Guide for the
elaboration of monographs on

RADIO-PHARMACEUTICAL PREPARATIONS

European Pharmacopoeia

EDQM

Edition 2018

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1. Monographs on radiopharmaceuticals

1.1. Introduction

This Guide for the elaboration of monographs on radiopharmaceutical preparations supplements the latest versions of both the Style guide of the European Pharmacopoeia and the Technical Guide for the elaboration of monographs. The general principles described herein do not differ from those applied to monographs on pharmaceutical substances. For this reason, in this guide, attention is given only to those subjects that are particular to radiopharmaceutical preparations. Unless specifically exempted, the requirements of the general monographs on *Substances for pharmaceutical use (2034)* and *Radiopharmaceutical preparations (0125)* apply to the individual monographs on radiopharmaceutical preparations. Where relevant, other general texts, for example, on dosage forms, also apply. To avoid any doubt this may in some cases be explicitly stated.

1.2. Monograph title

For a radiopharmaceutical preparation the title is given according to the INN nomenclature, provided this is available. The radionuclide symbol follows the name of the entity that is or contains the element. In the title the use of round parentheses is not intended to follow IUPAC rules (indicating an isotopically substituted compound) but simply a parenthetical comment to indicate that the preparation contains the isotopically modified entity.

Examples:

TECHNETIUM (^{99m}Tc) EXAMETAZIME INJECTION

FLUDEOXYGLUCOSE (^{18}F) INJECTION

If an INN is not available, the title is unambiguous and well known by the users. The radionuclide involved is stated as well as the position of the radionuclide in the molecule, if there is more than one possibility.

Example:

L-METHIONINE ([11C]methyl) INJECTION, instead of L-METHIONINE (11C) INJECTION

In addition to radiopharmaceutical preparations for direct clinical use there are two types of precursor that are included in the same section of the Pharmacopoeia. These are radionuclide precursors, which by definition are radioactive, and chemical precursors which are not radioactive.

In the case of a radionuclide precursor the name of the substance is completed by FOR RADIOLABELLING.

Example:

FLUORIDE (18F) SOLUTION FOR RADIOLABELLING

In the case of a chemical precursor the wording FOR RADIOPHARMACEUTICAL PREPARATIONS is added to the name of the substance. This allows the publication of precursor monographs in the section for radiopharmaceutical preparations and distinguishes between qualities that are suitable for radiopharmaceutical preparations and those that are not.

Example:

IOBENGUANE SULFATE FOR RADIOPHARMACEUTICAL PREPARATIONS

Non-radioactive, chemical precursors are not covered further in this guide as they are more appropriately covered by the more general Style and Technical Guides.

1.3. Definition

1.3.1. Formulae and names

It is not the intention of monographs on radiopharmaceutical preparations to indicate or prescribe whether the active substance, the radioactive chemical, is 'essentially carrier-free', 'no-carrier added' or, in IUPAC terms, 'labelled' or 'substituted'. In reality there will always be some molecules containing the non-radioactive, ground-state, natural nuclide. Where important on grounds of potential toxicity or target receptor saturation effects, the extent to which a preparation contains non-radioactive molecules of the 'active substance' is indicated by its specific radioactivity (Bq/g) or molar radioactivity (Bq/mole) or more simply by the amount of the non-radioactive compound per maximum recommended dose in millilitres (mg per *V*) in the Content section. In the Definition section, the name of the principal chemical compound is given according to IUPAC conventions. For the users of the Pharmacopoeia it is of little or no practical relevance whether a compound is substituted or labelled. The radioisotope symbol therefore appears in square brackets immediately before the radiolabelled entity assuming for this purpose that all radiopharmaceutical compounds are 'labelled' rather than 'substituted'.

Examples:

Sterile solution containing 2-[18F]fluoro-2-deoxy-D-glucopyranose (2-[18F]fluoro-2-deoxy-D-glucose) prepared by nucleophilic substitution

It is prepared by dissolving [[[(3-bromo-2,4,6-trimethylphenyl)carbamoyl] methyl]imino]diacetic acid (mebrofenin) in the presence of …

For well-defined radiolabelled substances, a graphic formula is given. This is a representation of a single radioactive molecule, the active substance in the preparation. In this case the radioactive atom is indicated without either brackets or parentheses.

