Technical guide for the ELABORATION OF MONOGRAPHS

European Pharmacopoeia

EDQM
8th Edition
2022

European Directorate for the Quality of Medicines & HealthCare | Direction européenne de la qualité du médicament & soins de santé

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I. INTRODUCTION

I.1 PURPOSE OF THE GUIDE

This document is a guide for the authors of monographs and also a means of communicating the principles for the elaboration and revision of monographs to the users of the European Pharmacopoeia (Ph. Eur.), especially industry, licensing authorities and official medicines control laboratories. Since the principles applied and guidance given for the elaboration and revision of monographs should be the same as those applied by licensing authorities, this Technical guide may also serve as a guideline in the elaboration of specifications intended for inclusion in marketing authorisation applications.

It is necessary to bear in mind that a monograph will be a mandatory standard and must be applicable in marketing authorisation procedures in all states parties to the Convention on the Elaboration of a European Pharmacopoeia (hereinafter the "European Pharmacopoeia Convention").

The term "elaboration" used hereinafter in this guide covers both "elaboration" and/or "revision".

I.2 ANALYTICAL PROCEDURES

The analytical procedures chosen for the identification tests, purity tests and assay(s) constituting the bulk of a pharmacopoeial monograph are preferably those already described and utilised in the Ph. Eur. In this context, the author of a monograph is referred not only to the General Chapters of the Ph. Eur. but also to published monographs on similar materials. The above considerations are intended to ensure a reasonable degree of harmonisation within the Ph. Eur. and only apply in cases where the procedures are found to be adequate for the specific purposes. However, due attention is also to be paid to the development of new procedures that offer significant improvements in terms of sensitivity, precision, accuracy or specificity/selectivity.

Analytical procedures included in monographs are validated as described in part III (ANALYTICAL VALIDATION) and other relevant specific parts of this guide. Validation reports are provided to the EDQM but are not published or otherwise provided to users.

The analytical procedures included in a monograph are validated and further verified in two or more laboratories. One of these may be the supplier of the procedure who initially validated it.

The laboratory reports on the validation and verification are to be provided to the EDQM to ensure future traceability.

The instructions for any analytical procedure cover all factors that may influence the results and that are deemed essential for an experienced analyst working according to acknowledged laboratory practices to be able to perform the analysis without necessarily having any prior knowledge of the investigation in question. Variations in the description of similar analytical procedures are to be avoided.

If it is expected that an analytical procedure will be used generally or if it requires a lengthy description and is used more than once, it may be proposed for inclusion in the general chapters of the Ph. Eur., to be referred to in the individual monographs. The procedures are prescribed on the scale conventionally applied in the Ph. Eur. except in cases where for reasons of availability of the material to be analysed, or because of its toxicity or its cost, work on a small scale would be advantageous.

I.3 EQUIPMENT

If the equipment utilised for an analytical procedure is not generally available in the states parties to the European Pharmacopoeia Convention, it must be possible to have it constructed according to its description in the Ph. Eur.

I.4 QUANTITIES

In prescribing the quantities (i.e. masses and volumes of substances, reagents and solvents to be taken for analysis), it is the practice of the Ph. Eur. to indicate, with the given number of significant figures, the exact target quantity value that is to be measured (see paragraph on Quantities in the *General Notices*). It is therefore necessary to take this aspect into consideration when drafting pharmacopoeial texts.

Table 1, which provides estimations of relative uncertainty, is to be consulted as a guide for minimising errors in the preparation of analytical solutions.

In order to avoid either the use of extremely low amounts or unnecessarily large quantities of solvents, a dilution series will often have to be prescribed for the preparation of dilute solutions used particularly for spectrophotometric measurement. In this case, not all combinations of (usually two or three) dilution steps will contribute equally to the random error of the dilution procedure. If critical for the purpose, the optimal dilution is prescribed in consideration of the relative errors (capacity tolerance divided by nominal volume) associated with the various sizes of volumetric pipettes and volumetric flasks commonly used for these operations The standard formula for estimating relative dilution error is the square root of the sum of the squares of individual relative errors.

Tables giving the optimal number and nature of dilution steps needed to achieve a given dilution ratio, based upon given specifications for the capacity tolerances of volumetric glassware, are available in the literature. For guidance, see Table 2 (note that these factors do not include reading errors).

Table 1 – Relative uncertainties in the preparation of analytical solutions

Concentration to be prepared	Preparation of solution	Percentage relative uncertainty		
		Mass	Volume	Total
10 g/1000 mL	10 g/1000 mL	< 0.01	0.05	0.05
	1 g/100 mL	0.02	0.12	0.12
	0.5 g/50 mL	0.04	0.17	0.17
	0.25 g/25 mL	0.08	0.23	0.24
	0.1 g/10 mL	0.02	0.50	0.54
1 g/1000 mL	1 g/1000 mL	0.02	0.05	0.05
	0.5 g/500 mL	0.04	0.07	0.08
	0.25 g/25 mL	0.08	0.23	0.24
	100 mg/100 mL	0.2	0.12	0.23
	50 mg/50 mL	0.4	0.17	0.43
	10 mg/10 mL	2.0	0.50	2.06
0.1 g/1000 mL	100 mg/1000 mL	0.2	0.05	0.21
	50 mg/500 mL	0.4	0.07	0.41
	25 mg/250 mL	0.8	0.08	0.80
	10 mg/100 mL	2.0	0.12	2.0
	5 mg/50 mL	4.0	0.17	4.0
	1 mg/10 mL	20.0	0.50	20.0
0.01 g/1000 mL	10 mg/1000 mL	2.0	0.05	2.0
	5 mg/500 mL	4.0	0.07	4.0
	1 mg/100 mL	20.0	0.12	20.0

An uncertainty of 0.2 mg for the weighing procedure has been assumed for the calculations of the percentage relative uncertainties.

Table 2 – Relative errors for dilution with analytical glassware (pipettes P/flasks F)

Concentration ratio	No. of steps	Step 1		Step 2		Relative error
		P	F	P	F	
1/2	1	25	50			0.16
1/2.5	1	20	50			0.18
1/5	1	20	100			0.17
1/10	1	25	250			0.13
1/12.5	1	20	250			0.16
1/30	1	15	500			0.20
1/50	1	20	1000			0.15
1/100	1	25	250	25	250	0.18
1/125	2	20	250	25	250	0.20
1/160	2	25	1000	25	100	0.19
1/200	2	25	500	25	100	0.18
1/250	2	20	250	25	500	0.20
1/400	2	25	250	25	1000	0.18
1/500	2	20	500	25	500	0.20
1/1000	2	20	1000	25	500	0.20

Adapted from R.B. Lam and T.L. Isenhour, Minimizing relative error in preparation of standard solutions by judicious choice of volumetric glassware, *Analytical Chemistry*, 1980, **53**, 1158-1161.

I.5 REAGENTS

When the quality of a reagent in one or more respects is critical for its intended use, it must be carefully defined by prescribing appropriate tests to demonstrate its suitability. Typically, analytical grade reagents are employed, in which case it is sufficient to give the name of the reagent, the CAS number and its formula.

Whenever possible, the reagent substances, reagent solutions, buffer solutions, volumetric solutions and standard solutions already described in Ph. Eur. general chapter *4. Reagents* are to be employed. Simple solutions of reagents that are prepared for single use are to be described in the monograph itself.

The use of reagents that are acknowledged to be extremely toxic or otherwise hazardous (e.g. carcinogenic) is to be avoided, especially in circumstances where their dangerous properties are difficult to control (e.g. when handled as fine powders or in spray reagents). The use of those substances that are prohibited or restricted in one or more of the states parties to the European Pharmacopoeia Convention is also to be avoided (mercury containing reagents, substances regulated through REACH regulation annex XIV, etc.). In monographs where these reagents are still described, the group of experts (GoE) concerned should initiate a revision of the relevant test with the objective of avoiding such reagents where possible.

I.6 COMMERCIAL NAMES

Commercial names for chromatography columns/plates and solvents/titrants/conditions for water determinations are always given as footnotes in draft monographs. Commercial names may also be provided for other products (test kits, reagents that are available from a single supplier or types of filter, etc.), depending on the perceived usefulness for analysts. These commercial names are transferred to the EDQM Knowledge Database after the monograph is adopted and are not published in the Ph. Eur.

I.7 REFERENCE STANDARDS

The general policy for Ph. Eur. reference standards is provided for information purposes in general chapter *5.12. Reference standards.* In addition to procuring candidates and establishing reference standards, the EDQM is responsible for storing and distributing reference standards. When candidate reference standards, notably impurity standards, are available only in limited quantities, the amount prescribed for the preparation of solutions is kept to a minimum. For the same reason, when a reference standard is introduced in a monograph or general chapter, consideration is to be given to its long-term sustainability. Before a monograph is published in *Pharmeuropa*, the required quantities of candidates should be supplied to the EDQM, who will advise on the best strategy for reference standards, while optimising the use of substances that are available in limited quantities (e.g. preparation of a spiked substance, use of a “dirty sample” or supply of the impurity alone). The EDQM aims to have the reference standards available at the date of publication of the monograph or, if this is not possible, by the time of implementation at the very latest. Having a sufficient amount of a suitable candidate reference available at the EDQM before the monograph is adopted is a pre-requisite for achieving this goal.

For infrared (IR) identification, preference is given to chemical reference substances (CRS) over reference spectra, except in special cases (e.g. when it is difficult to procure). In exceptional cases, for monographs on narcotic/psychotropic substances, the relevant GoE may decide to describe both a CRS and a reference spectrum in the identification test.

II. MONOGRAPHS ON SUBSTANCES FOR PHARMACEUTICAL USE [1]

Monographs are based on the specifications for substances used in medicinal products approved in member states. When a monograph is added to the work programme, enquiries are made by the EDQM to identify manufacturers of such substances and all data received are taken into account for the preparation of the monograph. Stakeholders are invited to collaborate on the elaboration of the monograph when the topic is added to the work programme so that their approved specifications can be taken in account.

Prior to the elaboration of a monograph, it is essential to gather as much information as possible on the substance in question. In particular, it is necessary to ascertain:

- whether the substance is of natural, synthetic or semi-synthetic origin;
- whether the substance is a mixture or a single entity;
- whether there are different crystalline forms, since the properties of the substance may vary in accordance with this parameter;
- whether both an enantiomer as well as the racemate or other mixtures of enantiomers are available;
- whether substances with a different degree of hydration (defined or variable) are available;
- whether the substance is available as a solvate (excluding hydrates);
- whether different entities (acid, base, salt, etc.) are available;
- where appropriate, the method(s) of preparation.

The Ph. Eur. and other relevant documents on the state of work must be consulted to see if monographs on similar substances exist or are being elaborated. If this is the case, it is important to ensure that similar monographs follow the same approach unless there are good reasons to deviate from it (e.g. developments in analytical techniques or different specifications).

When a substance exists both in a water-free form and in the form of one or more hydrates with different water contents, and if all these forms are used, they are normally treated as individual substances requiring separate monographs. The same rule applies for other solvates.

Substances that are to be described in a monograph may be members of a group of very similar substances (family). This holds true especially for excipients such as macrogols. In such cases, a master monograph (family monograph) is to be drafted clearly stating the attributes common to all members of the family and that can be used to identify single members of the family.

Most active substances and excipients are subject to the provisions of the general monograph

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[1] Where appropriate, the statements in this section apply to monographs on medicinal products, otherwise see other relevant technical guides such as the *Technical Guide for the elaboration of monographs on medicinal products containing chemically defined active substances*

Substances for pharmaceutical use (2034).

II.1 TITLE

The International Nonproprietary Name (INN) established by the World Health Organization (WHO) should be used wherever it is available, unless there are justifiable reasons for not doing so; it is supplemented as appropriate by the name of the anion or cation and by the degree of hydration. Anions and cations are indicated as “mono-, di-, tri-, etc.”, as appropriate.

The following rules apply for the degree of hydration:

- In the case of a well-defined hydrate, “hemi-, mono-, 1.5-, di-, tri-, etc. hydrate” is added to the title, whereas if the monograph covers more than one degree of hydration, the general term “hydrate” is used. In the latter case, a sentence is added to the DEFINITION section of the monograph (see part II.3). For monographs published prior to the 9th Edition of the Ph. Eur., retrospective introduction of the degree of hydration in titles would only be made after careful consideration.
- Since the 9th Edition of the Ph. Eur., monographs referring to “anhydrous” substances no longer specify this in their title, with the exception of a few monographs where this information has recognised added value and/or is used in common scientific language (e.g. *Ethanol, anhydrous*).
- No mention is added to the title of monographs covering substances that can be either water-free or with a defined or variable degree of hydration. This supplementary information is provided in the DEFINITION section of the monographs (see part II.3).

Where a substance is used in member states in approved medicinal products for veterinary use only, “for veterinary use” is included in the title.

II.2 FORMULAE, MASSES AND CAS NUMBERS

The chemical structure must be ascertained with the greatest possible care in order to establish the exact:

- graphic formula;
- empirical formula and relative molecular mass. The latter is calculated as follows: first, the relative atomic masses, or multiples thereof, are added together using all the figures of the International Table of Relative Atomic Masses; the total is then rounded off according to general rules and given to four significant figures if the molecular mass is below 600 or otherwise to three significant figures;
- the CAS number, included for information, wherever appropriate;
- chemical name (mentioned in the DEFINITION section of the monograph). This involves in particular:
    - investigating the possible existence of isomers so as to be able to specify which isomer is used or, failing that, to state that the product is a mixture of isomers;
    - in the case of a stereoisomer, it is not sufficient to take into account only the direction of the optical rotation. The absolute configuration is given by the appropriate IUPAC nomenclature at the asymmetrical centre(s), e.g. *R*/*S* system or any other appropriate

system, such as for carbohydrates and amino acids;
  - ascertaining the state of hydration so as to distinguish clearly between the well-defined hydrates (mono-, di-, tri-, etc. hydrate) and the products that contain variable quantities of water. In the latter case, the term "x-hydrate" is introduced in the chemical name.

II.3 DEFINITION

If the substance contains a variable quantity of water, or refers to both water-free and hydrate forms, a sentence is added to the DEFINITION section to explain the exact scope of the monograph.

Some chemical substances, particularly those obtained from raw materials of natural origin and substances produced by fermentation may not be easily separated from certain related substances (e.g. quinine salts). These may be treated as:

- a chemical product when obtained in a very pure state and when they can be assayed by a physico-chemical method;
- a substance accompanied by a certain proportion of related substances, giving an exact definition of the main component only (e.g. neomycin);
- a mixture of several components, sometimes difficult to define, where an overall description may suffice (e.g. nystatin).

Where applicable, the origin of the substance must be specified (name and strain of the organism from which the substance is derived). Where applicable, the monograph indicates that the substance is semi-synthetic and is derived from a fermentation product [to clarify application of the general monograph *Substances for pharmaceutical use (2034)*].

II.3.1. Combinations

In medicinal products, more or less well-defined chemical combinations (e.g. theophylline-ethylenediamine) or even mixtures are sometimes used. In such cases, it is necessary to specify each component of the combination or mixture precisely, with its chemical structure and the proportion in which it is present.

II.3.2. Content

The substance described by a monograph is never a wholly pure substance but contains a limited proportion of impurities. The content therefore forms an important part of the definition.

The content of the active substance must be within specified assay limits. These limits are established taking into account the following:

- the manufacturing process, which determines the degree of purity that may be reasonably achieved;
- the reproducibility and accuracy of the analytical procedure;
- current batch data of at least 10 production batches at release;
- the evaluation of stability data;
- a sufficient number of experimental results obtained on several batches (at least three),

if possible, of different origins and ages.

For a non-specific assay by titrimetry, the limits are set according to the table provided in part III.3.7 (i.e. usually 99.0-101.0%). Some monographs still include an assay by UV-Vis spectrophotometry, for which wider limits are generally set.

For a specific assay using a separation technique (for example, liquid or gas chromatography), the upper assay limit is normally 102.0%; the lower assay limit will take account of the impurities present based on the available batch/stability data and approved specifications. It may therefore be lower than 98.0%.

When the substance to be examined contains only impurities that do not interfere with the assay, or when it contains only a very low proportion of impurities interfering with the assay, the results of the assay can be used directly. It will then be stated that: "*[the substance] contains not less than x per cent and not more than the equivalent of y per cent* (at least 100.5%, but often a little more) *of [chemical definition of the pure product]*". The content of the substance is usually expressed with reference to the anhydrous or dried substance. According to the general monograph *Substances for pharmaceutical use (2034)*, the content of residual solvent is taken into account for calculation of the assay content of the substance, therefore no reference is made in the DEFINITION section of the individual monograph.

In cases where the water content is high (e.g. disodium phosphate dodecahydrate), content limits may be expressed with reference to the hydrate form of the substance, taking into account the molecular mass of the hydrate form (only for well-defined hydrates) or with reference to the substance on the anhydrous/dried basis in combination with determination of water content/loss on drying.

When the substance to be examined contains a relatively large proportion (a few %) of impurities that are determined at the same time as the active substance, appropriate wording is to be used (for instance, in the case of quinine salts: "*x per cent of total alkaloid salts, expressed as quinine salts*").

In exceptional cases, reference is made to only a part of the molecule or to an element (e.g. assay of magnesium oxide in light magnesium carbonate or assay of magnesium in magnesium stearate).

In the case of antibiotics determined by microbiological assays, the active substance content is expressed in International Units, where these exist, and only a minimum value is given.

See also part II.8.

II.4 PRODUCTION

Statements in the PRODUCTION section draw attention to particular aspects of the manufacturing process, but these are not necessarily exhaustive. They constitute mandatory requirements for manufacturers, unless otherwise stated. See the *General Notices* for further information.

II.5 CHARACTERS

As defined in the *General Notices*, statements in the CHARACTERS section of a monograph are not to be interpreted in a strict sense and are not regarded as requirements. The principal items that may be referred to under this section are outlined below.

II.5.1. Appearance

This description will typically cover colour and physical form. The term "white" is not used without qualification since, if viewed against a standard white material, very few pharmaceutical materials will appear truly white. Of course, it is not intended that such a comparison be made, but experience has shown that some users of the Ph. Eur. may insist on it as part of a purchasing contract. The term "white or almost white" is used instead. Where positive colours are to be described, this is done in terms of primary colours or combinations of primary colours.

