For PAT applications, when measuring moving materials or samples, ensure that there is no fouling of the sensor (e.g. no contamination or build-up of material).

Unless otherwise prescribed in the monograph, measure the absorbance using a path length of 1 cm at the prescribed wavelength. If a single value for the position of an absorption maximum or minimum is given in a monograph, the user must determine the wavelength position. The value obtained may differ by not more than ± 2 nm, unless otherwise prescribed.

Quantitative measurements relying on absorption values above 2.0 should be avoided.

Background correction. Select a suitable spectroscopic blank (e.g. air, blank solvent, solid material). Unless otherwise prescribed, all measurements are carried out with reference to the same solvent or the same mixture of solvents (blank).

Measure the blank and the sample within a short time-frame either in parallel in double-beam spectrophotometers or sequentially in single-beam spectrophotometers. The absorbance values of both blank and sample must be in the working range of the equipment as specified by the manufacturer.

For benchtop instruments, the absorbance of the solvent measured against air and at the prescribed wavelength must not exceed 0.4 and is preferably less than 0.2.

For chromatographic systems, the transmittance of the mobile phase may be used as the blank.

In some PAT applications, it may be impossible to remove the probe for background data collection. Various options are therefore to be considered, including the use of internal references, measurement of a blank using a second detector, etc. Only spectra measured against a blank possessing the same optical properties can be directly compared with one another.

For reflectance measurements, common reflectance blank samples include ceramics, fluoropolymers such as polytetrafluoroethylene (PTFE) and powders such as barium sulfate ($BaSO_4$) and magnesium oxide (MgO), but other suitable materials may also be used.

MATHEMATICAL TREATMENT OF SPECTRAL DATA

In the case of single wavelength analysis used to determine the concentration of an unknown sample (e.g. as prescribed in monographs), mathematical treatment consists in determining the regression of the photometric reading (absorbance) on the concentration of the standard samples.

In the case of full range spectra, data for both diffuse reflection and transmission modes may have to be treated before a classification or calibration model can be developed. The aim can be, for example, to reduce baseline drift or to correct for scatter caused by particle size changes in solid samples. For example, first-, second- or higher-order derivative spectra can typically be used to improve resolution or sensitivity. This pretreatment may be a useful means of simplifying the data and thereby reducing the variations that may cause interference in subsequently applied mathematical models.

A wide range of treatment methods, such as scaling, smoothing, normalisation and derivatisation, can be applied either singly or in combination. More information is available in general chapter *5.21. Chemometric methods applied to analytical data*.

CONTROL OF EQUIPMENT PERFORMANCE

Spectrophotometer performance is controlled (automatically or manually) at regular intervals as defined in the quality management system and dictated by the use of the equipment and the application. For example, equipment exposed to variations in temperature and humidity may need more frequent performance testing.

Requirements for control of equipment performance for the various measurement modes are summarised in Table 2.2.25.-1. Further such tests may be performed if appropriate.

Wavelength accuracy, absorbance accuracy and linearity are controlled using either certified reference materials such as solid filters or liquid filters in appropriate sealed cells, or solutions prepared in the laboratory as described below.

Table 2.2.25.-1. – *Minimum tests to be carried out for the control of equipment performance*

Purpose	Method	Wavelength accuracy	Absorbance accuracy	Photometric linearity	Stray light	Resolution/spectral bandwidth
Quantitative or limit test	Based on measurement of the absorbance at one or more identified wavelengths (e.g. assay or impurities test)	X	X	X	X	If required in the monograph
Identification test	Based on wavelength of absorption maxima and minima	X	-	-	X	-
	Based on absorption measurement and wavelength of absorption maxima	X	X	-	X	-
	Based on comparison of spectrum with that of reference substance	X	X	-	-	-

Control of wavelength accuracy. Control the wavelength accuracy of an appropriate number of bands in the intended spectral range using one or more reference materials; for example, use solid or liquid filters (e.g. *holmium perchlorate solution R*) to verify the position of absorption bands, or measure the emission from a light source to check emission-line position. Table 2.2.25.-2 shows examples of wavelengths used to check wavelength accuracy. When certified reference materials are used, the reference wavelength is that stated on the corresponding certificate.

Some instruments may have an automatic or inbuilt wavelength accuracy control feature.

Table 2.2.25.-2. – *Examples of wavelengths used for the control of wavelength accuracy*
*Note: the wavelength varies with the resolution of the instrument

Material		Wavelengths (nm)*
Solutions	Cerium in sulfuric acid	201.1; 211.4; 222.6; 240.4; 253.7
	Didymium in perchloric acid	511.8; 731.6; 794.2
	Holmium in perchloric acid	241.1; 287.2; 361.3; 451.4; 485.2; 536.6; 640.5
Solid filters	Didymium glass	513.5
	Holmium glass	279.3; 360.9; 453.4; 637.5
Lamps	Deuterium	486.0; 656.1
	Mercury (low pressure)	184.9; 253.7; 312.5; 365.0; 404.7; 435.8; 546.1; 577.0; 579.1
	Neon	717.4
	Xenon	541.9; 688.2; 764.2

For chromatographic systems, it is also possible to control wavelength accuracy by measuring the absorbance of a 0.05 mg/mL solution of *caffeine R* in *methanol R*; the absorption maximum is obtained at 272 nm and the minimum at 244 nm.