Examples:

[¹¹¹In]Indium oxine

1-(3-[¹²³I]iodobenzyl)guanidine or [¹²³I]iobenguane
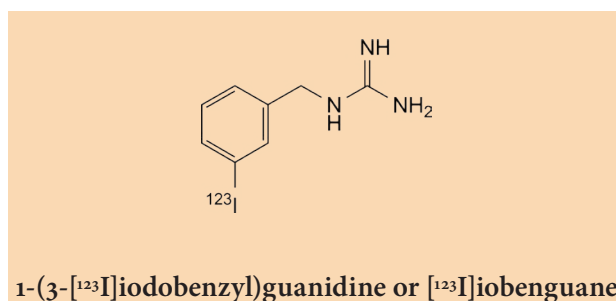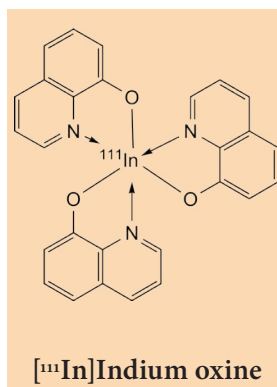
Similarly, the molecular formula and the relative molecular mass are stated for a single molecule.

Examples:

$C_{27}H_{18}{}^{111}InN_3O_3$ $\hspace{4cm}$ M_r 543.5
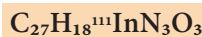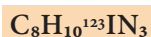
$C_8H_{10}{}^{123}IN_3$ $\hspace{4cm}$ M_r 271.2

The content section includes only statements that are essential to the substance or the preparation.

Example:

fluorine-18: 90 per cent to 110 per cent of the declared fluorine-18 radioactivity at the date and time stated on the label.

If necessary, the maximum content of the non-radioactive molecule in the radiopharmaceutical preparation is stated in order to give a lower limit for the specific radioactivity.

Example:

2-fluoro-2-deoxy-D-glucose: maximum 0.5 mg per maximum recommended dose in millilitres.

For preparations comprising a radionuclide and a complexing ligand, the maximum content of complexing ligand may be stated in cases, for example, where it may be pharmacologically active.

Example:

edotreotide: maximum 50 µg per maximum recommended dose in millilitres.

Content specifications are given only if the monograph allows their verification.

Content specifications are not given for substances which are considered as impurities.

If additives may be used these are stated, generally in a non-explicit manner.

Examples:

> **It may contain stabilisers and inert additives.**

> **The preparation may contain stabilisers such as ascorbic acid and edetic acid.**

> **It may contain a suitable buffer.**

If applicable, the definition states that the monograph applies to the substance obtained by a certain route of production. This information is not normally included in the title of the monograph.

Examples:

> **This monograph applies to an injection containing 6-[^{18}F]fluorolevodopa produced by electrophilic substitution.**

> **Sterile solution containing 2-[^{18}F]fluoro-2-deoxy-D-glucopyranose (2-[^{18}F]fluoro-2-deoxy-D-glucose) prepared by nucleophilic substitution.**

> **Sterile solution of a complex of technetium-99m with sodium hydroxy-methylenediphosphonate. It is prepared using *Sodium pertechnetate (^{99m}Tc) injection (fission) (0124)*, *Sodium pertechnetate (^{99m}Tc) injection (non-fission) (0283)* or *Sodium pertechnetate (^{99m}Tc) injection (accelerator-produced) (2891)*.**

1.4. Production

According to the *General notices (1.)*, the statements in this section constitute mandatory requirements, unless otherwise stated. These requirements are related to source materials, the manufacturing process itself and its validation and control, or to testing that is to be carried out by the manufacturer on the finished product, either on selected batches or on each batch before release. These statements may not necessarily be verified on a sample of the finished product.

Example:

> **Iodine-131 is obtained by neutron irradiation of tellurium or by extraction from uranium fission products.**

There is no need to give details of the production procedure. If various possibilities exist, then the procedures that were evaluated during the elaboration/revision can be given in the Knowledge database. The use of phrases such as 'may be produced by various reactions …' and 'the most frequently used method' is to be avoided. Such statements are included within the Definition or, if not directly relevant to the interpretation and use of the monograph, in the introductory note in *Pharmeuropa*.

1.5. **Characters**

The statements under the heading Characters are not to be interpreted in a strict sense and are not analytical requirements. The appearance of the preparation is given for information.

Examples:

Appearance: clear, colourless or slightly yellow solution.

Appearance: white or almost white suspension which may separate on standing.

Appearance: colourless gas.

Reference is also made to half-life and nature of the radiation of the radionuclide involved in the preparation.

Example:

Half-life and nature of radiation of fluorine-18: see general chapter *5.7. Table of physical characteristics of radionuclides*.

1.6. **Identification**

This section should provide assurance that the correct radionuclide is present and that the substance is present in the correct chemical form.

For radionuclide identification gamma-ray or beta-particle spectrometry is usually sufficient.

Examples:

A. *Gamma-ray spectrometry.*
Result: the energy of the most prominent gamma photon of iodine-123 is 0.159 MeV.

A. *Gamma-ray spectrometry.*
Result: the most prominent gamma photons of indium-111 have energies of 0.171 MeV and 0.245 MeV.