II.5.2. Taste

Taste is not to be taken into consideration.

II.5.3. Odour

In general, no reference is made to odour, especially for materials that would constitute a hazard if inhaled. Mention of odour in other cases must be justified.

II.5.4. Solubility

For solid materials, all solubilities are quoted in the general terms defined in general chapter *5.11. Characters section in monographs*, which also includes a procedure recommended for the estimation of solubility. For liquid materials, it is stated whether they are miscible or not. Solvents quoted are normally restricted to water, an alcohol and a lipophilic solvent (e.g. water, ethanol (96 per cent) or anhydrous ethanol, heptane). Solubilities in chloroform and ether are not mentioned and the use of hexane is discouraged. In exceptional cases, the solubility of different samples of a material may vary considerably, despite their composition still being within the limits set by the monograph. More than one solubility class is therefore given to cover the solubilities in the solvents affected (e.g. "*sparingly soluble to soluble in...*"). In some cases, it may be useful to specify solubility in alkalis or acids and, especially for materials that are very insoluble in the above-mentioned solvents, a special solvent may be indicated (e.g. dimethylformamide or dimethyl sulfoxide). It is not necessary to specify the solubility in every solvent that is used in performing the tests of the monograph itself. The solubilities or miscibilities in other solvents with which the material is often combined in practice (e.g. fatty oils) may also be mentioned.

II.5.5. Stability factors

Evidence of instability due to exposure to air, light and moisture is to be given (e.g. physostigmine sulfate turns red when exposed to air and light). Any such statement in the CHARACTERS section is given separately from the description of a pharmacopoeial material.

II.5.6. Hygroscopicity

A pragmatic method recommended for the determination of the tendency of a substance to take up atmospheric water (rather than a true determination of hygroscopicity) is given in general chapter *5.11. Characters section in monographs*. Some substances are hygroscopic or deliquescent, which results in difficulties for the analyst during weighing procedures. In such cases, this is indicated using the terminology defined in general chapter *5.11* and serves as an alert that the analyst should take necessary precautions when handling the substance. When a substance is hygroscopic, a STORAGE section is added ("*in an airtight container*").

II.5.7. Solid-state properties

Solid-state properties include crystallinity, polymorphism, density of solids, particle size of solids and specific surface area of solids. Solid-state properties, particularly polymorphism and pseudopolymorphism, may have an effect on the bioavailability of the substance and for the production of the medicinal product. General chapter *5.9. Polymorphism* should be consulted.

A procedure recommended for the determination of crystallinity is given in general chapter *5.11. Characters section in monographs*.

Solid-state properties of excipients that are relevant for functionality may be covered in the FUNCTIONALITY-RELATED CHARACTERISTICS section (see part II.12).

The inclusion of a statement of polymorphism in a monograph is intended to alert users to the need to evaluate this phenomenon during the development of a medicinal product, see also part on infrared absorption spectrophotometry (II.6.3).

Two cases are to be distinguished when polymorphism is known to exist:

- usually, the monograph does not exclude any of the possible crystalline forms;
- exceptionally, if the substance is only used in solid dosage forms and one form has been preferred for bioavailability reasons or by virtue of having a better safety/efficacy profile, then the monograph may be limited to that form by adding the following sentence: "*Preparation: examine the substances without prior treatment*". The techniques required to identify the form are included in the IDENTIFICATION section.

II.5.8. Other characteristics

Other physical characteristics that may be useful for information purposes, but which are not sufficiently precise to be defined under the IDENTIFICATION or TESTS sections, may be stated in the CHARACTERS section. This would typically apply to a melting point that is insufficiently precise to allow a range to be quoted; if a range can be quoted, the melting point may be included in the IDENTIFICATION section. Any potential for decomposition must be stated. Other general characteristics that may need to be stated in the CHARACTERS section include an indication of direction of optical rotation in a particular solvent or, in the case of radioactive materials, a statement of the half-life of the radionuclide and the type of radiation it emits.

II.5.9. Behaviour in solution

In cases where it is known that degradation may occur in solution, a warning is included in the text. In this context:

- "*Freshly prepared solution*" means that the solution is prepared each time the test/assay is to be carried out and is used within 24 h;

- "*Immediately before use*" indicates that the stability of the corresponding solution(s) was found to be critical during the elaboration of the text. The time between preparation and use must be kept to a minimum.

Furthermore, and where applicable in the tests, it should be indicated that the solutions are to be stored at a certain temperature and kept at a certain temperature in an autosampler.

II.6 IDENTIFICATION

II.6.1. General

The purpose of the IDENTIFICATION section of a monograph is to provide confirmation of the identity of the substance in question. Identification according to the Ph. Eur. is thus generally much more limited in scope than the identification and/or structural elucidation of an unknown substance or the determination of the composition of an unknown mixture. The task of identifying a material is not to be confused with the assessment of its purity or the determination of its strength, although ultimately all three aspects are complementary.

Thus, when taken together, the physical and/or chemical tests and reactions included in the IDENTIFICATION section ensure, as far as possible, specificity. The specificity of the identification should be such that active substances and excipients exhibiting similar structures are distinguished from each other. The tests must not be too sensitive (false reactions caused by the presence of tolerated impurities are to be avoided) and they must not require more experimental effort than necessary in order to differentiate the substance in question from other commercially available pharmaceutical substances. The time needed to perform a test is also taken into account when considering experimental effort.

Typically, a single set of identification tests is given; however, some monographs may give two or more alternative sets of identification tests that are equivalent and may be used independently. The intended purpose of the alternative sets of tests is the same (e.g. verification that the correct enantiomer is present).

In addition, for some substances used in community pharmacies or hospital pharmacies, a second series of identification tests is given (see part II.6.2). This second identification series should not be confused with the alternative sets mentioned above.

Some of the purity tests in a monograph may also be suitable for identification purposes, possibly in a modified form. A system of cross-references to the TESTS or ASSAY section can be used. This is particularly relevant if distinction between closely related materials depends on properties that are also parameters in purity or composition control (water content of different hydrates, chiral chromatography of enantiomers or optical rotation, viscosity of chain-length homologues of a polymer, etc.). Cross-reference to the ASSAY section often consists of identification via comparison of retention times and peak sizes (areas) of the substance to be examined with those of a reference substance. Acceptance criteria (e.g. permitted deviations in retention times) are not typically given in the monograph but should be defined in the internal quality management systems on the user's site. The IDENTIFICATION section in the monograph suffices to identify the article even if it includes cross-references to other sections.

The monograph of a substance must not be treated in isolation. When an identification series is being investigated, it is desirable that other similar substances, regardless of whether they are the subject of pharmacopoeial monographs, are examined at the same time to ensure that a particular combination of tests within a series will successfully distinguish between two similar substances.

In the case of a family monograph, identification of the type of substances may be supplemented by non-specific but discriminating tests to identify individual members of the family.

Examples of methods of identification are listed below and detailed guidance concerning some of them is given throughout part II.6.

Instrumental methods:

- Spectroscopic analysis, such as recording of infrared (IR) or nuclear magnetic resonance (NMR) spectra;
- Chromatographic examination by means of gas chromatography (GC) or liquid chromatography (LC).

Other methods may be used if appropriate:

- Determination of physical constants such as melting point, freezing point, boiling point, specific optical rotation, ultraviolet spectrum, specific absorbance, relative density, refractive index and viscosity.
- Chemical reactions such as colour or precipitation reactions (including formation of derivatives or degradation products, which may subsequently be subjected to physical examination) and determination of chemical values (saponification, ester, hydroxyl and iodine values).
- Chromatographic examination by thin-layer chromatography (TLC) or high-performance thin-layer chromatography (HPTLC).

II.6.2. First and second identification series

Some monographs have subdivisions (i.e. series) entitled “First identification” and “Second identification”.

The test(s) that constitute the “First identification” may be used in all circumstances. Second identification testing is only intended to be used by community pharmacies or hospital pharmacies that compound unlicensed pharmaceutical preparations provided it can be demonstrated that the substance is fully traceable to a batch certified to comply with all the requirements of the monograph and that this is documented in a certificate of analysis.

The implementation of the tests in the second identification series is subject to national regulation. A second identification series is not intended to be applied by manufacturers for quality control purposes for approved medicinal products (it is implied that good manufacturing practice is applied).

The aim of the tests in the second identification series is to confirm the identity of the substance using affordable analytical instrumentation and accessible implementation methods, rather than relying on complex technologies. Wherever possible, it is recommended to use the principles of mixed melting point, refractive index and, as required, miniature TLC complemented by wetchemical testing. Second identification tests should provide the user with at least two results that confirm the identity of the substance. These results can either be obtained by two independent tests or by a single test that provides two or more pieces of information about the identity of the substance. The combination of refractive index with relative density is an example of the former; a TLC with the application of a detection reagent is an example of the latter.

In order to introduce a second identification series, it should be assessed on a case-by-case basis whether concrete knowledge is available that the substance is used:

- in a magisterial formulary or a pharmacopoeia; or
- in formulations made for special target groups or distinct medicinal indications where no licensed product exists; or
- for pharmacy compounding (e.g. when they are offered for this purpose by suppliers).

II.6.3. Infrared absorption spectrophotometry

This is generally considered to be a satisfactory single method for verifying the identity of non-ionised organic substances other than salts of organic acids or bases. This analytical technique always requires the use of a reference substance or a reference spectrum. Reference substances are preferred to reference spectra; the latter are used where there are practical difficulties with providing a reference substance (e.g. in cases of particular toxicity or instability).

Organic salts of organic substances and some inorganic salts of organic substances (e.g. phosphates and sulfates) can readily be distinguished from each other. In the case of sulfates, however, it is necessary to extend the usual range of recording from 4000-650 cm^{-1} to 4000-400 cm^{-1}.

Since monographs do not typically prescribe a specific mode, all modes described in general chapter *2.2.24* (e.g. ATR mode, transmission mode) may be used. The type of sample preparation (disk, halide salt plate, mull, etc.) is not specified unless this has been found to be necessary during the elaboration of the monograph in order to obtain a satisfactory spectrum.

In certain cases, the infrared spectrum alone is not sufficient to confirm the identity of a substance and other tests must also be performed.

- *Salts of organic acids or bases*: for several ions or groups that form part of an organic substance (counter-ion), more than one identification test may be described in general chapter *2.3.1*. However, it is usually only necessary to use one of them.
- *Chemically related substances*: in the case of substances closely related to the substance to be examined where variations in the spectra are not considered sufficient for unambiguous identification, the infrared identification test is accompanied by another simple test (e.g. melting point or TLC with the use of a reference substance).
- *Polymorphism*: the sentence "*It shows polymorphism*" is added only when more than one crystalline forms is used in approved medicinal products and the different forms are available for testing.
  General chapter *2.2.24. Absorption spectrophotometry, infrared* allows for "recrystallisation" before recording of the spectrum.
  If a monograph mentions polymorphism, a method for "recrystallisation" is described, unless the intention is to limit the scope of the monograph to the crystalline form represented by the chemical reference substance, in which case the monograph indicates that the spectrum is recorded "without recrystallisation".

In exceptional cases, if the monograph describes a specific crystalline form or forms and when the IR spectrum is not characteristic, an additional test is introduced.

- *Optical isomers*: to identify a particular enantiomer or a racemate, see part II.6.6.

II.6.4. Absorption spectrophotometry (ultraviolet and visible)

Unlike IR spectroscopy, this method is usually not specific enough for identification purposes unless the absorption curve exhibits several maxima and minima, unusually strong or weak regions of absorption, etc.

Reference substances are not generally used for identification. The UV-Vis spectrum of a substance is therefore rarely used as the sole identification criterion.

The concentration of the solution to be examined is such that the absorbance preferably lies between 0.5 and 1.5, measured in a 1 cm cell.

The range of wavelengths to be explored must be stated; it does not typically extend to the region where end-absorption and solvent interference may be expected. The wavelengths of sharp maxima and minima are indicated by a single number, signifying ± 2 nm, while for broader bands a range is given. When it is considered necessary to mention the wavelength of shoulders, the term "about" may be used.

Specific absorbances are also given as a range (usually ± 5%) in order to cover variations in content of absorbing substance and experimental error. It is to be noted that the instrument tolerance for absorbance is ± 0.010 or 1%, whichever is greater, which means that the deviation due to this source of variability will depend on the absolute levels of absorbance. Furthermore, the content of absorbing substance will vary with the permitted content of water (or other solvents); when the latter does not exceed 1% or is within well-defined limits, it will usually be adequate to calculate the specific absorbance for the substance "as is" and to set the limits accordingly. When more than a single maximum is present in the spectrum, the ratio(s) between their absorbances can be substituted for the individual specific absorbances, providing the ratio is less than or equal to 5, thus avoiding having to correct the absorbances for the solvent content of the substance.

Care must be taken in the choice of solvents and solvent purity prescribed for UV spectrophotometry in order to avoid the presence of impurities, which may influence the absorbance of the substances to be examined.

In certain cases, the resolution of the instrument can be a critical factor in observing the required spectral features (e.g. benzenoid-type spectra showing a fine structure). The minimum resolution required may be indicated in the monograph. In order to determine this figure, the slit-width setting is deliberately varied to the point where the spectrum obtained is just adequate for the intended purpose. The resolution corresponding to this setting is then experimentally defined on the basis of an absorbance ratio for a 0.02% *V/V* solution of *toluene R* in *hexane R* or preferably *heptane R* as prescribed in general chapter *2.2.25. Absorption spectrophotometry ultraviolet and visible*. The minimum ratio is indicated in the monograph to two significant figures.

Table 3 indicates the approximate relationships to be expected between the spectral slit width and the absorbance ratio.

Table 3 – Resolution of spectrophotometers according to the slit width

Slit width (nm)	Ratio $A_{max\ 269\ nm}/A_{max\ 266\ nm}$
0.25	2.3
0.5	2.2
1.0	2.0
2.0	1.4
3.0	1.1
4.0	1.0

II.6.5. Melting point, freezing point and boiling point

These physical constants are of value to identification only if they are well defined and their determination is not accompanied by destruction to a degree that renders them extremely dependent on the actual mode of operation. The possible existence of polymorphism must also be taken into account; differences in the melting point must be indicated even when given in the CHARACTERS section. In exceptional cases, when the distinction of a specific crystalline form is necessary, determination of the melting point can aid in excluding the unwanted form(s).

However, it should be kept in mind that an apparent melting point may be observed: a solid-solid polymorphic transition may take place during testing and the melting point of the resultant form is measured.

For the first identification, neither the melting point alone nor the addition of a chemical reaction is sufficient to confirm the identity of a substance. However, combining one of these two tests with another identification test such as IR will often suffice. For the second identification, please refer to part II.6.2.

The melting point determined by the capillary method is defined in the Ph. Eur. (see general chapter *2.2.14. Melting point – capillary method*) as the last particle melting point (i.e. clear point or liquefaction point). It must not be confused with the melting interval even though both are given as a range.

II.6.6. Identification of substances that have one or more stereocentre(s)

When only the racemate monograph is available in the Ph. Eur., the angle of rotation will be given in the TESTS section, provided the specific optical rotation of the chiral form is known and is of sufficient magnitude to provide a meaningful test for racemic character.

When a monograph describes an enantiomer only, the monograph contains a test for enantiomeric purity in the TESTS section and a cross-reference in the IDENTIFICATION section. If this is not possible, a test for specific optical rotation is added in the TESTS section of the monograph and is cross-referenced in the IDENTIFICATION section.

If monographs exist for both the racemate and the enantiomer, the monograph of the racemate contains an optical rotation test in the TESTS section and a cross-reference in the IDENTIFICATION section. The use of an optical rotation test is discouraged in other situations due to its lack of specificity.

II.6.7. Thin-layer chromatography

This identification method requires the use of reference substances. Selectivity may be improved by combining TLC with chemical reactions *in situ* i.e. by employing appropriate spray or dipping reagents, in which case the same or a similar reaction is not to be repeated on a test-tube scale.

Although it is very important to ensure the separation of a critical pair in a related substances test, this plays a minor role in an identification test. The separation of a critical pair in the individual identification tests is no longer required but the separation of a critical pair in the TESTS section is maintained. However, during development and validation, separation of the substance from similar substances must be demonstrated.

A chromatographic separation test for TLC plates is usually described in general chapter *4.1.1. Reagents* to verify the performance of the plate type concerned. The test is intended to be a quality control procedure, carried out periodically by the TLC plate user. It is clear that such a general procedure is not appropriate for every thin-layer separation problem and that the description of a separation criterion might still be necessary to ensure the identification of the substance. In these exceptional cases, a separation criterion is described in the IDENTIFICATION section.

A TLC system applied to purity testing in a monograph is preferred for identification when suitable. In this case, the concentration of the solution to be examined and the corresponding reference solution are generally reduced so that 5-20 µg of each is deposited on the plate or sheet. It may also be necessary to switch to a more discriminating detection system.

For more technical requirements on these chromatographic methods, see part II.7.8.

II.6.8. Gas chromatography and liquid chromatography

The basic principles mentioned under thin-layer identification apply, taking account of the differences between the two. Gas and liquid chromatography are increasingly used for identification purposes; where they are, the IDENTIFICATION section simply refers to a test or assay that applies the method elsewhere in the monograph. These methods are used only if there is no suitable alternative; they are not used as the only identification test.

For more technical requirements on gas and liquid chromatography see part II.7.8.

II.6.9. Chemical reactions

Several commonly applied identification reactions of a chemical nature are included amongst the general chapters of the Ph. Eur., and these are to be used whenever appropriate. Where several reactions for an ion or group are given in general chapter *2.3.1. Identification reactions of ions and functional groups*, it is normally necessary to prescribe only one in the monograph. Note the need to specify the amount of material, or solution of it, to be taken for the identification test in question. The same holds true for tests that have to be described in full in the monograph. Identification reactions using toxic reagents (e.g. REACH reagents) are being slowly phased out; special care should be taken when choosing a chemical reaction to be added to a monograph.

Identification criteria that call for the recognition of an odour or a taste are to be avoided.

Each chemical reaction chosen must demonstrate the presence of a different part of the molecule to be identified.

To differentiate substances within a group (family) which differ either by the extent of condensation or by the length of the hydrocarbon chain (e.g. fatty acids), a cross-reference must be added to the appropriate purity test(s) where values are determined (e.g. iodine value, saponification value, etc.).