Acceptance criteria

It is recommended to test at least 2 wavelengths that bracket the intended spectral range.

For benchtop instruments, the tolerance for wavelength accuracy of UV-Vis spectroscopy in cuvettes is ± 1 nm at wavelengths below 400 nm and ± 3 nm at wavelengths of 400 nm and above.

For chromatographic systems, the tolerance for wavelength accuracy is ± 2 nm for the whole UV-Vis range.

For PAT applications, a tolerance of ± 2 nm for the UV-Vis range is recommended. However, wider tolerance intervals may be needed for some PAT applications, in which case the requisite wavelength accuracy must be defined by the user depending on the intended purpose, and using a risk-based approach.

The instrument parameters (especially the entrance optics such as slit-width or optical fibre diameter) influence the resolution and must be the same as those intended for the actual measurements.

Control of absorbance accuracy. Control the absorbance accuracy at an appropriate number of wavelengths in the intended spectral range, using suitable solid or liquid filters to check that the absorbance measured at the test wavelength matches the certified absorbance of the filter or the absorbance value that is calculated from a certified specific absorbance. *Nicotinic acid for equipment qualification CRS* may be used.

It is recommended to test absorbance accuracy at selected wavelengths using one or more solid or liquid filters with different absorbance levels; as a minimum, values at approximately the 2 limits of the expected absorbance range should be verified.

For chromatographic systems and PAT applications, the testing of absolute absorbance accuracy may not be necessary, providing that a standard curve is measured as required.

For measurements using *nicotinic acid for equipment qualification CRS*, the certified specific absorbance is given in the corresponding leaflet.

The solution of nicotinic acid can be prepared as follows: dissolve 57.0-63.0 mg of *nicotinic acid for equipment qualification CRS* in a 0.1 M hydrochloric acid solution prepared from *hydrochloric acid R* and dilute to 200.0 mL with the same acid solution; dilute 2.0 mL of the solution to 50.0 mL with the same acid solution to obtain a final concentration of 12 mg/L. These volumes can be adjusted to obtain nicotinic acid solutions with other concentrations (up to about 40 mg/L), for the purposes of testing different absorbance levels. The absorbance is measured at 213 nm and 261 nm.

Acceptance criteria

The difference between the measured absorbance and the absorbance of the certified material is ± 0.010 or ± 1 per cent, whichever is greater, for each combination of wavelength and absorbance assessed (applies to absorbance values not greater than 2). Tolerances for higher absorbance values should be defined on the basis of a risk assessment.

Control of photometric linearity. Control the photometric linearity in the intended spectral range. In the ultraviolet range, the filters used to control absorbance accuracy may be used, as can solutions of nicotinic acid or caffeine. In the visible range, neutral glass filters may be used. Prior to performing the test, ensure that the absorbance of the standards is compatible with the intended linear range.

Solutions with increasing concentrations (e.g. 5-40 mg/L) of *nicotinic acid for equipment qualification CRS* in a 0.1 M hydrochloric acid solution prepared from *hydrochloric acid R* may be used. The absorbance is measured at 213 nm and 261 nm.

For chromatographic systems, it is also possible to check photometric linearity using 0.5-50 mg/L solutions of *caffeine R* in *water for chromatography R*. The absorbance is measured at 273 nm.

Acceptance criterion

The coefficient of determination (R^2) is not less than 0.999.

Limit of stray light. Stray light is determined at an appropriate wavelength using suitable solid or liquid filters or solutions prepared in-house. The instrument parameters used for the test, such as slit-width and type of light source (e.g. deuterium or tungsten lamp), must be the same as those intended for the actual measurements.

Acceptance criterion

The acceptance criterion depends on the filters or solutions used, for example:

- the absorbance is not less than 3.0 when using a 10 g/L solution of *sodium iodide R* at 220 nm, a 10 g/L solution of *potassium iodide R* at 250 nm or a 50 g/L solution of *sodium nitrite R* at 340 nm and 370 nm;
- the absorbance is not less than 2.0 when using a 12 g/L solution of *potassium chloride R* at 198 nm.

These values apply when using a 1 cm cell and *water R* as the compensation liquid.

Control of resolution. Where prescribed in a monograph, measure the resolution of the equipment either using suitable certified reference materials, or by recording the spectrum

of a 0.02 per cent *V/V* solution of *toluene R* in *hexane R* or *heptane R*, with respectively *hexane R* or *heptane R* as the compensation liquid.

Acceptance criterion

For measurements taken with a solution prepared as described above, the minimum ratio of the absorbance at the maximum (269 nm) to that at the minimum (266 nm) is stated in the monograph.

SYSTEM SUITABILITY

System suitability tests may be required prior to sample measurement to verify critical parameters that may have an impact on the result.

These tests may cover wavelength accuracy, absorbance accuracy, stray light and photometric linearity. System functionality tests, for example those performed as part of equipment autotesting, may be considered part of the system suitability tests.