A. *Beta-particle spectrometry.*
Result: the maximum energy of beta particles due to yttrium-90 is 2.28 MeV.

For the identification of positron-emitting radionuclides, gamma-ray spectrometry is only contributory because the result 'the principal gamma photons have an energy of 0.511 MeV' applies to all and additional information about the physical half-life is therefore necessary. For preparations where the shelf-life is short relative to the time required for pre-release testing and an accurate determination of the half-life over a period corresponding to 3 estimated half-lives is not practical for the purposes of radionuclide identification, determination of the 'approximate half-life' is sufficient.

Example:

A. *Gamma-ray spectrometry.*
Result: the principal gamma photons have an energy of 0.511 MeV and, depending on the measurement geometry, a sum peak of 1.022 MeV may be observed.
B. *Approximate half-life*: 105 min to 115 min.

For identification of the chemical form, a specific chemical reaction and/or a separation technique might be used.

Examples:

> **B. Place 5-10 mg of *magnesium oxide R* in a glass container about 20 mm in internal diameter. Add 20 µL of the preparation to be examined. Examine in ultraviolet light at 365 nm. Bright yellow fluorescence is produced.**

> **B. To 100 µL of *silver nitrate solution R2* add 50 µL of the preparation to be examined. A white precipitate is formed.**

> **C. Examine the chromatograms obtained in test A for radiochemical purity (see Tests).**
> ***Result*: the principal peak in the radiochromatogram obtained with the test solution is similar in retention time/retardation factor to the principal peak in the chromatogram obtained with reference solution (a).**

> **C. Examine the chromatograms obtained in test A for radiochemical purity (see Tests).**
> ***Result*: the retardation factor of the principal peak in the radiochromatogram obtained with the test solution is 0.0 to 0.1.**

1.7. Maximum recommended dose in millilitres

Within the Pharmacopoeia a concept unique to radiopharmaceutical preparations is that of '*V*', i.e. the maximum recommended dose in millilitres. To achieve the desired outcome (diagnostic or therapeutic) from the administration of a radiopharmaceutical preparation a dose in becquerels is prescribed. Because of the inherent, and predictable, physical decay of radioactivity the 'potency' of a radiopharmaceutical preparation decreases with time and the required volume of an injection will increase in order to give the desired radioactivity dose. After the passage of one half-life, it will be necessary to administer twice the volume; after two half-lives the administered volume must increase four-fold. For this reason the administration volume of a radiopharmaceutical preparation is not defined but it must be subject to a maximum recommended volume in millilitres ('*V*'). This will be based on the radiopharmaceutical development studies of the quality, stability and safety of different formulations under a variety of conditions and subsequent analytical data from experimental batch production. It is expected that the maximum value of *V* is determined in process validation studies. In extreme cases this would be the entire volume of a multidose preparation, for example a reconstituted kit for radiopharmaceutical preparation or the formulated output of an extemporaneous synthesis process. Many tests on radiopharmaceutical preparations measure the analytes (related substances, bacterial endotoxins etc.) in terms of milligrams or units per millilitre, but the limits are specified in terms of milligrams or units per *V* to restrict their total administered amounts. Where reference solutions are necessary these are usually diluted to *V* mL before use. The methods will have been validated to include the range of volumes and concentrations likely to be encountered.

1.8. Tests

If relevant for the monograph, tests for sterility, bacterial endotoxins and residual solvents must be stated explicitly, as long as these tests are not covered by the general monograph *Radiopharmaceutical preparations (0125)*. The order of the tests follows that in the Style guide.

1.8.1. **pH**

The test is to be performed on the undiluted preparation, unless otherwise stated. The pH value may be determined by the use of potentiometry *(2.2.3)* or by the use of an appropriate reagent indicator solution *(2.2.4)* or strip, or by determination of acidity or alkalinity *(2.2.4)*. If a pH range is given with a precision of one decimal place, it may be useful to provide the name of an indicator strip found to be suitable. This would be included as a footnote to the monograph for information during elaboration and would be available on the EDQM Knowledge database.

Examples:

pH *(2.2.3)*: 4.5 to 8.5.

Acidity *(2.2.4)*: the solution is strongly acid.

Alkalinity *(2.2.4)*: the solution is strongly alkaline.

pH *(2.2.4)*: 4 to 7.

pH *(2.2.4)*: 4.5 to 7.0 [1]

Footnote: [1] Merck pH strips No. 109531 are suitable.