II.7 TESTS

II.7.1. General

The main purpose of the TESTS section is to limit impurities in chemical substances. General chapter *5.10. Control of impurities in substances for pharmaceutical use* gives details of the policy to be applied.

While the monograph must ensure adequate purity in the interests of public health, it is not the aim of the Ph. Eur. to impose excessive requirements that restrict unnecessarily the ability of manufacturers to produce compliant products.

In the interests of transparency, information is included wherever possible on:

- the impurities controlled by a test;
- the approximate equivalent (percentage, ppm, etc.) of the prescribed limit in terms of the defined impurities or class of impurities.

In addition to approved specifications in marketing authorisations, acceptance criteria and limits are set on the basis of analytical data at hand (i.e. batch results provided by manufacturers and data produced during monograph elaboration by the testing laboratories). In order to define limits for tests (loss on drying, residual water, etc.), the “3-sigma” rule may be used. In a normal distribution, 99.7% of values lie within three standard deviations of the mean. A minimum of 10 test results, obtained from one source, must be available to calculate the mean. However, it should be noted that the empirical rule is not applied systematically. This is especially true for the related substances test, where impurity limits should reflect more closely their real content in substances used in approved medicinal products.

Example 1: Determination of specification for water content (2.5.12)

- ➔ *Batch data provided by a manufacturer: 10 batches*
- ➔ *Min. value: 3.2%, max. value: 5.4%*
- ➔ *Mean + 3 sigma = 6.1%*

Conclusion: The limit for water is set at 6.1% according to the 3-sigma rule.

Example 2: Determination of specification for impurity X limit

- ➔ *Batch data for level of impurity X provided by a manufacturer: 57 batches*
- ➔ *52 batches around or less 0.05%, 4 batches about 0.08%, 1 batch 0.09%,*
- ➔ *Mean + 3 sigma = 0.11%*

Conclusion: The 3-sigma rule is not applied. The limit for impurity X is set at 0.10%, based on batch data.

Certain tests may apply to special grades (parenteral, dialysis solutions, etc.) or a test may have a special limit for a particular use: this is indicated within the test.

II.7.2. Title of tests

Wherever possible, the title includes the impurity or class of impurities limited by the test (Oxalic acid, Potassium, Copper, Chlorides, etc.). Non-specific tests carry a more general title appropriately chosen from the standard terminology of the Ph. Eur. (Appearance of solution, pH, Acidity or alkalinity, etc.) or a similar designation. Titles that merely refer to the methodology employed in the test (e.g. Absorbance) are to be avoided wherever possible.

II.7.3. Solution S

A solution of the substance to be examined, designated "Solution S", is prepared whenever this can be used to perform more than one test (and/or identification).

If necessary, several solutions S, (designated S1, S2, etc.) may be prepared in various ways, each being used for at least two tests.

For insoluble substances, solution S may be prepared by an extraction process.

The solvent used depends on the purpose of the tests and the solubility of the substance to be examined and that of its potential impurities. It may be:

- water (usually):
  - *carbon dioxide-free water R* in cases where the presence of carbon dioxide can appreciably influence the outcome of a test, e.g. for pH or Acidity or alkalinity (see part II.7.5);
  - *distilled water R* if solution S is used in the tests for barium, calcium and sulfates;
  - *carbon dioxide-free water R* prepared from distilled water when both previous cases apply;
- a dilute acid or an alkaline solution;
- more rarely, other solvents (alcohols, tetrahydrofuran, etc.) that give solutions with a narrower field of application than aqueous solutions.

The solvent must make it possible to carry out the specified tests, either directly or after suitable dilutions explicitly specified in each test. The concentration is around 20-50 g/L, but may be lower (e.g. 10 g/L) or higher (100 g/L, possibly more in exceptional cases). The quantity of solution S prepared must be sufficient to carry out each of the tests for which it has been prepared and should be adapted, if necessary, if the text is revised. If solution S is to be filtered, the loss on filtering must be taken into account, and if the insoluble portion thus separated is to be used for another test, this is clearly indicated.

While several tests may be carried out on the same portion of solution S, this is only done for substances where there are good reasons to economise (expensive products or products whose use is subject to restrictions) and this is then clearly indicated in the monograph.

Depending on the particular tests, the concentration of solution S is defined with varying levels of accuracy:

- for “Appearance of solution”, “pH” and some identifications, an accuracy of 5-10% is sufficient;
- for most limit tests, an accuracy of about 2% is appropriate;
- for some cases, such as the determination of specific optical rotation, specific absorbance, various chemical values and, more generally, tests where the result is obtained by calculation, a greater level of accuracy is needed.

The accuracy with which the concentration of solution S is defined is that required by the most exacting test for which it is intended. The description of the preparation of solution S thus specifies:

- the quantity of substance to be examined with the required accuracy (see *General Notices*);
- the volume, to one decimal place (10.0 mL, 25.0 mL, etc.) when the concentration must be known to within less than 1%, without a decimal (10 mL, 25 mL, etc.) when a lower accuracy is adequate.

II.7.4. Appearance of solution

This test makes it possible to ascertain the general purity of a substance through the detection of impurities insoluble in the solvent selected, or of coloured impurities.

The “Appearance of solution” test is practically always prescribed for substances intended for preparations for parenteral use. Apart from this, it is to be applied only if it yields useful information about specific impurities.

It can comprise one or both of the following tests:

- *Clarity and degree of opalescence of liquids* (*2.2.1*);
- *Degree of coloration of liquids* (*2.2.2*).

The two tests are practically always carried out on identical solutions, usually solution S, but they may be performed on different solutions.

The solvent employed is typically water but other solvents may be used depending on the solubility of the substance to be examined.

When an organic solvent is used to prepare solution S, it may be necessary to ensure that the solvent also complies with the test, especially where there is a very stringent requirement.

The more concentrated the solution the stricter the test. For very pure substances or those used in high doses, the concentration chosen is 50-100 g/L, whereas for less pure substances or substances administered in small doses the concentration is 10-20 g/L.

II.7.4.1. Clarity and degree of opalescence (2.2.1)

This test is mainly performed on colourless substances or those that give only slightly coloured solutions in order to permit valid comparison with reference suspensions. Newer instruments with ratio selection are capable of measuring coloured substances.

The quantity of solution required depends on the diameter of the comparison tubes used; it varies from 7-20 mL for tubes with a diameter of 15-25 mm prescribed in the general chapter. It is therefore necessary to take the larger volume into account.

Most often, the solution examined must be “clear” (as defined in the Ph. Eur.). However, in certain cases (e.g. substances that are not intended to be used in solution), a more marked opalescence may sometimes be permitted.

II.7.4.2. Degree of coloration of liquids (2.2.2)

This test applies to essentially colourless substances that contain, or may degrade to form, coloured impurities that can be controlled by limiting the colour of solution of the substance. Three methods are described in general chapter *2.2.2. Degree of coloration of liquids*:

- Method I only requires 2 mL of solution but is seldom prescribed except for substances that give highly coloured solutions;
- Method II, which is more discriminating and therefore more frequently used, requires the larger volume of solution employed for the clarity test;
- Method III describes the instrumental determination of the coloration and provides more objective data than the subjective viewing of colours by a small number of individuals.

The results given by these three methods are not necessarily the same, so the one to be used is specified in the monograph.

At present, the specifications indicated in the Ph. Eur. are all based on visual determination and an exact correlation between visual and instrumental results is not always possible, depending on the ability of the analyst to differentiate between colour grades (visual method) and on the equipment settings. Hence, when using chapter *2.2.2*, the analyst is asked to report the results together with the method used (I, II or III).

The solution is described as colourless when it is less coloured than reference solution B_9. When the solution is slightly coloured, the appropriate reference solution is given. When the shade of colour varies depending on the samples, two or more reference solutions of the same degree of colour may be mentioned, or even only the degree of coloration without specifying the actual colour.

For material intended for parenteral use and for highly coloured solutions, especially when the use of Method I is contemplated, it is preferable to apply a limit of absorbance measured with a spectrophotometer at a suitable wavelength (usually 400-450 nm). The concentration of the solution and the limit of absorbance must be stated. The conditions and limit must be based on knowledge of the absorbance curve in the range of 400-450 nm and on results obtained with appropriate samples, including stored and degraded samples, as necessary.

II.7.5. pH and Acidity or alkalinity

This test enables the limitation of acidic or alkaline impurities stemming from the method of preparation or purification or arising from degradation (e.g. from inappropriate storage) of the substance. The test may also be used to verify the stoichiometric composition of certain salts.

Two types of test for protolytic impurities are used in the Ph. Eur.: a semi-quantitative titration experiment using indicators or electrometric methods to define the limits (the Acidity or alkalinity test); or a pH measurement.

pH measurement is included if the material has buffering properties, otherwise a titrimetric procedure is recommended.

The question of whether to prescribe an Acidity or alkalinity test or a pH measurement in a pharmacopoeial monograph can be decided on the basis of an estimation of the buffering properties of the material. To this end, a titration curve can be constructed for an aqueous solution (or, if necessary, an extract) in the intended concentration (10-50 g/L) of a sample, preferably pure, of the substance to be examined, using 0.01 M hydrochloric acid and 0.01 M sodium hydroxide, respectively, and potentiometric pH measurement.

The inflexion point of the titration curve is the true pH of the solution and will, for a pure substance, be at the point of intersection with the pH-axis. The measure of the buffering capacity of the solution to be examined is the total shift in pH, (ΔpH), read from the titration curve as the result of adding 0.25 mL of 0.01 M sodium hydroxide to 10 mL of the solution and 0.25 mL of 0.01 M hydrochloric acid to a separate 10 mL portion of the same solution. The buffering capacity is inversely proportional to the ΔpH. For a sample that is not quite pure, carry out a parallel displacement of the titration curve so that the true pH of the solution is on the pH-axis before the ΔpH is read from the curve.

The magnitude of ΔpH of the solution to be examined determines the choice of method for the limitation of protolytic impurities according to the following scheme. The classification is based upon the observation that the colour change for most indicators takes place over a pH range of 2 units.

Class A	$\Delta pH > 4$	Acidity-alkalinity test using two appropriate indicators.
Class B	$4 > \Delta pH > 2$	Acidity-alkalinity test using a single appropriate indicator.
Class C	$2 > \Delta pH > 0.2$	Direct pH measurement.
Class D	$\Delta pH < 0.2$	The protolytic purity cannot be reasonably controlled. Substances that are salts consisting of ions with more than one acidic and/or basic function belong to this class and for these a pH measurement can contribute to ensuring the intended composition if the limits are sufficiently narrow.

It is evident that, by changing the concentration of the solution to be examined, the class of buffering properties as set out above into which the substance will fall can be altered to some extent, since the shape of the titration curve will also be modified as a result. The concentration range given above is not to be exceeded, however, unless poor water solubility means that a more dilute solution has to be used.

If a test for acidity-alkalinity cannot be performed with the use of indicators due to the coloration of the solution to be examined or other complications, the limits are then controlled electrometrically. If on the other hand, the addition of a standard acid or base leads to decomposition or precipitation of the substance to be examined, it may be necessary to prescribe a pH test regardless of the buffering properties.

If, for the reasons outlined above, a pH measurement has to be prescribed for solutions with little or no buffering capacity, the solution to be examined is prepared with *carbon dioxide-free water R*.

Conversely, it is not necessary to use *carbon dioxide-free water R* when preparing solutions that have sufficient buffering capacity to warrant a direct pH measurement because the required accuracy, which seldom exceeds 1/10th of a pH unit, will not be affected. When an acidity requirement corresponds to not more than 0.1 mL of 0.01 M sodium hydroxide per 10 mL of solution to be examined, the solution must be prepared using *carbon dioxide-free water R*. These considerations are to be borne in mind when prescribing the composition of solution S if it is to be used in a test for protolytic impurities.

II.7.6. Optical rotation (*2.2.7*)

Measurements of the optical rotation of an article, though sometimes useful for identification purposes, may be used as a purity test:

- either to assess the general purity of an optically active substance (a liquid or a solid in solution), by calculating the “Specific optical rotation” (title of the test);
- or to limit the presence of optically active impurities in any “optically inactive” mixture (racemate), provided that the specific optical rotation of the enantiomer at 589 nm is sufficient to ensure adequate sensitivity. In this case, the optical rotation of the liquid or of the solid in solution is measured under defined conditions (temperature, concentration, path length) and the range normally given should be − 0.10° to + 0.10° (covering the substances that are not true racemates).

In monographs on a single active enantiomer (eutomer), chiral chromatography (“Enantiomeric purity”) is preferred to control the other enantiomer (distomer) because specific optical rotation is generally not specific enough for an appropriate control. On the other hand, an achiral chromatographic procedure can generally be used to test for diastereoisomers.

Although the test is not suitable for highly coloured or opalescent solutions, filtration can sometimes make the determination possible for opalescent solutions. Shortening the path length can also help to measure particular samples (e.g. for some essential oils).

The following aspects are taken into account in describing the test:

- the solvent, which depends on the solubility of the substance to be examined and the observed optical rotation in that solvent. In the case of non-aqueous solvents, their purity and especially their water contents may need to be carefully defined;
- the quantity of substance to be used, determined with sufficient accuracy (generally 1%), and the volume to be prepared (given to one decimal place). Although the volume depends on the apparatus used, 25.0 mL is usually prescribed because it rarely exceeds that amount. The concentration of the solution must be high enough to give a reliable reading of the angle of rotation;
- the degree of hydration or organic solvation of the substance (for the calculation of the result);
- the result is the mean of at least five measurements when evaluated visually, with an instrument allowing readings to the nearest 0.01°;
- measured angles of optical rotation are given to two decimal places;
- specific optical rotation values are given to two or three significant figures: values below 10 are given to two significant figures, while values of 10 and over are given to three

significant figures;

- composition limit for racemates.

The value of the specific optical rotation is calculated with reference to the dried or anhydrous substance.

II.7.7. Absorption spectrophotometry (ultraviolet and visible) (*2.2.25*)

The absorption of electromagnetic radiation may be used in purity tests as a limit test for certain impurities. The typical case is that of impurities that absorb in a region where the substance to be examined is transparent, in which case the absorbance of a solution of the substance to be examined is measured. This test may be performed in the following ways:

- by direct measurement on the solution, where the absorbance measured is a maximum absorbance at a given wavelength or over a wavelength range;
- after carrying out a chemical reaction that forms, with the impurity, a substance that absorbs at a wavelength where the substance to be examined is transparent, a maximum value at the given wavelength being prescribed.

For measurements in the ultraviolet region, it is advisable to avoid measuring at wavelengths below 230 nm as more interferences and more stray light are observed in this region.

It is important to describe precisely the operational conditions to be observed, in particular the preparation of solutions prepared by successive dilutions.

II.7.8. Related substances

The policy on control of impurities is described in general chapter *5.10. Control of impurities in substances for pharmaceutical use* and in the general monograph *Substances for pharmaceutical use (2034)*. Monographs should be elaborated accordingly. Monographs are designed to take account of substances used in approved medicinal products in member states and should provide adequate control of all impurities occurring in these substances, insofar as the necessary information and samples (substance and impurities) are available from the manufacturers. Such impurities are controlled in a test for related substances and any other individual test for impurities (e.g. “Impurity X” or “Enantiomeric purity”). Where the required information and samples are not provided for a substance synthesised by a given method, the monograph will not necessarily cover the corresponding impurity profile.

The provisions for related substances in the general monograph *Substances for pharmaceutical use (2034)* and general chapter *5.10* apply to all active substances and excipients, unless otherwise stated therein.

If an exception is to be made for a particular substance normally covered by these provisions, the following statement is included in the specific monograph: “*The thresholds indicated under Related substances (Table 2034.-1) in the general monograph Substances for pharmaceutical use (2034) do not apply*”. It is recommended to provide the reason for the deviation in a footnote during the *Pharmeuropa* stage. This explanation will be transferred to the EDQM Knowledge Database once the monograph is published in the Ph. Eur.

Monographs should include acceptance criteria for:

- each specified impurity;
- unspecified impurities (previously referred to as "any other impurities"), normally set at the identification threshold;
- the total of impurities.

Impurities to be controlled include intermediates and by-products of synthesis, co-extracted substances in products of natural origin and degradation products. Monographs on organic chemicals usually have a test entitled "Related substances" (or a test with equivalent purpose under a different title), designed to control organic impurities. Where applicable, inorganic impurities are usually covered by other tests. Residual solvents are covered by specific provisions [see below and in general chapter *5.4. Control of residual solvents* and the general monograph *Substances for pharmaceutical use (2034)*].

DNA-reactive (mutagenic) impurities. *ICH guideline M7 on assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk* (EMA/CHMP/ICH/83812/2013) entered into force on 1 January 2016.

The following pragmatic approach is in line with the ICH M7 guideline and should be followed when elaborating or revising monographs related to substances for human use. A DNA-reactive impurity is covered in the individual monograph **only** where there is study data demonstrating mutagenicity of the impurity by a recognised toxicity test. The existence of structural alerts alone is considered <u>insufficient</u> to trigger follow-up measures. Following a decision by the Ph. Eur. Commission (November 2016), DNA-reactive impurities should be addressed in individual monographs in:

- the PRODUCTION section, by a statement, when no specific test or limit is known to the GoE at the time of elaboration/revision of a monograph or when the technique is so special that it is not available to a majority of users;
- the TESTS section, when the analytical procedure and the limit are known and the technique is widespread.

Additional information and requirements for specific types of DNA-reactive impurities is provided in the general monograph *Substances for pharmaceutical use (2034).*

If a new synthetic route is used that may give rise to different DNA-reactive impurities or to higher levels of previously recognised ones, the evaluation by a Competent Authority should be used as the basis for the impurity in question.

If an issue concerning a DNA-reactive impurity is raised by a Competent Authority (notably for revision of a monograph or in comments on a *Pharmeuropa* draft), this will be dealt with on the basis of data provided to the Ph. Eur. Commission by the Competent Authority.

Control of impurities. The most common and preferred method for controlling organic impurities is LC; GC or CE may be the preferred method in some instances. Although there are still some monographs that prescribe TLC, this technique should be reserved for controlling specific impurities that cannot conveniently be controlled by LC or GC. Existing TLC tests that do not follow this recommendation will be replaced gradually as soon as information on suitable LC or GC tests becomes available.