In the case of UV-Vis detection for chromatographic systems, additional system suitability tests are applicable if prescribed in the monograph and/or in general chapter *2.2.46. Chromatographic separation techniques*.

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2.2.26. PAPER CHROMATOGRAPHY

ASCENDING PAPER CHROMATOGRAPHY

Apparatus. The apparatus consists of a glass tank of suitable size for the chromatographic paper used, ground at the top to take a closely fitting lid. In the top of the tank is a device which suspends the chromatographic paper and is capable of being lowered without opening the chamber. In the bottom of the tank is a dish to contain the mobile phase into which the paper may be lowered. The chromatographic paper consists of suitable filter paper, cut into strips of sufficient length and not less than 2.5 cm wide; the paper is cut so that the mobile phase runs in the direction of the grain of the paper.

Method. Place in the dish a layer 2.5 cm deep of the mobile phase prescribed in the monograph. If prescribed in the monograph, pour the stationary phase between the walls of the tank and the dish. Close the tank and allow to stand for 24 h at 20 °C to 25 °C. Maintain the tank at this temperature throughout the subsequent procedure. Draw a fine pencil line horizontally across the paper 3 cm from one end. Using a micro pipette, apply to a spot on the pencil line the volume of the solution prescribed in the monograph. If the total volume to be applied would produce a spot more than 10 mm in diameter, apply the solution in portions allowing each to dry before the next application. When more than one chromatogram is to be run on the same strip of paper, space the solutions along the pencil line at points not less than 3 cm apart. Insert the paper into the tank, close the lid and allow to stand for 1 h 30 min. Lower the paper into the mobile phase and allow elution to proceed for the prescribed distance or time. Remove the paper from the tank and allow to dry in air. Protect the paper from bright light during the elution process.

DESCENDING PAPER CHROMATOGRAPHY

Apparatus. The apparatus consists of a glass tank of suitable size for the chromatographic paper used, ground at the top to take a closely fitting glass lid. The lid has a central hole about 1.5 cm in diameter closed by a heavy glass plate or a stopper. In the upper part of the tank is suspended a solvent trough with a device for holding the chromatographic paper. On each side of the trough, parallel to and slightly above its upper edges, are two glass guide rods to support the paper in such a manner that no part of it is in contact with the walls of the tank. The chromatographic paper consists of suitable filter paper, cut into strips of sufficient length, and of any convenient width between 2.5 cm and the length of the trough; the paper is cut so that the mobile phase runs in the direction of the grain of the paper.

Method. Place in the bottom of the tank a layer 2.5 cm deep of the solvent prescribed in the monograph, close the tank and allow to stand for 24 h at 20 °C to 25 °C. Maintain the tank at this temperature throughout the subsequent procedure. Draw a fine pencil line horizontally across the paper at such a distance from one end that when this end is secured in the solvent trough and the remainder of the paper is hanging freely over the guide rod, the line is a few centimetres below the guide rod and parallel with it. Using a micro-pipette, apply on the pencil line the volume of the solution prescribed in the monograph. If the total volume to be applied would produce a spot more than 10 mm in diameter, apply the solution in portions, allowing each to dry before the next application. When more than one chromatogram is to be run on the same strip of paper, space the solutions along the pencil line at points not less than 3 cm apart. Insert the paper in the tank, close the lid, and allow to stand for 1 h 30 min. Introduce into the solvent trough, through the hole in the lid, a sufficient quantity of the mobile phase, close the tank and allow elution to proceed for the prescribed distance or time. Remove the paper from the tank and allow to dry in air. The paper should be protected from bright light during the elution process.

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2.2.27. THIN-LAYER CHROMATOGRAPHY

Thin-layer chromatography is a separation technique in which a stationary phase consisting of an appropriate material is spread in a uniform thin layer on a support (plate) of glass, metal or plastic. Solutions of analytes are deposited on the plate prior to development. The separation is based on adsorption, partition, ion-exchange or on combinations of these mechanisms and is carried out by migration (development) of solutes (solutions of analytes) in a solvent or a suitable mixture of solvents (mobile phase) through the thin-layer (stationary phase).

APPARATUS

Plates. The chromatography is carried out using pre-coated plates as described under *Reagents* (*4.1.1*). The particle size of the silica gel is indicated after the name of the reagent in the tests where it is used.

Pre-treatment of the plates. It may be necessary to wash the plates prior to separation. This can be done by migration of an appropriate solvent. The plates may also be impregnated by procedures such as development, immersion or spraying. At the time of use, the plates may be activated, if necessary, by heating in an oven at 120 °C for 20 min.

Chromatographic tank with a flat bottom or twin trough, of inert, transparent material, of a size suitable for the plates used and provided with a tightly fitting lid. For horizontal development the tank is provided with a trough for the mobile phase and it additionally contains a device for directing the mobile phase to the stationary phase.

Micropipettes, microsyringes, calibrated disposable capillaries or other application devices suitable for the proper application of the solutions.

Fluorescence detection device to measure direct fluorescence or the inhibition of fluorescence.