1.8.2. **Non-radioactive substances and related substances**

Example:

Alovudine and related substances. Liquid chromatography *(2.2.29).*

This section consists of tests for specific non-radioactive substances and known or potential non-radioactive impurities. If the definition prescribes limits for the specific radioactivity or for the non-radioactive substance of the preparation, then a test must be given to determine the content of the non-radioactive substance of the preparation. Within the text of a monograph, impurities (chemical and radiochemical) are referred to as 'Impurity A', 'Impurity B', etc. These are defined in the Impurities section at the end of the monograph applying the terminology of the glossary of chapter *5.10. Control of impurities in substances for pharmaceutical use*. In the text, the titles of tests for impurities will refer to 'Impurity A', 'Impurity B', etc. However, the first time an impurity is mentioned (for example in the preparation of reference solutions), the name of the reagent is used, followed by the impurity's identification in parentheses.

Example:

Dissolve 1.0 mg of *2-chloro-2-deoxy-D-glucose R* **(impurity A) in** *water R.*

The limits are set based on routine batch analysis results and taking into account toxicology and / or efficacy data and the capabilities of the prescribed methods.

The following example also serves as a guide to the standard style for the description of such a test.

Example:

2-Fluoro-2-deoxy-D-glucose and impurity A. Liquid chromatography *(2.2.29).*
Test solution. **The preparation to be examined.**

Reference solution (a). Dissolve 1.0 mg of *2-fluoro-2-deoxy-D-glucose R* in *water R* and dilute to 2.0 mL with the same solvent. Dilute 1.0 mL of the solution to V with *water R*, V being the maximum recommended dose in millilitres.

Reference solution (b). Dissolve 1.0 mg of *2-chloro-2-deoxy-D-glucose R* (impurity A) in *water R* and dilute to 2.0 mL with the same solvent. Dilute 1.0 mL of the solution to V with *water R*, V being the maximum recommended dose in millilitres.

Reference solution (c). Dissolve 1.0 mg of *2-fluoro-2-deoxy-D-mannose R* in *water R* and dilute to 2.0 mL with the same solvent. Mix 0.5 mL of this solution with 0.5 mL of reference solution (a).

Column:

– *size*: l = 0.25 m, Ø = 4.0 mm;

– *stationary phase: strongly basic anion exchange resin for chromatography R* (10 μm).

Mobile phase: 4 g/L solution of *sodium hydroxide R* in *carbon dioxide-free water R* protected from atmospheric carbon dioxide.

Flow rate: 1 mL/min.

Detection: suitable detector for carbohydrates in the required concentration range, such as a pulse amperometric detector and a radioactivity detector connected in series.

Injection: 20 μL.

Run time: twice the retention time of 2-fluoro-2-deoxy-D-glucose.

Relative retention with reference to 2-fluoro-2-deoxy-D-glucose (retention time = about 12 min): 2-fluoro-2-deoxy-D-mannose = about 0.9; impurity A = about 1.1.

System suitability: reference solution (c) using the carbohydrate detector:

– *resolution*: minimum 1.5 between the peaks due to 2-fluoro-2-deoxy-D-mannose and 2-fluoro-2-deoxy-D-glucose;

– *signal-to-noise ratio*: minimum 10 for the peak due to 2-fluoro-2-deoxy-D-glucose.

Limits: in the chromatogram obtained with the carbohydrate detector:

– *2-fluoro-2-deoxy-D-glucose*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.5 mg/V);

– *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.5 mg/V).

In some tests a number of related substances may be detected; in these cases a limit on their total and a disregard limit may be specified.

Example:

Limits: in the chromatogram obtained with the spectrophotometer

– *fluoromisonidazole*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.1 mg/V);

– *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 mg/V);

– *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 mg/V);

– *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 mg/V);

– *disregard limit*: 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.03 mg/V).

The column temperature is mentioned only if absolutely necessary or if it is not room temperature (15 °C to 25 °C). Unless otherwise stated, it is assumed that the column temperature is to be constant.

1.8.3. Residual solvents

These are usually limited according to general chapter *5.4. Residual solvents*. For preparations where the shelf-life is short relative to the time required for pre-release testing and distribution, the statement 'The preparation may be released for use before completion of the test' may be added.

Example:

Residual solvents: limited according to the principles defined in general chapter *5.4*. The preparation may be released for use before completion of the test.

Where ethanol is present in a radiopharmaceutical preparation as an excipient limits are given on its concentration or total quantity per administration. The methods described in *2.4.24. Identification and control of residual solvents* may be applicable but because of the higher permissible levels and the range of possible administration volumes it may be necessary to validate alternative methods.