Where the counter-ion of an active substance is formed from a lower organic acid, a test for related substances of the organic moiety is usually not considered necessary (e.g. magnesium lactate used as a source of magnesium).

Monographs frequently have to be designed to cover different impurity profiles because of the use of different synthetic routes and purification procedures by manufacturers. The usual practice is to include a general LC test, supplemented where necessary by other tests (LC, GC, CE, TLC or other techniques) for specific impurities. However, it is becoming increasingly impractical in some cases to design a single general test; in such cases, more than one general test is included and the scope of the different tests is defined in the tests themselves with a cross-reference in the IMPURITIES section.

Monographs cover a number of specified impurities listed in the IMPURITIES section. Specified impurities are those that occur in current batches of the substances used in approved products and for which an individual acceptance criterion is provided. Wherever feasible, monographs also have an acceptance criterion for other impurities (at the identification threshold for the substance) and a limit for the total of impurities (or a limit for the total of impurities other than a number of identified specified impurities) above the reporting threshold. The acceptance criterion for specified impurities may be set at the identification threshold for the substance.

The acceptance criteria for specified impurities take account of both:

- approved limits;
- recent batch data and stability data, with the acceptance criteria being set to take account of routine production conditions; data is provided by the manufacturer for typical batches and verified experimentally during elaboration of the monograph on at least three batches.

If several approved limits exist, the highest is taken.

When a monograph describes the salt form of the substance, then, for the purpose of calculation and specification setting and unless otherwise prescribed, the impurity is assumed to be present in the same salt form.

All decisions on impurity acceptance criteria should be based on the real impurity content (meaning after application of correction factors (CFs), where applicable) in representative batches examined.

Impurities must be specified and located appropriately in the chromatogram if the reported batch values for an impurity are:

- above the applicable limit for unspecified impurities before correction and cross this limit downwards when corrected (overestimation, CF<1); or
- below the limit for unspecified impurities before correction and cross this limit upwards when corrected (underestimation, CF>1).

Usually, no correction factor will be given if the reported batch values for an impurity are below the applicable limit for unspecified impurities before correction and below the reporting threshold (disregard limit) after correction.

In any case, CFs between 0.8 and 1.25 (corresponding to response factors of 1.2-0.8) are not given in monographs. Additional information on the indication of CFs is given in part II.7.8.2.b.

Response and correction factors. According to general chapter *2.2.46. Chromatographic separation techniques*, the relative detector response factor (commonly referred to as the “response factor”) expresses the sensitivity of a detector for a given substance relative to a standard substance. The correction factor given in the monograph is the reciprocal value of the response factor.

The response factor can be determined by preparing solutions of defined concentrations of the impurity and the substance to be examined and measuring them by LC/UV at a given wavelength and flow rate. The concentration of the impurity and that of the substance to be examined should be of the same order of magnitude and the measurement should be carried out using a calibration curve determined at several points around the concentration which corresponds to the acceptance criterion of the impurity. For the calculation, the mean of the area ratios over the whole range of linearity or the ratio of the slopes of the respective linearity regression equations may be used. The response factor can be calculated using the following formula:

$$RRF = \frac{A_i}{A_s} \times \frac{C_s}{C_i}$$

RRF = (relative) response factor;

A_i = area of the peak due to the impurity;

A_s = area of the peak due to the substance to be examined;

C_s = concentration of the substance to be examined in milligrams per millilitre;

C_i = concentration of the impurity in milligrams per millilitre.

It is also important to consider the form (base/acid or salt) of both the impurity and the substance to be examined used when determining the response factor and to apply an additional correction for the molecular mass ratio when they are present in different forms. This correction can be done by ensuring that C_i is expressed with respect to the same form as the substance to be examined (i.e. as base/acid or salt) provided the impurity can actually be present in that form.

Preferably, the response factor should be determined in two laboratories using the same protocol. If different UV-Vis detector types (diode array detector (DAD) and variable wavelength detector (VWD)) are available, these may also be considered for this measurement.

The weighings of impurity and substance to be examined should both be corrected for their respective purity. If the available amount of impurity does not allow any experimental determination, values from the certificate of analysis may be used. If enough material is available, the chromatographic purity and water/solvent content of the impurity and the substance to be examined should be determined beforehand. A provisional value might be assigned on the basis of the following formula:

$$content(\%) = [100 - (water + solvents)] \times \frac{chromatographic\ purity\ (\%)}{100}$$

where the chromatographic purity is determined by normalisation or using a dilution of the test solution or of a solution of the impurity.

When only a small amount of the impurity is available, analytical procedures with low sample amounts may be preferred (e.g. thermogravimetric analysis for water/solvents, coulometry for water and LC to estimate purity by injecting a concentrated solution of the impurity). Suitable alternative approaches such as a combination of qNMR and LC data or a comparison of LC-UV and LC-CAD may be employed.

Separation methods. For pharmacopoeial purposes, the objective of a purity test using a separation method will usually be the control of impurities derived from one or more known manufacturing processes and decomposition routes. However, the experimental conditions, especially the detection system, are chosen specifically so as not to make the test unnecessarily narrow in scope. Chromatographic purity tests may often be the best means of providing a general screening of organic impurities derived from new methods of manufacture or accidental contamination. It may be advantageous to supplement a chromatographic test with other chromatographic or non-chromatographic tests.

As mentioned in part II.6.8, a chromatographic system applied to purity testing may, when suitable, be applied also for identification.

When a related substances test based on a chromatographic technique is carried out, a representative chromatogram is published with the monograph in *Pharmeuropa*. Although the chromatogram will not ultimately be published in the Ph. Eur., it will be transferred to the EDQM Knowledge Database.

When a mixture of impurities with or without the substance to be examined is available as a reference substance (e.g. peak identification CRS, impurity mixture CRS or system suitability CRS), a representative chromatogram, if mentioned in the monograph, will be supplied with the reference substance.

Monographs should provide a reliable means of locating the impurities used for the system suitability test (SST) and all specified impurities on the chromatogram. Identification of impurities at or below the limit for unspecified impurities is necessary if a correction factor is to be applied. In such cases, these impurities are listed as specified impurities.

Peaks may be located using:

- a reference standard or a reagent for each impurity;
- a reference standard containing some or all of the impurities, (e.g. peak identification CRS, system suitability CRS).

Location by relative retention is not generally considered sufficient for pharmacopoeial purposes, especially for gradient elution. Where a reference standard containing one or several impurities, with or without the substance to be examined, is to be used, a sample of each specified impurity should be provided to the EDQM to enable the establishment of the reference standard.

In general, relative retention is given to one decimal place. However, it is given to two decimal places where necessary to indicate the elution order of closely eluting peaks. The following general considerations apply to separation techniques:

- high concentrations/loadings are normally used since the symmetry of the principal peak or shape of the spot is not critical in impurity testing, so long as there is no interference. When using an external standard in quantitative determinations, the response of the principal peak in the chromatogram obtained with the test solution does not need to be in the linear range of the detector;
- in general tests for related substances, the substance to be examined should not be chemically modified (e.g. derivatisation) before purity testing since the impurity pattern may be modified;

- similarly, extraction of the free base or acid prior to impurity testing is to be avoided;
- t_R of the principal peak is determined using the diluted test solution (to increase accuracy while avoiding saturation effects).

II.7.8.1. Thin-layer chromatography (2.2.27) and high-performance thin-layer chromatography for herbal drugs and herbal drug preparations (2.8.25)

TLC methods should only be used to control a specified impurity and where LC, GC or CE methods are not appropriate (usually due to a lack of a suitable detection system). More information on HPTLC can be found in the *Technical guide for the elaboration of monographs on herbal drugs and herbal drug preparations*.

Commercially available pre-coated plates, described in general chapter *4.1.1. Reagents*, are to be used; the trade name of the plate found to be suitable during the elaboration of the monograph is indicated in a footnote to the draft monograph and added to the EDQM Knowledge Database after the monograph is adopted. In addition to information on the coating material used (type of coating material, type of binder), general chapter *4.1.1. Reagents* describes a suitability test procedure under *TLC silica gel plate R*. The monograph must describe the type of plate, including the particle size for HPTLC plates, and include a system suitability requirement. It is often the case that the substances that would be best suited for a SST will not be readily available individually, in which case a sample of the substance to be examined containing them as contaminants or even a deliberately spiked sample may then be prescribed. Permissible adjustments to the different parameters are indicated in general chapter *2.2.46. Chromatographic separation techniques*.

If any pre-treatment is required or if the chromatography is carried out in unsaturated conditions for the satisfactory conduct of the test, then this information is included in the text of the monograph. This especially applies to the use of reverse-phase plates.

One or more dilutions of the substance to be examined will often prove adequate for reference purposes, provided the impurities to be compared exhibit a similar behaviour under the chosen chromatographic conditions. This implies that the spots to be compared must be sufficiently close in terms of their R_F value to minimise errors introduced by different diffusion of the substances during their migration. Otherwise, reference solutions containing the specified impurities are to be employed. It may be necessary to instruct the analyst to disregard a spot – often due to the non-migrating counter-ion of a salt – remaining on the starting line.

Summation of the responses exhibited by each individual spot is only acceptable when appropriate equipment is prescribed. It is not recommended to set a limit or limits for the concentration of impurities without a limit on their number, otherwise the total theoretical impurity level would be unacceptably high. This situation may be counteracted by limiting the impurities on two or more levels, allowing only a defined number to be at the higher level and the rest below the lower level. As examples, the test may specify that no contaminant may exceed a relative concentration of 1% and that only one may exceed 0.25%, or that no contaminant may exceed a relative concentration of 1%, only one contaminant above 0.5% and no more than four contaminants above 0.25%.

II.7.8.2. Liquid chromatography (2.2.29)

Defining the appropriate chromatographic system will often be one of the major problems to be dealt with when developing a pharmacopoeial purity test based on chromatography. In LC, the matter is further complicated by the existence of numerous variants of stationary phases, especially amongst the chemically bonded reverse-phase materials for which not only brand-to-brand but occasionally also batch-to-batch variations occur, all of which can influence a given separation. Once the type of stationary phase tested has been found to show a satisfactory separation, it must be defined by selecting the appropriate reagent entry. Correspondence tables between the trade name of the LC columns and the description of the stationary phases are available on the Extranet, General Information for Experts section. Particle size (µm) is stated in the analytical procedure; for size-exclusion chromatography, particle size (µm) and pore size (nm) are stated. The trade name of the column(s) found to be suitable during the elaboration of the monograph is indicated in a footnote to the draft monograph and is transferred to the EDQM Knowledge Database after the monograph is adopted.

The following are given when describing the chromatographic system: the column dimensions (length and internal diameter), nature of the stationary phase (as detailed previously) including any steps to prepare or pre-treat it, composition and flow rate of the mobile phase including gradient programme (if any), column and autosampler temperature (if differing from room temperature or especially if thermostated), method of injection (if important), injection volume and method of detection.

If a pre-column is deemed useful during the elaboration of the monograph and the validation data has been obtained using the pre-column, its use is normally stated in the monograph.

Depending on the detection wavelength selected, the analyst should choose a suitable grade of solvent when preparing the mobile phase. The following guidance applies to the most frequently used solvents, methanol and acetonitrile. If water is used as a component of the mobile phase, w*ater for chromatography R* should be used.

Wavelength intervals	**Acetonitrile grade**	**Methanol grade**
$\lambda \geq 250$ nm	*Acetonitrile R*	*Methanol R*
220 nm $\leq \lambda <$ 250 nm	*Acetonitrile for chromatography R*	*Methanol R1*
$\lambda <$ 220 nm	*Acetonitrile R1*	*Methanol R2*

Permissible adjustments to the different parameters are indicated in general chapter *2.2.46. Chromatographic separation techniques*.

Wherever possible, test and reference solutions are prepared using the mobile phase as the solvent in order to minimise peak anomalies.

Unlike solutions for quantitative use, the quantities prescribed in reference solutions for qualitative use only are described without an extra decimal place.

Since many active substances are synthesised by a number of synthetic routes, the list of potential impurities to be limited may be large and the analytical challenge to separate them is great. For the sake of robustness and reproducibility, isocratic elution is to be preferred when setting up a pharmacopoeial procedure. However, because isocratic liquid chromatographic methods may not be sufficiently selective, there is an increasing need to employ gradient methods.

When a gradient system is described, all necessary parameters must be clearly given (composition of mobile phases, equilibrium conditions, gradient conditions (linear or step), etc.). In general, the return to the initial conditions and re-equilibration are not prescribed in monographs since this is considered to be instrument specific. Should this information be considered important (e.g. ion-exchange chromatography), it may be added as a note to the draft monograph and transferred later to the EDQM Knowledge Database.

For gradient elution in LC, an important parameter to be considered is the volume between the solvent mixing chamber and the head of the column. This volume is referred to as the dwell volume, "D" (other terms employed include effective system delay volume, dead volume and delay volume). The dwell volume is dependent on the configurations of the pumping system including the dimensions of the capillary tubing, the solvent mixing chamber and the injection loop. Large differences in dwell volume from one pumping system to another will result in differences in elution of peaks. The greatest effect of differing dwell volumes on retention times is for those substances that are not strongly retained. Thus, gradient systems should be designed with an initial isocratic phase so that analytes do not elute too close to the injection peak, making it possible to correct for marked differences in dwell volume between different gradient pumping systems. The minimum time for the initial isocratic step will depend on the dwell volume of the system and will allow equilibration of the system after sample injection. When the initial validation has been performed without an initial isocratic step, it may not be necessary to revalidate a procedure to which an isocratic step has been added if analytes do not elute too close to the injection peak. The dwell volume of the pumping system employed to develop the procedure should be equal to or less than 1.0 mL. If the procedure is developed using a system with a dwell volume greater than 1.0 mL, then a suitable initial isocratic step is essential. Experts' reports should indicate the dwell volume of the instrument used for their experimental work. This dwell volume will be stated in a footnote in the draft text and will be transferred to the EDQM Knowledge Database after the monograph is adopted. A method for determining the dwell volume is provided in general chapter *2.2.46. Chromatographic separation techniques*.

II.7.8.2.a. System suitability criteria

One or more system suitability criteria are to be included in the test. Requirements given in general chapter *2.2.46. Chromatographic separation techniques* are also applicable.

Separation capacity. This criterion is necessary when separation techniques are employed for assays and tests for related substances. The following approaches, most of which require the separation or partial separation of a critical pair, are acceptable for a SST for selectivity:

- **Resolution.** As calculated by the formula given in general chapter *2.2.46. Chromatographic separation techniques* using two closely eluting peaks (critical pair). In cases where several closely eluting impurities are present, it may be useful to describe more than one resolution requirement, particularly in gradient systems. The resolution test described should ensure that all the impurities controlled by the procedure and not just the critical pair are separated from each other and from the principal peak. Peaks of different heights may be used to calculate the resolution provided the detector is not saturated.
- **Peak-to-valley ratio.** This can be employed when complete separation between two adjacent peaks cannot be achieved (i.e. when the resolution is less than 1.5). The minimum requirement for peak-to-valley ratio should not be less than 1.5. Better separation is often necessary to ensure a meaningful integration of impurity peaks. When the quantitative composition of a reference standard used in this test changes (replacement batch), it is necessary to check whether the SST requirements need to be adjusted.

When gradient elution is described, describing a system suitability requirement for each critical gradient step is desirable.

In situ degradation such as oxidation, hydrolysis, *Z-E* isomerisation or ring closure offers an alternative approach for defining the suitability of the system, provided that the solution of the substance can be degraded, in mild “stress” conditions within a reasonably short time, to produce decomposition products. The peaks of these products can then be used to determine a resolution or a peak-to-valley ratio. This may be a useful alternative to using impurity reference standards.

In exceptional cases, a chromatogram of an impure or preferably “spiked” substance can also be employed to define the system. In this case, a chromatogram is usually supplied with the reference substance (for system suitability or for peak identification) or the peak identification is described in the text of the test for related substances (e.g. when only one impurity is to be identified).

The use of a spiked (or impure) substance requires procurement of sufficient material to establish the reference substance used and, in the future, replacement of the SST material with material exhibiting the same characteristics.

It should be noted that retention times or relative retention values are given only for information and do not constitute alternative system suitability criteria.

Sensitivity. The disregard limit/reporting threshold serves a dual purpose:

- decision criterion for whether a peak area or a corrected peak area of an impurity is to be included in the total of impurities;
- general criterion for determining compliance of the actual chromatographic system with the requirement of general chapter *2.2.46. Chromatographic separation techniques* (signal-to-noise (S/N) ratio ≥ 10 at the disregard limit/reporting threshold).

Typically, the disregard limit for substances covered by a monograph is set in accordance with the reporting threshold given in Table 2034.-1 (see *Substances for pharmaceutical use (2034)*). However, disregard limits are only described when the comparative style is used for the related substances test; new and revised monographs should be written in the quantitative style and include a reporting threshold. This threshold helps compensate for differences in sensitivity that can be observed when different analytical systems are being employed.

When the normalisation procedure is used for quantitation, a reporting threshold is always included in the test.

When external standardisation is used, if several impurities are limited and a limit for total impurities is prescribed, a reporting threshold is included in the test. When only one impurity is limited, no reporting threshold is included, but if the sensitivity is borderline, a minimum S/N requirement may be added to the monograph.

For specified impurities with CFs > 1.25 (i.e. response factors < 0.8), the peak should be quantifiable not only at its limit, but also down to the disregard limit/reporting threshold, which is important for determining of the sum of impurities. Therefore, if the general signal-to-noise requirement of 10 is not applicable, it may be necessary to add a specific sensitivity criterion (S/N) for this impurity.