Example:

Ethanol (*2.4.24* or another suitable, validated method): maximum 10 per cent *V/V* and maximum 2.5 g per administration, taking the density (*2.2.5*) to be 0.790 g/mL

1.8.4. Physiological distribution

Tests involving animals should be avoided. Some radiopharmaceutical preparations may comprise a mixture of radiolabelled components of varying composition not readily determined by other analytical methods. Where the physico-chemical test(s) for radiochemical purity is (are) not adequate to completely define and control the radiochemical species in a radiopharmaceutical preparation, a physiological distribution test may be required. General guidance on the performance of the test is given in the general monograph *Radiopharmaceutical preparations (0125)* but the wording of the test and limits will depend on the precise nature of the test, although harmonisation with similar texts is desirable.

1.8.5. Sterility

The general monograph *Radiopharmaceutical preparations (0125)* requires that 'Radiopharmaceutical preparations for parenteral administration comply with the test for sterility'. It is therefore not necessary to specify a test for sterility in individual monographs on radiopharmaceutical preparations except in the following case:

A short shelf-life, compared with the duration of analysis, then it is allowed to release the preparation before completion of the test. A statement would be indicated in the monograph:

Sterility. It complies with the test for sterility prescribed in the monograph *Radiopharmaceutical preparations (0125)*. The preparation may be released for use before completion of the test.

1.8.6. **Bacterial endotoxins**

The general monograph on *Radiopharmaceutical preparations (0125)* requires that 'Radiopharma-ceuticals for parenteral administration comply with the test for bacterial endotoxins (*2.6.14*) or with the test for pyrogens (*2.6.8*)'. Further, 'The limit for bacterial endotoxins is indicated in the individual monograph or calculated according to general chapter *5.1.10. Guidelines for using the test for bacterial endotoxins'*. The latter indicates that for intravenous radiopharmaceuticals the limit should be 2.5 IU of endotoxin per kilogram body mass. That is, for an average 70 kg subject, 175 IU per maximum recommended dose in millilitres. It is therefore not necessary to specify a test for bacterial endotoxins in individual monographs on radiopharmaceutical preparations except in the following cases:

- If the preparation has a short shelf-life, compared with the duration of analysis, then the preparation may be released for use before completion of the test. In such cases the following statement is indicated in the monograph:

Example for a ready-to-use preparation:

> **Bacterial endotoxins (*2.6.14*): less than 175/*V* IU/mL, *V* being the maximum recommended dose in millilitres. The preparation may be released for use before completion of the test.**

Examples for a solution for radiolabelling:

> **Bacterial endotoxins (*2.6.14*): less than 20 IU/mL, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins. The preparation may be released for use before completion of the test.**

> **Bacterial endotoxins (*2.6.14*): less than 175/*V* IU/mL, *V* being the maximum recommended dose in millilitres. The preparation may be released for use in manufacturing before completion of the test.**

> **Bacterial endotoxins (*2.6.14*): less than 175 IU/*V*, *V* being the maximum volume to be used for the preparation of a single patient dose, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins. The solution may be released for use before completion of the test.**

- If the limit differs from the general limit, then this other limit is to be indicated.

Example:

> **Bacterial endotoxins (*2.6.14*): less than 14/*V* IU/mL, *V* being the maximum recommended dose in millilitres.**

- If the method of preparing the sample is not the usual one, then the sample preparation is to be given in detail.
- If the gel clot test will not work, then another test method is indicated.

1.9. **Radionuclidic purity**

This section prescribes a maximum limit for the content of radionuclidic impurities and a minimum content of the radionuclide in question.

Example (for an iodine-123 labelled preparation):

RADIONUCLIDIC PURITY

The preparation may be released for use before completion of the test.

Iodine-123: minimum 99.7 per cent of the total radioactivity.

Gamma-ray spectrometry.

Determine the relative amounts of iodine-123, iodine-125, tellurium-121 and other radionuclidic impurities present. For the detection of tellurium-121 and iodine-125, retain the preparation to be examined for a sufficient time to allow iodine-123 to decay to a level which permits the detection of radionuclidic impurities. No radionuclides with a half-life longer than that of iodine-125 are detected.

Radionuclidic impurities with a half-life longer than that of the radionuclide in the preparation may be determined after a suitable period of decay. In this case, indications are given on how long a sample is to be retained before starting the measurement of the remaining longer-lived impurities and it is stated if the preparation may be released for use before completion of this part of the test.

Example (for a fluorine-18 labelled preparation):

RADIONUCLIDIC PURITY

The preparation may be released for use before completion of test B.

Fluorine-18: minimum 99.9 per cent of the total radioactivity.

A. Gamma-ray spectrometry.

Limit: **peaks in the gamma-ray spectrum corresponding to photons with an energy different from 0.511 MeV or 1.022 MeV represent not more than 0.1 per cent of the total radioactivity.**

B. Gamma-ray spectrometry.

Determine the amount of fluorine-18 and radionuclidic impurities with a half-life longer than 2 h. For the detection and quantification of impurities, retain the preparation to be examined for at least 24 h to allow the fluorine-18 to decay to a level that permits the detection of impurities.

Result: **the total radioactivity due to radionuclidic impurities is not more than 0.1 per cent.**

For radiopharmaceutical preparations labelled with technetium-99m a radionuclidic purity test is not described because these are prepared with *Sodium pertechnetate (⁹⁹ᵐTc) injection (fission) (0124)*, *Sodium pertechnetate (⁹⁹ᵐTc) injection (non-fission) (0238)* or *Sodium pertechnetate (⁹⁹ᵐTc) injection (accelerator-produced) (2891)* for which radionuclidic requirements are already defined.

1.10. Radiochemical purity

This section is one of the most important and contains specific tests for a radiopharmaceutical preparation. It is also the most difficult for which to provide a standard text. The tests in this section ensure that the radionuclide in question is present in the desired chemical form. The name(s) of impurity(ies) tested for is(are) used as the title of the test whenever possible. Limits are expressed as a minimum percentage of the total radioactivity of the radionuclide concerned in the desired chemical form. In some circumstances limits may also be prescribed for the maximum percentage of individual or combined radiochemical impurities.

Example (for a fluorine-18 labelled preparation):

RADIOCHEMICAL PURITY

[¹⁸F]Alovudine. Liquid chromatography (2.2.29) as described in the test for alovudine and related substances. If necessary, dilute the test solution with *water R* to obtain a radioactivity

concentration suitable for the radioactivity detector.

Examine the chromatogram recorded using the radioactivity detector and locate the peak due to [^{18}F]alovudine by comparison with the chromatogram obtained with reference solution (a) using the spectrophotometer.

Limit:

– *[^{18}F]alovudine*: minimum 95 per cent of the total radioactivity due to fluorine-18.

Impurity D. Thin-layer chromatography (2.2.27).

Test solution. The preparation to be examined.

Plate: *TLC silica gel plate R*.

Mobile phase: *water R*, *acetonitrile R* (5:95 V/V).

Application: about 5 µL.

Development: over ⅔ of the plate.

Drying: in a current of warm air.

Detection: suitable detector to determine the distribution of radioactivity.

Retardation factors: impurity D = about 0; [^{18}F]alovudine = about 0.7.

Limit:

– *impurity D*: maximum 5 per cent of the total radioactivity due to fluorine-18.

Example (for a technetium-99m labelled preparation using only paper or thin-layer chromatography):

RADIOCHEMICAL PURITY

Impurity A. Thin-layer chromatography (2.2.27).

Test solution. The preparation to be examined.

Reference solution (a). To 1 mL of a 1 g/L solution of *stannous chloride R* in a 5.15 g/L solution of *hydrochloric acid R* in a closed vial, add 2 mL of *Sodium pertechnetate (^{99m}Tc) injection (fission) (0124)*, *Sodium pertechnetate (^{99m}Tc) injection (non-fission) (0283)* or *Sodium pertechnetate (^{99m}Tc) injection (accelerator-produced) (2891)* containing 100-400 MBq. Use within 30 min.

Reference solution (b). To a vial of *medronate for radiochemical purity testing CRS* add 2 mL of *Sodium pertechnetate (^{99m}Tc) injection (fission) (0124)*, *Sodium pertechnetate (^{99m}Tc) injection (non-fission) (0283)* or *Sodium pertechnetate (^{99m}Tc) injection (accelerator-produced) (2891)* containing 100-400 MBq. Allow to stand for 15 min.

Plate: *TLC silica gel plate R*; use a glass-fibre plate.

Mobile phase: 136 g/L solution of *sodium acetate R*.

Application: about 2 µL.

Development: immediately, over ⅘ of the plate.

Drying: in air.

Detection: suitable detector to determine the distribution of radioactivity.

Retardation factors: impurity A = 0.0-0.1; impurity B and [^{99m}Tc]technetium medronate = 0.9-1.0.

Impurity B. Thin-layer chromatography (2.2.27).

Test solution. The preparation to be examined.

Reference solution (a). *Sodium pertechnetate (^{99m}Tc) injection (fission) (0124)*, *Sodium pertechnetate (^{99m}Tc) injection (non-fission) (0283)* or *Sodium pertechnetate (^{99m}Tc) injection (accelerator-produced) (2891)*.

Reference solution (b). Reference solution (b) of the test for impurity A.

Plate: *TLC silica gel plate R*; use a glass-fibre plate.

Mobile phase: *methyl ethyl ketone R*.

Application: about 2 µL.

Development: immediately, over ⅘ of the plate.

Drying: in air.

Detection: suitable detector to determine the distribution of radioactivity.

Retardation factors: impurity A and [⁹⁹ᵐTc]technetium medronate = 0.0-0.1 and impurity B = 0.9-1.0.