Example: impurity X is specified at 0.15% with a correction factor of 5 and a general disregard *limit/reporting threshold at 0.05%. For the impurity X under consideration, the sensitivity of the procedure is sufficient if:*

- *(1) a S/N ratio of minimum 10 is obtained with a 0.05% (relative to the test solution) solution of impurity X, when impurity X is available as a reagent/CRS and used as external standard; or*
- *(2) a S/N ratio of minimum 50 (10 x 5 for the correction factor) is obtained with a 0.05% solution of the active substance when impurity X is not available.*

Option (2) is preferred when only limited amounts of the isolated impurity are available and the correction factor of the specified impurity is between 0.2 and 5. Outside this range, it is preferable to use the impurity as external standard to avoid the additional uncertainty introduced by the multiplication factor. In the case of option (2), since the correction factor of impurity X is 5 (i.e. the response factor is 0.2) and a dilution of the test solution is used for the quantitation, it is recommended to verify the sensitivity of the procedure during its validation. The S/N ratio of the impurity peak at the reporting threshold should be at least 10 to be quantifiable. To take account of different sensitivities of equipment used, a minimum S/N ratio should be described in monographs where the observed S/N of the impurity peak is not higher than 50 at the reporting threshold. The introduced S/N ratio requirement should be at least 10 times the correction factor (e. g. correction factor is 4, then S/N requirement should be at least 40).

Example 1: Rosuvastatin calcium: Impurity C, correction factor 1.4, limit 0.8%, reporting threshold 0.05%, quantified using a dilution of the test solution of 0.2% (ref. sol. (b)).

- ➔ *S/N of impurity C is 55 at the reporting threshold (minimum requirement of 10 to be quantifiable, but a S/N minimum 50 should be obtained to take account of the sensitivity of different equipment);*
- ➔ *S/N of principal peak in ref. sol. (b) is 361, i.e. ≈ 90 at the reporting threshold of 0.05% (minimum requirement at the reporting threshold: 10 × 1.4 (CF) = 14).*

<u>Conclusion</u>: the procedure is very sensitive so a minimum S/N is not required in the monograph.

Example 2: Correctoprolol (theoretical case): Impurity A, correction factor 2.2, limit 0.2%, reporting threshold 0.05%, quantified using a dilution of the test solution of 0.1% (ref. sol. (b)).

- ➔ *S/N of impurity A is 35 at the reporting threshold (minimum requirement of 10 to be quantifiable, but a S/N minimum 50 should be obtained to take account of the sensitivity of different equipment)*
- ➔ *S/N of principal peak in ref. sol. (b) is 154, i.e. 77 at the reporting threshold of 0.05% (minimum requirement at the reporting threshold 10 × 2.2 (CF) = 22).*

<u>Conclusion</u>: based on these results, the sensitivity is sufficient but the minimum requirement might not be met if less sensitive equipment is used; the recommendation is to include in the monograph a minimum requirement for S/N of 44 for reference solution (b) (22 × 2 since ref. sol. (b) at 0.10%).

For tests for impurities which are limited at ppm level (e.g. DNA-reactive impurities), the SST may include a minimum S/N ratio requirement, such as S/N minimum 10 at 50% of the stated limit for quantitative tests and S/N minimum 10 at the stated limit for limit tests.

Repeatability. In LC with UV detection, it is commonly accepted that the relative standard deviation of the peak area obtained on a minimum of three injections of a reference solution corresponding to 0.1% of the test solution is not more than 5.0%.

II.7.8.2.b. Quantitation

Quantitation is required for limits applied to specified impurities, unspecified impurities and total impurities. It is most commonly achieved using an external standard and less commonly by the normalisation procedure. The use of the normalisation procedure is discouraged because linearity problems may be observed.

External standard. A dilution of the test solution/substance to be examined is used, unless there is a large difference in the detector response of a specified (or exceptionally an unspecified) impurity that necessitates the use of a specific external standard, which may be:

- a solution of the impurity, normally in the form of a reference standard (preferred option);
- a solution of the substance to be examined containing a known amount of the impurity.

Where a dilution of the substance to be examined is used as the external standard, experts should determine CFs for the impurities, which are indicated in monographs only if they are outside a range of 0.8-1.25 (i.e. the corresponding response factors are outside a range of 0.8-1.2) and considered relevant in light of the batch results (see part II.7.8). CFs are normally given to only one decimal place. The "whole" substance (active moiety, counter-ion and solvate) is taken into account (e.g. *Donepezil hydrochloride monohydrate (3067)*: "*Calculation of percentage content: for each impurity, use the concentration of donepezil* ***hydrochloride monohydrate*** *in reference solution (a)"*).

It is recommended not to apply CFs of less than 0.2 or greater than 5 for specified impurities, but to use external standards in these cases where possible.

In order to take account of different responses, it is possible to use a wavelength that is different from the default wavelength for the control of particular impurities. It is understood that the test and the reference solutions are recorded at the same wavelength unless otherwise prescribed.

The acceptance criteria for related substances tests may be expressed either in terms of comparison of peak areas (the historically used "comparative test style") or as numerical values (the "quantitative test style" that is preferred for new texts or major revisions).

Based on the requirements of the general monograph *Substances for pharmaceutical use (2034)*:

- in monographs using the comparative style (acceptance criteria expressed as a comparison of peak areas), a *disregard limit* is usually set with reference to a dilution of the test solution;
- in monographs referring to numerical values for acceptance criteria, a *reporting threshold* is defined as a numerical value (%).

Normalisation procedure. Quantitation by (area) normalisation requires that all the solutes are known to be eluted and detected, preferably with uniform response factors, and that the detector response is linear up to about 120% of the concentrations employed. This must be validated.

As indicated in general chapter *2.2.46. Chromatographic separation techniques*, peaks due to solvents or reagents or arising from the mobile phase or the sample matrix, and those at or below the reporting threshold, are excluded before calculating the percentage content of a substance by normalisation. An additional reference solution is prescribed to determine the reporting threshold.

The corresponding numerical value (%) is stated in the monograph.

II.7.8.3. Gas chromatography (2.2.28)

The difficulties encountered when defining the appropriate chromatographic system in GC purity tests are similar to those mentioned under LC (part II.7.8.2), although the emphasis may be elsewhere. The experimental details to be described in a pharmacopoeial test must, therefore, also be worded as an example so that the chromatographic parameters can be varied to obtain the required performance. Once the type of stationary phase tested has been found to show a satisfactory separation, it must be defined by selecting the appropriate reagent entry (*4.1.1*). Correspondence tables between the trade name of the GC columns and the reagent stationary phase description are available on the Extranet, *General Information for Experts* section. The film thickness (in µm, capillary columns) or the particle size (in µm, packed columns, in older procedures) is given after the reagent name. The trade name of the column(s) found to be suitable during elaboration of the monograph is indicated in a footnote to the draft monograph and is transferred to the EDQM Knowledge Database after the monograph is adopted.

The chromatographic system must be described in essentially the same way as for LC, with the appropriate adjustments made (temperature programme (if any) instead of elution programme, injection port and detector temperatures, etc.). The use of packed columns should be avoided. Permissible adjustments of the different parameters are provided in general chapter *2.2.46. Chromatographic separation techniques*.

For reasons of robustness and reproducibility, isothermal operating conditions are preferred. Quantitation is usually based on an internal standard technique or on the (area) normalisation procedure. The same limitations concerning summation of peak responses as mentioned for LC apply here.

For the expression of acceptance criteria, the principles defined in part II.7.8.2.b for LC are to be applied.

II.7.8.4. Capillary electrophoresis (CE) (2.2.47)

CE is increasingly employed to separate and control a large number of impurities of vastly different polarities. It is also suitable for controlling the content of the unwanted enantiomer in chiral therapeutic substances. The problem encountered in reverse-phase LC of varying performance from different stationary phases is avoided if the separation is conducted in a fused-silica capillary.

Joule heating occurs during a run. To obtain satisfactory reproducibility, a defined temperature is maintained using a thermostat; for instruments without a thermostat, a low voltage should be used.

The limit of detection is adversely affected by the small injection volume and the small detection pathway in the capillary, even when stacking techniques are applied. For the control of impurities or assays, it is recommended to use an internal standard to achieve appropriate precision. Otherwise, the guidance for the use of this technique is similar to that given previously for LC.

For chiral analysis, a chiral reagent is added to the running buffer. The chiral reagent should be carefully described in the monograph or as a reagent, particularly for cyclodextrin derivatives. Since many of the cyclodextrin derivatives are randomly substituted, it is important to give the exact or average degree and location of substitution. More than one batch of the cyclodextrin derivative should be used for the validation of the analytical procedure.

Experimental parameters to be considered for inclusion in the monograph:

- instrumental parameters: voltage, polarity, temperature, capillary size (diameter and length – total and effective – to the detector);
- coating material of the capillary (where applicable);
- buffer: pH, molarity, composition;
- sample solvent;
- separation: pole outlet, voltage (*U*), current (*I*);
- injection: time (*t*), voltage (*U*) for electrokinetic injection or pressure difference Δp for hydrodynamic injection;
- detection: wavelength, instrumentation;
- temperature;
- shelf life of solutions;
- rinsing procedures (time, reagents, Δp) needed to stabilise the migration times and the resolution of the peaks:
    - pre-conditioning of a new capillary;
    - pre-conditioning of the capillary before a series of measurements;
    - between-run rinsing.

The following information is provided in a footnote and transferred to the EDQM Knowledge Database after the monograph is adopted:

- if a coated capillary is used, the trade name of the capillary found suitable during the elaboration of the monograph;
- for chiral separations, the trade name of the chiral reagent (cyclodextrin or other) found to be suitable during the elaboration of the monograph.

In order to minimise the electro-osmotic flow signal, test and reference solutions are, wherever possible, prepared using *water for chromatography R* or the running buffer as the solvent.

II.7.9. Readily carbonisable substances

The value of this non-specific test has greatly diminished through the introduction of chromatographic tests providing more information on organic impurities. A test for readily carbonisable substances is often highly sensitive, which can be a major advantage if this is required. However, it should be noted that those impurities that produce a coloration under the conditions of the test will often respond equally well to a test for colour in simple aqueous or alcoholic solution, and in such cases unnecessary duplication is to be avoided.

If, during the elaboration of a monograph, it appears that impurities may be present that are not accounted for by other tests, then this test is carried out and, if appropriate, included in the monograph.

II.7.10. Foreign anions and/or cations

Since strong inorganic acids and bases are widely used in synthesis, the contents of foreign anions and/or cations in a substance can be indicative of the extent to which it has been purified. They can also reveal whether contamination with closely related substances has taken place. At the same time, impurities that are typically ionic can often be removed from poorly water-soluble substances by treatment with water without necessarily removing the organic impurities. As a result, tests for anions and cations cannot replace a test for related substances in organic substances but they may constitute a useful supplement for water-soluble organic substances. For inorganic substances, which are usually prepared from other inorganics, a much broader range of tests for foreign ions must be considered.

When considering the introduction of tests for foreign anions in organic substances, a single test, either for chlorides, sulfates or – less commonly – nitrates, will usually suffice, even if several could theoretically be present. The test is then to be carried out on the most abundant anion. When a test for chlorides is considered (up to 0.10%) a limit test should be used instead of titration.

Certain cations must be stringently limited because of their toxicity or catalytic activity. These are treated separately in part II.7.11. In organic substances, the majority of cations are adequately controlled via a determination of sulfated ash, unless there are special reasons for limiting their presence, either individually or in smaller groups (see part II.7.18).

II.7.11. Elemental Impurities

Since the scope of the ICH guideline covers all medicinal products for human use on the market, a cross-reference to general chapter *5.20* (linked to the ICH Q3D guideline) has been introduced in general monograph *Pharmaceutical preparations (2619)*, rendering the guideline mandatory.

Since the 9th Edition of the Ph. Eur., all the tests for heavy metals (*2.4.8*) have been deleted from individual monographs on substances for both human and veterinary use. As of the 11th Edition, tests for heavy metals will also be deleted from individual monographs on substances for veterinary use only. In both cases, no such test will be included in new monographs. For products within the scope of ICH Q3D, users are expected to apply the guidance laid down in the guideline, and analytical procedures may be developed with the help of general chapter *2.4.20. Determination of elemental impurities*.

A different policy is applied for monographs that describe specific tests for elemental impurities. It is decided on a case-by-case basis if tests are kept for these monographs, particularly for those on excipients of natural origin.

II.7.12. Loss on drying *(2.2.32)*

It should be noted that the loss on drying test covers both water and other substances that are volatile at the prescribed drying temperature.

Generally, only an upper limit for loss on drying is given. If the substance is defined as a hydrate (or solvate), upper and lower limits are indicated. Drying is carried out to constant mass, unless a drying time is specified in the monograph. However, it should be noted that any indicated drying time may not necessarily lead to a dry substance. When a drying time is prescribed, adequate validation data must be provided. Where the drying temperature is indicated using a single value, a tolerance of ± 2 °C is understood. For temperatures higher than 105 °C, a greater tolerance has to be indicated in the monograph.

Based on agreements reached in the Pharmacopoeial Discussion Group (PDG), 105 °C is generally prescribed for chemicals as the temperature of choice for this test.

General chapter *2.2.32. Loss on drying* includes four sets of standard conditions that are referred to in monographs using conventional expressions:

a) "in a desiccator" (over 100 g of *molecular sieve R* at atmospheric or reduced pressure and at room temperature);

b) "*in vacuo*" (over *molecular sieve R* at a pressure not exceeding 2.5 kPa at room temperature);

c) "*in vacuo* within a specified temperature range" (over *molecular sieve R* at a pressure not exceeding 2.5 kPa within the temperature range specified in the monograph) [NOTE: the drying capacity of desiccants decreases when the temperature increases];

d) "in an oven within a specified temperature range" (the preferred specified temperature is 105 °C, for harmonisation with the Japanese and US pharmacopoeias, with an implied tolerance of ± 2 °C).

If other conditions are used, in particular lower pressures (e.g. for antibiotics), these are described in the monograph. A molecular sieve 0.5 nm is the preferred drying agent.

Limits below 10% should be given to two significant figures and limits of 10% or greater to three significant figures. The sample size is chosen to give a difference of 5-50 mg before/after drying and is given to four significant figures.

The test can be carried out on a semi-micro scale, in which case the accuracy with which the test sample is to be weighed should be specified accordingly.

Method d) is to be preferred when the product is sufficiently stable at 105 °C. Otherwise, method b) or c) is usually applied. It is important to remember, however, that organic solvents are not always easily removed (e.g. organic solvents in colchicine).

II.7.13. Thermogravimetry *(2.2.34)*

This method can be used to determine loss on drying when the amount of substance has to be restricted, to reduce analyst exposure to toxic substances (e.g. vincristine sulfate and vinblastine sulfate) or if the substance is only available in limited quantities.

II.7.14. Semi-micro determination of water (*2.5.12)* – volumetric Karl-Fischer

The commercial name of the titrant and the solvent used during elaboration of the monograph should be indicated in a footnote to the monograph; it will be transferred to the EDQM Knowledge Database after the monograph is adopted.

Limits below 10% should be given to two significant figures and limits of 10% or greater to three significant figures. If water content is less than 0.5%, it is recommended to switch to micro determination of water. The sample size is chosen to obtain a titration volume of about 1 mL and should be given to three significant figures; it may be necessary to lower the strength of the titrant when testing samples with low water content.

In the case of well-defined hydrates, water content is specified as a range, whereas a maximum content is generally prescribed for products containing variable quantities of water. When more than one form is identified, a cross-reference to the water test is placed in the IDENTIFICATION section of the monograph.

II.7.15. Micro determination of water (*2.5.32)* – coulometric Karl-Fischer

No detailed description for the composition of the electrolyte (anolyte and catholyte) reagent is given in this general chapter since almost all laboratories use commercially available, ready-to-use reagents.

The commercial name of the titrant (electrolyte reagent) used during elaboration of the monograph should be indicated in a footnote to the monograph; it will be transferred to the EDQM Knowledge Database after the monograph is adopted.

The method of sample preparation must be described. If dissolution in a water-free solvent is necessary, the solvent and the volume must be given. When the oven technique is used to release the water from the sample, the heating temperature is stated in the monograph. The selected gas and gas flow rate are indicated in a footnote and transferred to the EDQM Knowledge Database. The heating time may also be indicated, depending on the instrument used. The direct introduction of solid material in the reaction vessel should only be prescribed in exceptional cases (e.g. no suitable solvent found, degradation of the substance upon heating).

Limits should be expressed to two significant figures. In the case of well-defined hydrates, water content is specified as a range, whereas a maximum content is generally prescribed for products containing variable quantities of water. When more than one form is identified, a cross-reference to the water test is placed in the IDENTIFICATION section of the monograph.

The sample size is normally chosen to have a water content of 100 µg to 10 mg. Titrations down to 10 µg are prescribed only where the water content is very low or the sample size is limited by the cost of the substance. The calculation is based on the maximum value as stated in the monograph. The sample size should be stated to three significant figures.

II.7.16. Gas chromatographic determination of water

This method, using a thermal conductivity detector (TCD), may also be used for the determination of water.

II.7.17. Determination of water by distillation (*2.2.13*)

This method is used mainly for herbal drugs. It is applicable to a quantity of substance capable of yielding 2-3 mL of water.

II.7.18. Sulfated ash (*2.4.14*)

This test is usually intended for the determination of total foreign cations present in organic substances and in those inorganic substances which themselves are volatilised under the conditions of the test. Due to the resulting high bias, the test will be of little value as a purity requirement for the majority of inorganic salts of organic substances.

The limit in a test for sulfated ash is usually set at 0.1%, unless otherwise justified. The amount of substance prescribed for the test must be such that a residue corresponding to the limit will not be less than 1 mg (calculated by mass difference) and the prescribed mass of substance is then given to the appropriate number of significant figures (1.0 g). If the substance tested contains fluorine, the monograph should describe the use of a platinum crucible.

II.7.19. Residue on evaporation

The amount of a liquid material prescribed for the test is such that a residue corresponding to the limit will weigh at least 1.0 mg. The appropriate mass or volume of the substance will normally be in the range of 10-100 g (or mL).

II.7.20. Residual solvents (*2.4.24*)

Control of residual solvents is covered in general chapter *5.4. Residual solvents* and in the general monograph *Substances for pharmaceutical use (2034)*, which apply the ICH Q3C guideline. A procedure included in general chapter *2.4.24* must be validated if it is quantitatively applied to control residual solvents in a substance. Suitable validated procedures may be used instead of those described in general chapter *2.4.24*.

A test for a Class 1 solvent is included in the monograph if it is potentially present in an approved product.

Tests for Class 2 solvents are not included in monographs since the limit may be set using option 2 of general chapter *5.4. Residual solvents*, whereby all the ingredients in a medicinal product are taken into account.

A test for a Class 3 solvent is included if it is potentially present in an approved product at a level higher than 0.5%, otherwise a test for loss on drying is generally prescribed.

Where a quantitative determination of a residual solvent is carried out and a test for loss on drying is not carried out, the content of residual solvent is taken into account when calculating the assay content of the substance, the specific optical rotation and the specific absorbance.

II.7.21. Bacterial endotoxins

When a substance for pharmaceutical use is intended for injection or irrigation, the substance has to comply with the test for bacterial endotoxins. Guidance on how to establish limits is given in general text *5.1.10. Guidelines for using the test for bacterial endotoxins*. In principle, the test is no longer added to new monographs. Compliance with the test is requested via the general monograph *Substances for pharmaceutical use (2034).* A test is included only where a specific procedure has to be described (e.g. if a specific sample preparation has to be used or if a specific method of general chapter 2.6.14 has to be applied). If a test is included in the monograph, no limit is given.

For monographs under revision, the decision whether or not to delete the test and/or the limit is made on a case-by-case basis.

During the elaboration and, if applicable, revision of a monograph, data are gathered and examined in order to decide whether there is a need to give a specific sample preparation procedure in the individual monograph or whether it can be considered that the topic of bacterial endotoxins is adequately covered by the general monograph *Substances for pharmaceutical use (2034).* These data include but are not limited to: validation of the bacterial endotoxin test, batch data and demonstration of absence of interference of the substance with the test.

If a test for pyrogens is replaced by a test for bacterial endotoxins, the decision concerning whether to include a test in the monograph follows the considerations described above. The information on the replacement of the testing procedures is given in the EDQM Knowledge Database.

II.8 ASSAY

Assays are included in monographs unless:

- all the foreseeable impurities can be detected and limited with sufficient accuracy and precision;
- certain quantitative tests, similar to assays, are carried out with sufficient accuracy and precision (specific optical rotation, specific absorbance, etc.);
- specific profiles of relevant substances such as composition of the fatty acid fraction (see general chapter *2.4.22. Composition of fatty acids by gas chromatography*) or composition of the sterol fraction of a fat or fatty oil (see general chapter *2.4.23. Sterols in fatty oils*) have been established;
- the tests performed are sufficient to establish the quality of the substance (typically for non-active substances, e.g. ethanol or water).

More than one assay may be necessary if:

- the substance to be examined consists of a combination of two parts that are not necessarily present in absolutely fixed proportions, so that the assay of only one of the two constituents does not make it possible to determine the substance as a whole correctly (e.g. theophylline and ethylenediamine);
- the results of the quantitative tests do not fully represent the therapeutic activity, in which case a biological assay is included.

In the case of well-defined salts, the assay of only one of the ions, preferably the pharmacologically active moiety, is generally considered sufficient. It is only rarely necessary to determine all the ions and, in any case, it is considered superfluous to determine one of these by two methods even when these rely on different analytical principles.

When the identification and purity tests are sufficiently specific and selective, a non-specific but precise assay may be used (e. g. by volumetric titration), rather than a specific and less precise assay. When an active substance is covered by a monograph and a monograph on the corresponding medicinal product already exists or is being elaborated, the same chromatographic assay procedure should ideally be described.

Every assay procedure proposed must be validated according to the procedures described for the different techniques in part III.

II.8.1. Absorption spectrophotometry (utraviolet and visible) (*2.2.25*)

UV-Vis spectrophotometric assays may be carried out directly or after a suitable chemical reaction. Other techniques are usually preferred. When monographs containing an assay based solely on UV-Vis spectrophotometry are revised, it is recommended to replace it with a chromatographic-separation-based assay or a titration.

II.8.1.1. Direct measurement

This is not specific but may be of acceptable accuracy and precision and is usually performed without a reference substance: the absorbance of the solution is measured at the specified absorption maximum, and the content of the substance to be examined is calculated on the basis of the specific absorbance stated in the monograph.

The specific absorbance value must be verified for a new substance. The manufacturer must supply validation data supporting the acceptance of the “true” value, otherwise this value needs to be validated by the (co-)rapporteur. These validation data include, for example, the purity of the substance used to determine the value, which is demonstrated by employing several methods (separation techniques, absolute methods, the response factors of likely impurities, solvents, etc.).

With a reference substance, the active substance content is calculated by comparing the absorbance of the solution to be examined with that of a solution of the reference substance.

For experimental details and results, see general chapter *2.2.25. Ultraviolet and visible absorption spectrophotometry*.

II.8.1.2. Measurement after a colour reaction

This measurement is carried out by comparison with a reference substance. The results may be less accurate and precise due to the sample treatment.

II.8.2. Volumetric analysis

The amount of the substance taken for the assay is such that the final titration, using automatic titration equipment, will consume less than 10 mL – preferably 7-8 mL – of titrant in order to permit the use of standard titration equipment. In the case of back-titration, the fixed volume of the first titrant added must also be adequate so that the result of the assay will not be based upon volumes that are too similar.

Blank tests are to be prescribed whenever necessary, unless already stipulated in the corresponding general chapter. A blank test can be avoided when the composition of the medium in which a volumetric solution is standardised is the same as that in which it is to be used.

Either potentiometric end-point detection or a visual colour change indicator can be specified in the monograph, when an acid-base or redox titration is described. The potentiometric mode of end-point detection (*2.2.20. Potentiometric titration*) is clearly applicable in almost all cases. Determination by visual colour change should be avoided, except for complexometric titrations, where this is generally not possible. Where potentiometric detection is specified, the appropriate indicator electrode for that purpose is to be given in the text only if necessary (special type of electrode). The number of inflexion points to be evaluated is given. Other modes of detection may be specified, such as the amperometric method (*2.2.19. Amperometric titration*) or the voltametric method (*2.2.65. Voltametric titration*). Whichever mode is used, it must be known to be appropriately reproducible and preferably stoichiometrically exact. When a visual indicator is specified, the colour change is given only when it is different from that described in general chapter *4.1.1. Reagents*.

The following methods are recommended for the titration of halide salts of organic bases and some quaternary ammonium substances:

a) Alkalimetric titration in an alcoholic medium. This is the preferred option for the volumetric titration of halide salts. When carrying out alkalimetric titration, it may be necessary to add 5 mL of *0.01 M hydrochloric acid* before the titration and to measure the volume of titrant required between the two points of inflexion. However, it is advisable to test the feasibility of the titration before adding *0.01 M hydrochloric acid*.

b) Titration with perchloric acid, the sample being dissolved in anhydrous acetic acid before adding acetic anhydride or a mixture of acetic anhydride and anhydrous formic acid.

c) Argentimetry.

d) Methods a) (with the addition of 5 mL of *0.01 M hydrochloric acid*) and b) are often suitable for quaternary ammonium substances.

II.8.3. Chromatography-based techniques

In pharmacopoeial practice, the chromatographic techniques on which assays may be based are normally limited to LC and GC. The recommendations contained in part II.7.8 on related substances for LC and GC will also be valid for developing assays based on these techniques. The use of an external standard in LC and the addition of an internal standard in GC are recommended. Such methods require the use of a CRS with an assigned content (see part I.7. Reference Standards).

II.8.4. Determination of nitrogen by sulfuric acid digestion (*2.5.9*)

Any substance to be assayed by this method has a digestion time assigned after determination of its digestion profile.

The digestion profile may be determined as follows. Several individually weighed portions of the prescribed amount of substance are assayed in accordance with the general chapter while varying the time for which the reaction mixture is boiled, normally up to 120 min, after the mixture has cleared. By plotting the resulting nitrogen content against the boiling time, it is possible to determine the minimum digestion time necessary to obtain constant values. In cases where the necessary digestion time exceeds 30 min, the time required is indicated in the monograph.

II.9 STORAGE

Although the statements given under this heading in a monograph of the Ph. Eur. do not constitute pharmacopoeial requirements, the appropriate information to safeguard the quality of a pharmacopoeial material during storage is to be given here where appropriate.

The terminology given in the *General Notices* and in general chapter *3.2. Containers* should be used. Protection against loss or uptake of constituents via the gas phase requires an “airtight container". A "sealed container" is also "tamper-evident", while the converse is not necessarily true.

Manufacturers should be requested to provide stability data. In considering the guidance to be given in the monograph, the behaviour of the material towards exposure to atmospheric air, various degrees of humidity, different temperatures and actinic light are to be taken into account. Where a substance is described in the CHARACTERS section as hygroscopic, deliquescent or sensitive to air, "airtight container" is indicated. When a substance is known to be sensitive to actinic light, "protected from light" is indicated.

In this context, it must be borne in mind that the method given in general chapter *5.11. Characters section in monographs* for hygroscopicity is not to be used to define storage conditions. This is a rapid method that gives an indication of the hygroscopicity of the substance as an aid to the analyst so that the proper handling precautions can be taken when examining the substance in laboratory conditions.

II.10 LABELLING

Since the labelling of medicine is subject to international agreements and supranational and national regulations, the indications given under LABELLING are not exhaustive: they consist of both mandatory statements (necessary for the application of the monograph) and other statements that are included only as recommendations. In general, for bulk active substances, the requirements given in this section of a pharmacopoeial monograph are confined to those essential for the correct interpretation of the other requirements in the monograph. When, for example, a starting material has to comply with additional requirements (e.g. sterility), the label must state, where appropriate, that the contents of the container are suitable for that use. Furthermore, when the inclusion of certain stabilisers or other additives is authorised by the monograph, their presence will generally have to be declared on the label.

II.11 IMPURITIES

Monographs on organic chemicals should have an IMPURITIES section defining the impurities that are known to be detected by the prescribed tests and that have been considered in defining the acceptance criteria for related substances. Subheadings are given for "Specified impurities" and "Other detectable impurities". All specified impurities covered by the monograph are included in this section. In addition, it may be useful to include information on other detectable impurities, (impurities whose detection by the monograph tests is known and has been experimentally verified) but that are not known to occur in current production batches above the identification threshold).

The IMPURITIES section gives a list showing the chemical structure and chemical nomenclature (of the base/acid/neutral substance, not as the salt) for each impurity. Impurities are designated by a capital letter (A, B, C, D, etc.). Trivial names may be included in parenthesis in cases where they are considered to be informative.

The IMPURITIES section may also give information on the tests that limit a given impurity, for example where this test is not a "Related substances" test (e.g. enantiomeric purity) or where there is more than one "Related substances" test.

II.12 FUNCTIONALITY-RELATED CHARACTERISTICS

Monographs on excipients may have a section on FUNCTIONALITY-RELATED CHARACTERISTICS (FRCs). This is introduced by a standard paragraph indicating the non-mandatory status. The uses for which each FRC is relevant are also stated. FRCs may be presented by:

- giving simply the name;
- giving the name and a recommended method from the general chapters of the Ph. Eur.;
- giving the name, a recommended method and typical values;
- giving the name and a cross-reference to a test present in the mandatory part of the monograph.

III. ANALYTICAL VALIDATION

This section describes the procedures to be carried out to validate the tests that are intended to be described in a Ph. Eur. monograph. These tests include tests for identification, instrumental and non-instrumental tests for the control of impurities, and the assay procedure. The validation requirements vary according to the type of test and the technique employed. This section contains the texts on Analytical Validation adopted by the ICH in 1994, the Extension of the ICH text “Validation of Analytical Procedures” which includes valuable information concerning validation requirements for registration applications and specific guidelines for the validation of pharmaceutical procedures using different analytical techniques.

III.1 <u>DEFINITIONS AND TERMINOLOGY</u>

[ICH document. Text adopted and published by the *International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use* (1994)].

III.1.1. Introduction

This document presents a discussion of the characteristics for consideration during the validation of the analytical procedures included as part of registration applications submitted within the EC, Japan and USA. This document does not necessarily seek to cover the testing that may be required for registration in, or export to, other areas of the world. Furthermore, this text presentation serves as a collection of terms and their definitions, and is not intended to provide direction on how to accomplish validation. These terms and definitions are meant to bridge the differences that often exist between various compendia and regulators of the EC, Japan and USA.

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. A tabular summation of the characteristics applicable to identification, control of impurities and assay procedures is included. Other analytical procedures may be considered in future additions to this document.

III.1.2. Types of analytical procedures to be validated

The discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures:

- Identification tests;
- Quantitative tests for impurities' content;
- Limit tests for the control of impurities;
- Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.

Although there are many other analytical procedures, such as dissolution testing for drug products or particle size determination for drug substance, these have not been addressed in the initial text on validation of analytical procedures. Validation of these additional analytical procedures is equally important to those listed herein and may be addressed in subsequent documents.

A brief description of the types of tests considered in this document is provided below:

- identification tests are intended to ensure the identity of an analyte in a sample. This is normally achieved by comparison of a property of the sample (e.g. spectrum, chromatographic behaviour, chemical reactivity, etc.) to that of a reference standard;
- testing for impurities can be either a quantitative test or a limit test for the impurity in a sample. Either test is intended to accurately reflect the purity characteristics of the sample. Different validation characteristics are required for a quantitative test than for a limit test;
- assay procedures are intended to measure the analyte present in a given sample. In the context of this document, the assay represents a quantitative measurement of the major component(s) in the drug substance. For the drug product, similar validation characteristics also apply when assaying for the active or other selected component(s). The same validation characteristics may also apply to assays associated with other analytical procedures (e.g. dissolution).

III.1.3. Validation characteristics and requirements

The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated. Typical validation characteristics that should be considered are listed below:

- Accuracy;
- Precision;
  - Repeatability;
  - Intermediate precision;
- Specificity;
- Detection limit;
- Quantitation limit;
- Linearity;
- Range.

Each of these validation characteristics is defined in the attached Glossary. The table lists those validation characteristics regarded as the most important for the validation of different types of analytical procedures. This list should be considered typical for the analytical procedures cited but occasional exceptions should be dealt with on a case–by-case basis. It should be noted that robustness is not listed in the table but should be considered at an appropriate stage in the development of the analytical procedure.

Furthermore revalidation may be necessary in the following circumstances:

- changes in the synthesis of the drug substance;
- changes in the composition of the drug product;
- changes in the analytical procedure.

The degree of revalidation required depends on the nature of the changes. Certain other changes may require validation as well.

	TYPE OF ANALYTICAL PROCEDURE			
	IDENTIFICATION	TESTING FOR IMPURITIES		ASSAY
		Quantitative test	Limit test	Dissolution Measurement only Content / potency
CHARACTERISTIC				
Accuracy	–	+	–	+
Precision				
Repeatability		+	–	+
Intermediary Precision		+*	–	+*
Specificity**	+	+	+	+
Detection Limit	–	-***	+	–
Quantitation Limit	–	+	–	–
Linearity	–	+	–	+
Range	–	+	–	+

– signifies that this characteristic is not normally evaluated.
\+ signifies that this characteristic is normally evaluated.
* in cases where reproducibility (see Glossary) has been performed, intermediate precision is not needed.
** lack of specificity of one analytical procedure, could be compensated by other supporting analytical procedure(s).
*** may be needed in some cases.

III.1.4. Glossary

Analytical procedure. The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the preparation of reagents, use of the apparatus, generation of the calibration curve, use of the formulae for the calculation, etc.

Specificity. Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradation products, matrix, etc.

Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s).

This definition has the following implications:

- Identification: to ensure the identity of an analyte.
- Purity tests: to ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc.
- Assay (content or potency): to provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample.

Accuracy. The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

Precision. The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample, it may be investigated using artificially prepared samples or a sample solution.

The precision of analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

Intermediate precision expresses variations within laboratories: different days, different analysts, different equipment, etc.

Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardisation of methodology).

Detection limits. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Quantitation limits. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of substances in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

Linearity. The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

Range. The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Robustness. The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

III.2 METHODOLOGY

[ICH document. Text adopted and published by the *International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use* (1996)].

III.2.1. Introduction

This document is complementary to the parent document which presents a discussion of the characteristics that should be considered during the validation of analytical procedures. Its purpose is to provide some guidance and recommendations on how to consider the various validation characteristics for each analytical procedure. In some cases (for example, demonstration of specificity) the overall capabilities of a number of analytical procedures in combination may be investigated in order to ensure the quality of the drug substance or drug product. In addition, the document provides an indication of the data which should be presented in a new drug application.

All relevant data collected during validation and formulae used for calculating validation characteristics should be submitted and discussed as appropriate.

Approaches other than those set forth in this guideline may be applicable and acceptable. It is the responsibility of the applicant to choose the validation procedure and protocol most suitable for their product. However, it is important to remember that the main objective of validation of an analytical procedure is to demonstrate that the procedure is suitable for its intended purpose. Due to their complex nature, analytical procedures for biological and biotechnological products in some cases may be approached differently than in this document.

Well-characterised reference materials, with documented purity, should be used throughout the validation study. The degree of purity required depends on the intended use.

In accordance with the parent document and for the sake of clarity, this document considers the various validation characteristics in distinct parts. The arrangement of these parts reflects the process by which an analytical procedure may be developed and evaluated.

In practice, it is usually possible to design the experimental work such that the appropriate validation characteristics can be considered simultaneously to provide a sound, overall knowledge of the capabilities of the analytical procedure, for instance: specificity, linearity, range, accuracy and precision.

III.2.2. Specificity

An investigation of specificity should be conducted during the validation of identification tests, the determination of impurities and the assay. The procedures used to demonstrate specificity will depend on the intended objective of the analytical procedure.

It is not always possible to demonstrate that an analytical procedure is specific for a particular analyte (complete discrimination). In this case a combination of two or more analytical procedures is recommended to achieve the necessary level of discrimination.

III.2.2.1. Identification

Suitable identification tests should be able to discriminate between substances of closely related structures which are likely to be present. The discrimination of a procedure may be confirmed by obtaining positive results (perhaps by comparison with a known reference material) from samples containing the analyte, coupled with negative results from samples which do not contain the analyte. In addition, the identification test may be applied to materials structurally similar to or closely related to the analyte to confirm that a positive response is not obtained. The choice of such potentially interfering materials should be based on sensible scientific judgement with a consideration of the interferences which could occur.

III.2.2.2. Assays and impurity tests

For chromatographic procedures, representative chromatograms should be used to demonstrate specificity and individual components should be appropriately labelled. Similar considerations should be given to other separation techniques.

Critical separations in chromatography should be investigated at an appropriate level. For critical separations specificity can be demonstrated by the resolution of the two components which elute closest to each other.