Limit:

– [⁹⁹ᵐTc]*technetium medronate*: minimum 95 per cent of the total radioactivity due to technetium-99m.

Calculate the percentage of radioactivity due to [⁹⁹ᵐTc]technetium medronate using the following expression:

$$100 - (A + B)$$

A = percentage of radioactivity due to impurity A determined in the test for impurity A under Radiochemical purity;

B = percentage of radioactivity due to impurity B determined in the test for impurity B under Radiochemical purity.

For determination of radiochemical purity using liquid chromatography the potential for retention of radioactivity on the column must be considered. This is reflected in a formula for the calculation of the limits.

Example:

Calculate the percentage of radioactivity due to [⁹⁹ᵐTc]technetium sestamibi using the following expression:

$$\frac{(100 - B) \times T}{100}$$

B = percentage of radioactivity due to impurity B determined in the test for impurity B under Radiochemical purity;

T = percentage of the radioactivity due to [⁹⁹ᵐTc]technetium sestamibi in the chromatogram obtained with the test solution.

1.11. Radioactivity

This section corresponds to the assay section in monographs of chemical substances.

Example:

RADIOACTIVITY
Determine the radioactivity using a calibrated instrument.

1.12. Storage

Information regarding storage is included in the general monograph on *Radiopharmaceutical Preparations (0125)*. If additional information is necessary for the interpretation of the requirements, this is specified in the individual monograph.

Examples:

STORAGE
In an airtight container, protected from light, at a temperature of 2 °C to 8 °C.

STORAGE
Protected from light, at 25 °C or below.

1.13. Labelling

Information regarding labelling is included in the general monograph *Radiopharmaceutical Preparations (0125)*. If additional information is necessary for the interpretation of the requirements, this is specified in the individual monograph.

Examples:

LABELLING
The label states:
– that the solution is not for direct administration to humans;
– that it is the user's obligation to verify that the content of metal impurities and strontium-90 is sufficiently low for the intended application
– where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

LABELLING
The label states the percentage content of ethanol in the preparation.

LABELLING
The label states the specific radioactivity expressed in GBq of iodine-123 per gram of iobenguane base.

1.14. Impurities

See general chapter *5.10. Control of impurities in substances for pharmaceutical use*.

Where there are potential chemical, radiochemical or radionuclidic impurities limited by the prescribed tests these are listed with a graphical formula where possible.

Examples:

Specified impurities: A, B, C, D, E.
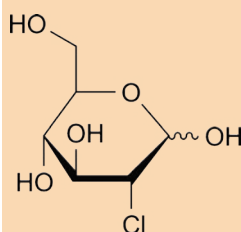
A. 2-chloro-2-deoxy-D-glucopyranose (2-chloro-2-deoxy-D-glucose),
E. [^{18}F]fluoride.

A. [^{99m}Tc]technetium in colloidal form,
B. [^{99m}Tc]pertechnetate ion

A. iodine-125,

B. tellurium-121,

C. [^{123}I]iodate ion.

Non-radioactive inorganic impurities (e.g. metals) are not listed in the impurities section.

2. Analytical validation

This section describes the aspects to be considered in the validation of tests foreseen for inclusion in monographs of radiopharmaceutical preparations in the Ph. Eur. It includes the assessment of tests for identification, instrumental and non-instrumental tests for the control of radiochemical and radionuclidic impurities, and the methods for the determination of radioactivity. The validation requirements vary according to the type of test and the technique employed.

This section discusses the analytical procedures with a special emphasis on the determination and measurement of radioactivity. Tests not involving the determination or measurement of radioactivity, but for which the radioactivity potentially has an influence on the result, are also discussed in this section.

2.1. Definitions and terminology

The text is based upon the existing ICH Q2 (R1) text, which is given also in the Section Analytical Validation of the more overarching Technical Guide for the Elaboration of Monographs.

2.1.1. Introduction

This document presents an overview of the characteristics for consideration during the validation of analytical procedures required for monograph elaboration and may also provide valuable guidance for registration applications. The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose.

A tabular summary of the characteristics applicable to identification, control of impurities and assay procedures with a special emphasis on determination and detection of radioactivity is included.

2.1.2. Types of analytical procedures to be validated

The discussion of the validation of analytical procedures is directed to the following types of analytical procedures:
- identification tests;
- quantitative tests for impurity content;
- limit tests for the control of impurities;
- quantitative tests for determination of radioactivity (the equivalent of the Assay in a monograph of a chemical substance).

A brief description of the types of tests considered in this document is provided below:
- Identification tests are intended to show that the radionuclide is the correct one and that it is present in the stated chemical form.
- Tests for radionuclidic impurities are intended to give information on the identity and content of potential radionuclidic impurities and thus on the overall radionuclidic purity of the preparation.