In cases where a non-specific assay is used, other supporting analytical procedures should be used to demonstrate overall specificity. For example, where a titration is adopted to assay the drug substance, the combination of the assay and a suitable test for impurities can be used.

The approach is similar for both assays and impurity tests:

Impurities are available

- for the assay, this should involve demonstration of the discrimination of the analyte in the presence of impurities and/or excipients; practically, this can be done by spiking pure substances (drug substance or drug product) with appropriate levels of impurities and/or excipients and demonstrating that the assay result is unaffected by the presence of these materials (by comparison with the assay result obtained on unspiked samples);
- for the impurity test, the discrimination may be established by spiking the drug substance or drug product with appropriate levels of impurities and demonstrating the separation of these impurities individually and/or from other components in the sample matrix. Alternatively, for less discriminating procedures it may be acceptable to demonstrate that these impurities can still be determined with appropriate accuracy and precision.

Impurities are not available

If impurity or degradation product standards are unavailable, specificity may be demonstrated by comparing the test results of samples containing impurities or degradation products to a second well-characterised procedure, e.g. pharmacopoeial procedure or other validated analytical procedure (independent procedure). As appropriate, this should include samples stored under relevant stress conditions: light, heat, humidity, acid/base hydrolysis and oxidation.

- For the assay, the two results should be compared.
- For the impurity tests, the impurity profiles should be compared.

Peak purity tests (e.g. diode array, mass spectrometry) may be useful to show that the analyte chromatographic peak is not attributable to more than one component.

III.2.3. Linearity

Linearity should be established across the range (see part III.2.4) of the analytical procedure. It may be demonstrated directly on the drug substance (by dilution of a standard stock solution) and/or separate weighings of synthetic mixtures of the drug product components using the proposed procedure. The latter aspect can be studied during investigation of the range.

Linearity should be established by visual evaluation of a plot of signals as a function of analyte concentration or content. If there is a linear relationship, test results should be evaluated by appropriate statistical methods, for example, by calculation of a regression line by the method of least squares. In some cases, to obtain linearity between assays and sample concentrations, the test data may have to be subjected to a mathematical transformation prior to the regression analysis. Data from the regression line itself may be helpful to provide mathematical estimates of the degree of linearity. The correlation coefficient, y-intercept, slope of the regression line and residual sum of squares should be submitted. A plot of the data should be included. In addition, an analysis of the deviation of the actual data points from the regression line may also be helpful for evaluating linearity.

Some analytical procedures, such as immunoassays, do not demonstrate linearity after any transformation. In this case the analytical response should be described by an appropriate function of the concentration (amount) of an analyte in a sample.

For the establishment of linearity, a minimum of five concentrations is recommended. Other approaches should be justified.

III.2.4. Range

The specified range is normally derived from linearity studies and depends on the intended application of the procedure. It is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision when applied to samples containing amounts of analyte within or at the extremes of the specified range of the analytical procedure.

The following minimum specified ranges should be considered:

- for the assay of a drug substance or a drug product: from 80 to 120% of the test concentration;
- for the determination of an impurity: from the quantitation limit (QL) or from 50% of the specification of each impurity, whichever is greater, to 120% of the specification;
- for impurities known to be unusually potent or to produce toxic or unexpected pharmacological effects, the detection/quantitation limit should be commensurate with the level at which the impurities must be controlled. *Note: for validation of impurity test procedures carried out during development, it may be necessary to consider the range around a suggested (probable) limit;*
- if assay and purity are performed together as one test and only a 100% standard is used, linearity should cover the range from QL or from 50% of the specification of each impurity, whichever is greater, to 120% of the assay specification;
- for content uniformity, covering a minimum of 70 to 130% of the test concentration, unless a wider more appropriate range, based on the nature of the dosage form (e.g. metered dose inhalers) is justified;
- for dissolution testing: ± 20% over the specified range, e.g. if the specifications for a controlled released product cover a region from 20%, after 1 hour, up to 90%, after 24 hours, the validated range would be 0-110% of the label claim.

III.2.5. Accuracy

Accuracy should be established across the specified range of the analytical procedure.

III.2.5.1. Assay

Drug substance (Active pharmaceutical ingredient). Several methods of determining accuracy are available:

- application of an analytical procedure to an analyte of known purity (e.g. reference material);
- comparison of the results of the proposed analytical procedure with those of a second well-characterised procedure, the accuracy of which is stated and/or defined (independent procedure);
- accuracy may be concurrently determined when precision, linearity and specificity data are acquired.

Drug product. Several methods for determining accuracy are available:

- application of the analytical procedure to synthetic mixtures of the drug product components to which known quantities of the drug substance to be analysed have been added;
- in cases where it is impossible to obtain samples of all drug product components, it may be acceptable either to add known quantities of the analyte to the drug product or to compare the results obtained from the second, well-characterised procedure, the accuracy of which is stated and/or defined (independent procedure);
- accuracy may be concurrently determined when precision, linearity and specificity data are acquired.

III.2.5.2. Impurities (quantitation)

Accuracy should be assessed on samples (drug substance/drug product) spiked with known amounts of impurities.

In cases where it is impossible to obtain samples of certain impurities and/or degradation products, it is acceptable to compare results obtained by an independent procedure. The response factor of the drug substance can be used.

III.2.5.3. Recommended data

Accuracy should be assessed using a minimum of nine determinations over a minimum of three concentration levels covering the specified range (e.g. three concentrations/three replicates each).

Accuracy should be reported as percent recovery by the assay of a known added amount of analyte in the sample or as the difference between the mean and the accepted true value together with the confidence intervals.

III.2.6. Precision

Validation of tests for assay and for quantitative determination of impurities includes an investigation of precision.

III.2.6.1. Repeatability

Repeatability should be assessed using:

- a minimum of nine determinations covering the specified range for the procedure (e.g. three concentrations/three replicates each), *or*
- a minimum of six determinations at 100% of the test concentration.

III.2.6.2. Intermediate precision

The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used. The applicant should establish the effects of random events on the precision of the analytical procedure. Typical variations to be studied include days, analysts, equipment, etc. It is not necessary to study these effects individually. The use of an experimental design (matrix) is encouraged.

III.2.6.3. Reproducibility

Reproducibility is assessed by means of an inter-laboratory trial. Reproducibility should be considered in case of the standardisation of an analytical procedure, for instance, for inclusion of procedures in pharmacopoeias. These data are not part of the marketing authorisation dossier.

III.2.6.4. Recommended data

The standard deviation, relative standard deviation (coefficient of variation) and confidence interval should be reported for each type of precision investigated.

III.2.7. Detection limit

Several approaches for determining the detection limit are possible, depending on whether the procedure is a non-instrumental or instrumental. Approaches other than those listed below may be acceptable.

III.2.7.1. Based on visual evaluation

Visual evaluation may be used for non-instrumental methods but may also be used with instrumental methods.

The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

III.2.7.2. Based on signal-to-noise ratio

This approach can only be applied to analytical procedures which exhibit baseline noise. Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. A signal-to-noise ratio between 3 or 2:1 is generally acceptable.

III.2.7.3. Based on the standard deviation of the response and the slope

The detection limit (DL) may be expressed as:

$$DL = \frac{3.3\sigma}{S}$$

σ = the standard deviation of the response,

S = the slope of the calibration curve.

The slope S may be estimated from the calibration curve of the analyte. The estimate of σ may be carried out in a variety of ways, for example:

- Based on the standard deviation of the blank. Measurement of the magnitude of analytical background response is performed by analysing an appropriate number of blank samples and calculating the standard deviation of these responses.
- Based on the calibration curve. A specific calibration curve should be studied using samples containing an analyte in the range of DL. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

III.2.7.4. Recommended data

The detection limit and the method used for determining the detection limit should be presented.

In cases where an estimated value for the detection limit is obtained by calculation or extrapolation, this estimate may subsequently be validated by the independent analysis of a suitable number of samples known to be near or prepared at the detection limit.

III.2.8. Quantitation limit

Several approaches for determining the quantitation limit are possible, depending on whether the procedure is non-instrumental or instrumental. Approaches other than those listed may be acceptable.

III.2.8.1. Based on visual evaluation

Visual evaluation may be used for non-instrumental methods, but may also be used with instrumental methods.

The quantitation limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

III.2.8.2. Based on signal-to-noise ratio

This approach can only be applied to analytical procedures which exhibit baseline noise.

Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and by establishing the minimum concentration at which the analyte can be reliably quantified. A typical signal-to-noise ratio is 10:1.

III.2.8.3. Based on the standard deviation of the response and the slope

The quantitation limit (QL) may be expressed as:

$$QL = \frac{10\sigma}{S}$$

σ = the standard deviation of the response,

S = the slope of the calibration curve.

The slope S may be estimated from the calibration curve of the analyte. The estimate of σ may be carried out in a variety of ways for example:

- Based on the standard deviation of the blank. Measurement of the magnitude of analytical background response is performed by analysing an appropriate number of blank samples and calculating the standard deviation of these responses.
- Based on the calibration curve. A specific calibration curve should be studied using samples containing an analyte in the range of QL. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

III.2.8.4. Recommended data

The quantitation limit and the method used for determining the quantitation limit should be presented. The limit should be subsequently validated by the analysis of a suitable number of samples known to be near or prepared at the quantitation limit.

III.2.9. Robustness

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters.

If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure. One consequence of the evaluation of robustness should be that a series of system suitability parameters (e.g. resolution test) is established to ensure that the validity of the analytical procedure is maintained whenever used.

Typical variations are:

- stability of analytical solutions;
- different equipment;
- different analysts.

In the case of LC, typical variations are:

- influence of variations of pH in a mobile phase;
- influence of variations in mobile phase composition;
- different columns (different lots and/or suppliers);
- temperature;
- flow rate.

In the case of GC, typical variations are:

- different columns (different lots and/or suppliers);
- temperature;
- flow rate.

III.2.10. System suitability testing

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analysed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated. See Pharmacopoeias for additional information.

III.3 SPECIFIC APPLICATION TO ANALYTICAL PROCEDURES USED IN THE PH. EUR.

The following parts describe a number of points that are important for the validation of analytical procedures employing specific analytical techniques. These guidelines are to be used in conjunction with the general chapters of the Ph. Eur. and the validation requirements given previously in the ICH documents.

III.3.1. Optical rotation (*2.2.7*)

III.3.1.1. Introduction

The solvent should be chosen in order to obtain an angle of rotation that is as great as possible. The stability of the angle of rotation of the solution should be checked over a period of at least 2 hours. If necessary, the use of a freshly prepared solution may be prescribed. In exceptional cases, it may be necessary to prescribe an equilibration period before the measurement is carried out. Whenever possible, the use of a wavelength corresponding to the D-line of sodium (i.e. 589 nm) is prescribed.

III.3.1.2. Identification

When the substance examined is an enantiomer, the specific optical rotation is used for the identification.

If the specific optical rotation is used for identification only, the result does not have to be calculated on the dried substance or the solvent-free substance. The limits prescribed should take into account any variation in content and purity of samples of different origin that comply with the monograph.

III.3.1.3. Tests

Specific optical rotation may be used to verify the optical purity of an enantiomer. This method is less sensitive than chiral LC. In the case where one enantiomer is to be limited by the measurement of specific optical rotation, then it is to be demonstrated that under the conditions of the test, the enantiomer has sufficient optical activity to be detected. The result is calculated on the dried substance or the solvent-free substance. Whenever possible, the influence of potential impurities should be reported. Limits for the specific optical rotation should be chosen with regard to the permitted amount of impurities. In the absence of information on the optical activity of related substances and when insufficient amounts of the related substances are available, the limits are usually arbitrarily fixed at ± 5% around the mean value obtained for samples that comply with the monograph. Samples of different origin should be examined whenever possible. It is also worthwhile examining samples that are close to the expiry date to obtain information on the influence of normal ageing.

Measurement of the angle of rotation may be used to verify the racemic character of a substance. In that case limits of − 0.10° to + 0.10° are usually prescribed.

If possible, it is to be demonstrated that, under the conditions of the test, the enantiomer has sufficient optical activity to be detected.

III.3.2. Absorption spectrophotometry (ultraviolet and visible) (*2.2.25*)

In all cases, the suitability of the operating conditions (solvents employed and their quality, pH of the solution, etc.), must be demonstrated.

In normal use, ultraviolet spectrophotometry is a technique of limited discrimination power. The use of 1^{st}- and 2^{nd}-order derivative techniques may increase discrimination power.

III.3.2.1. Identification

Ultraviolet spectrophotometry is rarely the only procedure described for identification. When it is included in an identification series, discrimination power must be demonstrated by comparing the spectrum of the analyte with spectra of similar substances. Discrimination power can be increased by using absorbance ratios rather than absorbance values.

III.3.2.2. Limit test

When ultraviolet spectrophotometry is used for a limit test, it is to be demonstrated that at the appropriate wavelength, the related substance to be limited makes a sufficient contribution to the measured absorbance. The absorbance corresponding to the limiting concentration of the related substance must be established.

III.3.2.3. Assay

When ultraviolet spectrophotometry is used for the assay, the contribution to the absorbance of the known impurities must be evaluated. The use of specific absorbance values for assays is discouraged, but may be possible in dissolution tests in monographs on medicinal products (see the Technical guide for the elaboration of monographs on medicinal products containing chemically defined active substances). If specific absorbance values are prescribed, they must be evaluated by an inter-laboratory trial using a batch of known purity. Purity is to be estimated by applying a variety of techniques including separation techniques and absolute techniques.

III.3.3. Non-instrumental limit tests

III.3.3.1. Appearance of solution (2.2.1 and 2.2.2)

These simple visual tests compare the colour (or opalescence) of the test solution against a series of standards. Typically, the test solution should be clear and colourless. These tests are intended to give an assessment of the general purity of the substance. When degrees of colour (or opalescence) are permitted, the impurity and the level to which the degree of coloration (or opalescence) corresponds are often unknown. Validation is based on the examination of batch data supplied by the manufacturer(s). However, when the impurity causing the opalescence or colour is known, it may be possible to validate the visual test by comparison with a more sophisticated analytical technique.

III.3.3.2. Acidity or alkalinity

This is a general test of the purity of a substance. It is a non-specific test used for the control of protolytic impurities. The appropriate use of this test is described above.

III.3.3.3. Limit tests for anions/cations (2.4)

These are simple and rapid tests but they are to be shown to be appropriate by recovery experiments and/or comparison with other more sophisticated techniques.

Sulfated ash (*2.4.14*). The sulfated ash test is intended as a global determination of cations present in organic substances but is obviously not applicable to inorganic salts of acidic organic substances. The limit is normally 0.1%. This gravimetric test controls the content of foreign cations to a level appropriate to indicate the quality of production. This method can be considered to be well established and no further validation is required.

Colour or precipitation reactions. Limit tests are also described for individual cations and anions which are based on visual comparison of a colour or opalescence. It is essential that it is demonstrated that:

- the colour or opalescence is visible at the target concentration (limit);
- the recovery of added ion is the same for the test and reference solutions (by visual observation and if possible by absorbance measurement);
- the response is sufficiently discriminating around the target value (50%, 100% and 150% of the target value) by measuring the absorbances at an appropriate wavelength in the visible region;
- a recovery experiment at the target value is carried out six times and the repeatability

relative standard deviation (RSD) calculated. Recovery should be greater than 80% and the repeatability RSD should be not more than 20%.

It would be desirable, when appropriate, to compare the results obtained from a recovery experiment using the proposed limit test procedure with a quantitative determination using a different technique (e.g. atomic absorption spectrophotometry for cations or ion chromatography for anions). The results obtained by the two techniques are to be similar.

III.3.4. Atomic absorption spectrometry (*2.2.23*)

Atomic absorption spectrometry is exclusively employed in tests to determine the content of specific elements that are present in substances as impurities. The following validation requirements are particularly pertinent to atomic spectrometric methods. More validation requirements are given in the general chapter.

III.3.4.1. Specificity

In principle, this technique is specific, using the appropriate source and wavelength, for the element to be determined since the atom emits or absorbs radiation at discrete spectral lines. However, interferences may be encountered due to optical and/or chemical effects. Thus it is important to identify the interferences and, if possible, reduce their effect by using appropriate means before starting the validation programme.

Such interferences may result in a systematic error if a direct calibration procedure is employed or may reduce the sensitivity of the technique. The most important sources of error in atomic spectrometry are associated with errors due to the calibration process and to matrix interference (care must be taken to avoid memory effects).

III.3.4.2. Calibration

Aqueous standards are prepared and analysed at different concentration levels, spread over the calibration range.

The number of concentration levels at which standards must be prepared depends on the calibration model used. To demonstrate the applicability of a straight-line regression model, standards should be prepared at a minimum of four concentration levels. A parabolic regression model also requires at least four concentration levels. Preferably, the concentration levels are evenly distributed over the calibration range.

Generally, it is recommended to perform at least five measurements at each concentration level.

Calibration problems can often be detected visually. However, these plots alone cannot be used as proof of the suitability of the calibration procedure.

- The measured absorbances are plotted as a function of the concentration, together with the curve that describes the calibration function and its confidence interval. This curve should fit the data points.
- The residuals (i.e. the difference between the measured and the estimated absorbance) are plotted as a function of the concentration. When a suitable calibration procedure is applied, the residuals are randomly distributed around the x-axis.

When the variance of the signal increases with the concentration, as is often the case with atomic spectrometry and shown from either a plot of the residuals or with a one-tailed *t*-test, a weighted calibration model is better suited. Both linear and quadratic weighting functions are applied to the data to find the most appropriate weighting function to be employed.

For a weighted model, the weighted residuals (i.e. the weight multiplied by the residual) are plotted as a function of the concentration:

- the measured absorbances are plotted as a weighted function of the concentration, together with the curve that describes the calibration function and its confidence interval;
- the weighted residuals are plotted as a function of the concentration.

It must be demonstrated that the data accurately fit the model. Application of a straight-line regression model implies that the linearity of the calibration line is investigated.

III.3.4.3. Matrix effects

When aqueous reference solutions are used to estimate the calibration function, it must be ensured that the sensitivities obtained with the sample solution and the aqueous solutions are similar. When a straight-line calibration model is applied, differences in sensitivity can be detected by comparing the slopes of standard addition and aqueous reference solutions calibration graphs. The quality of the estimation of the slopes of both regression lines depends on the number and distribution of the measurement points. Therefore, it is recommended to include sufficient measurement points (always > 5) in both regression lines, and to concentrate these points mainly on the extremes of the calibration range.

The slopes of the standard addition line and the aqueous calibration line are compared, by applying a *t*-test, to check whether slopes of both regression lines are significantly different. If that is the case, then Method II (standard additions) is to be applied; if it is not the case, Method I (direct calibration) can be applied.

III.3.4.4. Detection and quantitation limit (based on the standard deviation of the blank)

To estimate the detection and quantitation limit, representative blanks are prepared and analysed. Preferably, matrix blanks are used, which contain every component of the sample except the analyte. However, when no matrix blanks are available, reagent blanks, containing all reagents and prepared in the same manner as the sample solution, can be used.

Other aspects of the validation programme are covered above.

III.3.5. Separation techniques

The different chromatographic procedures (TLC, GC and LC) may be employed in the IDENTIFICATION section, in the TESTS section for the limitation of related substances and in the ASSAY section to determine the content of the active substance. The analytical procedures are to be validated according to the principles described previously, but there are specific aspects of the different chromatographic techniques that are to be taken into consideration. These are described below.

III.3.5.1. Thin-layer chromatography (2.2.27)

This chromatographic technique is widely employed in the Ph. Eur. for identification using a reference substance and for the limitation of impurities with or without the use of a reference substance. When impurities are to be determined quantitatively, appropriate instrumentation must be employed. For the most part, silica is employed as the stationary phase but reverse-phase stationary phases (e.g. silanised silica gel) or cellulose stationary phases are also employed. Nonetheless, the following points are common to the application of thin-layer chromatographic techniques whether used for identification or for a test for related substances.

- Specificity: it is accepted that for an identification test, specificity cannot be attained using this technique alone but good discrimination can be expected. It must be accompanied by other tests which together assure specificity. Selectivity may not be attainable for a limit test, in which case one or more additional tests must be described to control the impurities not separated. Discrimination power is to be demonstrated. For an identification test, improvement in discrimination power can sometimes be achieved using a spray reagent that differentiates similar substances by colour.
- Stationary phase: it is to be demonstrated that the test is applicable using plates of the same type but of different origin. Separations that can only be achieved on one particular type of plate are to be avoided, if possible.
- Performance test (SST): such a test is generally performed to verify the separation of two closely eluting substances, the substance itself and a similar substance (critical pair). It is to be demonstrated that the separation of the chosen substances will guarantee the suitability of the chromatographic system. This performance criterion is essential for a test for related substances.

Additional aspects that require further documentation when TLC is applied to a test for related substances include:

- Detection: the use of specific spray reagents must be avoided when applying a related substances test unless the test is designed to limit a named impurity using a reference substance.
- Detection limit: when applying a quantitative instrumental procedure, one of the described methods for the calculation of the DL applies. When a visual method is applied, it is to be demonstrated that the quantity corresponding to the specified limit is detectable.
- Response factors: if the known impurities are available, then the similarity of response factors (relative to the substance itself) is demonstrated using the given detection conditions. For a limit test, differences in response can be shown by comparison of the visual detection limits.
- Quantitation limit, linearity, range and repeatability: data are also required when an instrumental quantitative TLC procedure is applied.

III.3.5.2. Liquid chromatography (2.2.29)

LC is usually applied to limit the content of impurities in a substance (employing an external standard, usually an appropriate dilution of the test solution), to determine the content of a substance (employing an external standard), and occasionally as an identification by cross-reference to one of the aforementioned procedures. Attention is to be paid to a number of aspects peculiar to LC.

III.3.5.2.a. Identification

It is accepted that for an identification test, specificity may not be attained using this technique but good discrimination can be expected. It must be accompanied by other tests that together ensure specificity. Discrimination power must be demonstrated with retention times, relative retentions or mass distribution ratio of similar substances, and the substance itself, being reported. Such information is to be supplied for a variety of stationary phases of a similar type.

III.3.5.2.b. Limit test

- Specificity:
  - *Discrimination power of the separation*: separation of known and potential impurities from the substance itself and if possible, from each other, must be demonstrated. Specificity may be ensured by detection by mass spectrometry. Impurities not separated from the substance must be controlled by another procedure. The retention times, relative retention times or mass distribution ratio of the substance and the impurities must be reported. Such information is to be supplied for a variety of stationary phases of a similar type.
  - *Discrimination power of the detection system*: the choice of the detector or the detector conditions employed must be justified (e.g. change in the detection wavelength when using UV detection) while specificity can be ensured by the use of detection by mass spectrometry.
- Response factors: it is essential to demonstrate the similarity of response of the substance and known impurities (at the wavelength of detection for UV detection but applies also to other detection systems, e.g. conductimetry). A response factor of a known impurity that is greater than 1.2 or less than 0.8 compared to that of the substance to be examined may require the use of either CFs or of that individual impurity as an external standard when the proposed limit is 0.1% or greater.
- Detection and quantitation limits: these limits must be determined for the external standard, which is either a dilution of the substance to be examined or a known impurity. When a peak of an impurity elutes close to the peak of the substance, particularly if it elutes after the peak due to the substance, detection and quantitation limits are to be determined on that impurity. One of the methods for calculation of both the DL and the QL is applied.
- Stability: data should be provided demonstrating the period of use of reference and test solutions.
- Recovery: when an extraction procedure is employed, a recovery experiment using known and available impurities is to be carried out under optimal conditions and the results reported. It is to be demonstrated that the recovery shows an acceptable accuracy and precision.
- Derivatisation: when pre- or post-column derivatisation is employed, it is important to establish the optimal reaction conditions (time and temperature) and also to investigate the stability of the derivative under normal conditions of use.
- System suitability test: as described for TLC. The use of the S/N ratio is only required when the DL and the specified limit are similar.

III.3.5.2.c. Assay

- Specificity: this is preferable but not essential provided that the interfering impurity is present at a low level and is controlled by another test.

- System suitability test: as described in general chapter *2.2.46. Chromatographic separation techniques*. Table 2.2.46.-1 can be extended as follows:

	Number of individual injections				
	3	**4**	**5**	**6**	**10**
B (%)	**Maximum permitted relative standard deviation**				
1.0	0.21	0.30	0.37	0.42	0.60
1.5	0.31	0.44	0.55	0.64	0.90
2.0	0.41	0.59	0.73	0.85	1.20
2.5	0.52	0.74	0.92	1.06	1.51
3.0	0.62	0.89	1.10	1.27	1.81
3.5	0.72	1.04	1.22	1.48	2.11
4.0	0.83	1.19	1.46	1.70	2.41
4.5	0.93	1.33	1.65	1.91	2.71
5.0	1.04	1.48	1.83	2.12	3.01

Limit tests and assays must be validated as described above (see part III.2) for linearity, repeatability and reproducibility.

III.3.5.3. Gas chromatography (2.2.28)

III.3.5.3.a. Identification

Specificity: as described for LC.

III.3.5.3.b. Limit test

- Specificity: as described for LC.
- Response factors: as described for LC; response factors relative to the substance itself must be provided. This is particularly important when using selective detectors (ECD, NPD, etc.).
- Detection and quantitation limits: as described for LC.
- Stability: as described for LC.
- Derivatisation: as described for LC.
- Internal standard: it is to be demonstrated that under the chromatographic conditions employed, the peak due to the internal standard does not interfere with the impurity peaks or that due to the substance itself.
- Recovery parameters: as described for LC.

III.3.5.3.c. System suitability test

Details that are to be provided of chromatographic criteria to which a user must conform to successfully apply the test are as follows.

- The S/N ratio is usually determined for a signal that is equal to or greater than the DL.
- Resolution between the peak due to the substance and a closely eluting peak of an impurity or between the peak due to the substance and the peak due to the internal

standard. It is also useful to give the acceptable range of values for the symmetry factor when it is different from the accepted range of 0.8-1.8 as given in general chapter *2.2.46*. This is particularly important when employing packed columns and when the peak of an impurity to be controlled elutes immediately after the principal peak. Verification of performance using a similar column, when possible, is recommended.

- Head-space injection technique: this type of injection is employed for highly volatile substances. It is important to demonstrate that the temperature and time of pre-heating of the injection vial results in equilibrium conditions. The presence or absence of a matrix effect should also be demonstrated. One way of validating head-space injection conditions is to carry out multiple head-space extractions (after each injection, the head space is vented and the vial is re-equilibrated before re-injection of the gaseous phase). The pre-requisite for good conditions is that the relationship of the logarithms of the areas of the analyte peak to the number of extractions is linear with a coefficient of regression of 1.0. Matrix effects can be overcome by the use of the standard addition technique.

III.3.5.3.d. <u>Assay</u>

- Specificity: as described for LC.
- System suitability test: as described in general chapter *2.2.46. Chromatographic separation techniques* (see also part III.3.5.2.c).

Limit tests and assays must be validated as described above (see part III.2) for linearity, repeatability and reproducibility.

III.3.5.3.e. <u>Identification and control of residual solvents (*2.4.24*)</u>

The sample preparation and GC systems employed are to be validated for the substance to be examined by applying the criteria given above with particular respect to:

- specificity;
- detection and quantitation limits;
- recovery;
- repeatability;
- linearity, when employed quantitatively.

III.3.6. Semi-micro determination of water (*2.5.12*)

A number of commercial Karl Fischer reagents are available so it is important to ensure their suitability for use by means of a validation procedure such as standard addition.

Standard addition

Determine the water content of the sample under the proposed conditions. Then, under airtight conditions, add a suitable volume of a standardised solution of water in *methanol R* and determine the water content m_{H_2O} as mg water. Repeat this step at least five times.

Calculate the regression line of the cumulative water determined against the water added. Calculate slope *b*, intercept with the ordinate *a* and intersection of the extrapolated calibration line with the abscissa *d*.

The slope b is to be between 0.975 and 1.025 (deviation $\pm$ 2.5%) to be acceptable. The percentage errors e_1 and e_2 are lower than $\pm$ 2.5%.

$$e_1 = \frac{a - m_{H_2O}}{m_{H_2O}} \times 100 \qquad\qquad e_2 = \frac{|d| - m_{H_2O}}{m_{H_2O}} \times 100$$

Calculate the recovery of each standard addition step. The mean recovery is to be within 97.5% and 102.5% to be acceptable.

III.3.7. Volumetric titrations (*2.5.11*, *2.2.19*, *2.2.20*)

When developing a new volumetric assay procedure, it is recommended to titrate at least seven different quantities under the prescribed conditions in a randomised order to give end-point volumes in the range of 20-90% of the volume of the burette employed. Subsequently, the data are treated statistically and a number of criteria are to be fulfilled to permit acceptance of the titration procedure.

The relative error in reading of the mass on the balance and of the volume at the end-point is to be less than 0.5% of the values found.

The results, as end-point volumes V_i in dependence of mass m_i, are evaluated by linear regression. The regression line is calculated and characterised by the slope b_{obs}, the extrapolated intercept a_{obs} and the precision as $\sigma(V)$.

1st Criterion – Proportional Systematic Error (Bias)

The calculated slope b_{obs}, taking into account the titre of the standardised volumetric solution, is within 0.3% for potentiometric titrations (0.5% for visual titrations) compared to the theoretical value given as titration constant b_{theor}.

$$\left(\frac{b_{obs} - b_{theor}}{b_{theor}}\right) \times 100 \leq 0.3\% \ (0.5\% \ for\ visual\ determination)$$

where $b_{theor} = \frac{Z}{M_r C_r}$

M_r is the relative molecular mass, Z is the stoichiometric factor of the chemical reaction and C_r is the molar concentration of the titrant.

2nd Criterion – Additional Systematic Error (Bias)

The extrapolated intercept a_{obs} is less than 0.4% for potentiometric titrations and 0.6% for visual titrations of the expected or target titration volume. This criterion may not be fulfilled if the titration is carried out too rapidly (potentiometric titration) or an unsuitable indicator has been employed (visual titration).

$$\left(\frac{a_{obs}}{V_T}\right) \times 100 \ < 0.4\% \ (0.6\% \ for\ visual\ determination)$$

where a_{obs} is the extrapolated intercept of the regression line at zero and V_T is the expected or target titration volume.

3rd Criterion – Precision (Statistical Error)

The remaining estimated standard deviation $\sigma(V)$ is less than 0.3% for potentiometric titrations (0.5% for visual indicator titrations) of the mean titration volume of end-point using the titration procedure to be introduced in the monograph.

$$\left(\frac{\sigma(V)}{V_T}\right) \times 100 \; < 0.3\% \; (0.5\% \; for \; visual \; determination)$$

where $\sigma(V)$ is the estimated standard deviation.

$$\sigma(V) = \sqrt{\frac{\sum(V_i - a_{obs} - b_{obs} m_i)^2}{n-2}}$$

where V_i is the titration volume, m_i is the mass of the substance and n is the number of titrations performed.

4th Criterion – Practical Relative Error

Some titration procedures may not fulfil the first and second criteria but exhibit low and acceptable bias at the target titration volume (8 mL ± 1 mL for a 10 mL burette). Thus, if the first and/or the second criteria given above are not met, then calculate the relative accuracy at the target titration volume.

$$\left|\left(\frac{a_{obs}}{V_T} + \frac{b_{obs} - b_{theor}}{b_{theor}}\right)\right| \times 100$$

However, when the volumetric titration procedure is well established, it is sufficient to verify that the repeatability and accuracy of the titration (minimum 6 replicates) are not greater than the limits given in the table and decision tree below.

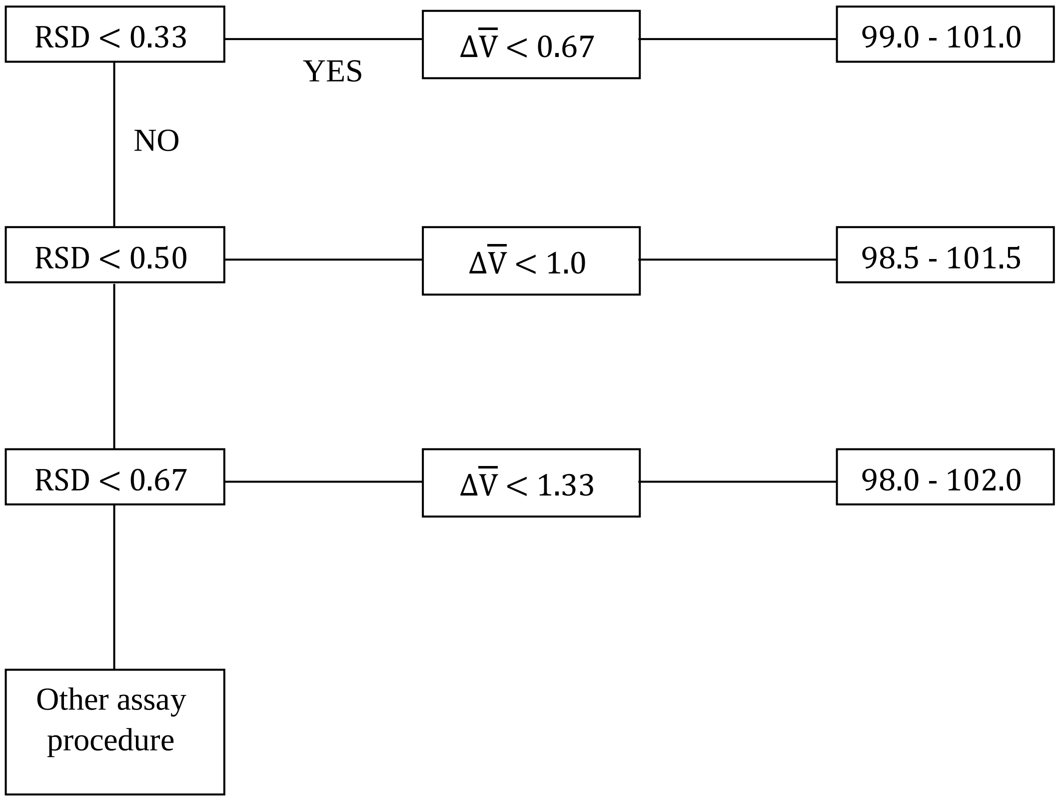
VOLUMETRIC TITRATION	**CONTENT LIMITS (%)**	**REPEATABILITY (RSD)**	**RELATIVE ACCURACY (%)**
Acid/base	± 1.0	0.33	± 0.67
Non-aqueous	± 1.0	0.33	± 0.67
Conjugate acid of base	± 1.0	0.33	± 0.67
Redox	± 1.5	0.5	± 1.0
Argentometric	± 1.5	0.5	± 1.0
Complexometric	± 2.0	0.67	± 1.33

The figures in the table are given as guidance and it may be demonstrated that stricter limits can be applied. The use of volumetric titrations is applicable only when it has been demonstrated that impurities are present at low levels, otherwise other assay methods are to be introduced.

Decision tree for validation of volumetric titrations

Repeatability: Relative standard deviation (RSD) over six replicate measurements (n = 6)

Relative accuracy: $\Delta\overline{V} = \frac{\overline{V} - V_{theory}}{V_{theory}}$



III.3.8. Peptide identification by nuclear magnetic resonance spectrometry (*2.2.64*)

The following factors should be addressed in procedure validation.

- Spectral consistency, to demonstrate that, within reasonable ranges, the spectrum obtained is independent of sample quantity, sample pH, analysis temperature (calibration error or recalibration changes) or mis-setting of spectral acquisition parameters such as pulse width. The effects of small changes in sample preparation procedures, such as deuterium exchange, should be considered. Analysis of a number of different batches of the test product should be included to demonstrate consistent spectra.
- Specificity: the spectrum of the test sample should be compared with those of other similar products handled on the same manufacturing site and shown to be distinctive, with notes of obvious spectral differences. The spectra of potential impurities could be assessed (especially specified impurities). These may be deamidated forms, variants containing a "wrong" amino acid enantiomer, or forms with an incorrect sequence. This approach should be similar to that used when assessing the specificity of chromatographic identity tests.
- Other variability:
    - operator-to-operator variability, expected to be small; it should be confirmed if more

than one operator will undertake the test;
  - spectrometer drift over time, probably negligible.

Minor revalidation will be required after probe servicing or console servicing, software upgrades or purchase of new spectrometer components; this can often be achieved using reference samples supplied with the spectrometer.

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