- Tests for radiochemical impurities are intended to give information on the identity and content of potential radiochemical impurities related to the synthesis or preparation of the active moiety of the finished product.
- Testing for non-radioactive 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; this is all described in the parent document ICH Q2 (R1) Guideline.
- Determination of radioactivity (assay, content or potency) is intended to measure the radio-activity (number of disintegrations per unit of time) of the concerned radionuclide in the intended chemical form (the active substance). Radioactivity is expressed in terms of *total radioactivity* (e.g per dosage unit – like a capsule – or per container – like a vial) or as *radioactive concentration* (per volume unit of the preparation). It is expressed at a given date and time.

2.1.3. **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;
- Quantification limit;
- Linearity;
- Range.

Each of these validation characteristics is defined in the following Glossary (section 2.1.4). 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 and justified. 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 process, e.g. changes in the radionuclide production, synthesis of the precursor, synthesis of the radioactive compound, etc.;
- changes in the composition of the finished product;
- changes in the analytical procedure.

The degree of revalidation required depends on the nature of the changes. Other changes may require validation as well. The decision which degree of revalidation is needed, is based upon the outcome of a risk assessment.

Methods used in stability tests to establish the shelf life of products should be included in valida-tion plans.

2.1.3.1. *Modified 'ICH table', adapted to the specific class of radiopharmaceuticals*

Characteristic	Radionuclide identity (approx. T½)	Radionuclide identity (spectrometry)	Radiochemical identity (LC/TLC/PC)	Radionuclidic purity (limit test)	Radionuclidic purity (spectrometry)	Radiochemical purity* (LC/TLC/PC)	Radioactivity (assay)
Accuracy	–	–	–	–	+	+	+
Precision							
Repeatability	+	–	–	–	(+)	(+)	+
Intermediate Precision	–	–	–	–	(+)	(+)	–
Specificity	+	+	+	+	+	+	+
Detection Limit	–	–	–	+	–	–	–
Quantification Limit	–	–	–	–	+	+	–
Linearity	+	–	–	–	+	+	+
Range	+	–	–	–	+	+	+

* Radioenantiomeric purity measurements should be validated analogously.

(+): not always possible (e.g. short half-life, see text).

LC = liquid chromatography; TLC = thin-layer chromatography; PC = paper chromatography

2.1.4. Glossary

Analytical procedure. The analytical procedure refers to the way of performing the analysis. The steps necessary to perform each analytical test should be described in detail. 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, elemental impurities, residual solvents content, etc.
- *Radioactivity* (the equivalent of the Assay in a monograph of a chemical substance): for non-spectrometric methods of measurement of radioactivity, e.g. using ionization chambers, solid-state detectors (scintillation or semiconductors) and liquid scintillation, the detectors are in general unable to fully discriminate all radiations coming from different radionuclides. Then, the reliability of these radioactivity measurement methods requires the assurance of the absence of interfering radionuclides (radionuclidic purity) or knowledge of their relative contribution to the measurement results.

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

Quantification limit. The quantification 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 quantification 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.

2.2. Methodology

As indicated before, general methodology of validation is described in ICH Q2 (R1) Validation of Analytical procedures, part II Methodology and is not necessarily repeated in this document. The validation of the methods used for the analysis of radiopharmaceutical preparations is done in accordance with ICH Q2(R1) principles as well, but the standard ICH approach might not be applicable, or may need adaptation due to the fact that a radionuclide decays with time, that the determination techniques are not the same as those used in traditional chemical analysis and that radioactive preparations, due to their radiation, cannot easily be shipped from one place to another. This document focuses on such situations.

2.2.1. **Introduction**

The purpose of the section 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 capabilities of a number of analytical procedures in combination may be investigated in order to ensure the overall quality of the radiopharmaceutical preparation.

All relevant data collected during validation and formulae used for calculating validation characteristics should be available.

The physical property of radioactive decay leads to the fact that the required administration volume of a radiopharmaceutical will change throughout its period of validity (shelf-life). For extemporaneously prepared radiopharmaceuticals and reconstituted 'cold kits', the volume to be administered may range from a fraction of a production run or reconstitution soon after completion (for example, a few millilitres) to its entire volume at the end of its shelf-life (for example, 15 mL or 20 mL). This range of volumes should always be considered when designing protocols for the validation of analytical procedures.

Approaches other than those set forth in this guide may be applicable and acceptable. It is up to the experts in charge of the monograph elaboration to choose the validation procedure and protocol most suitable for the preparation concerned. 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.

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.

2.2.2. **Specificity**

An investigation of specificity should be conducted during the validation of identification tests, the determination of impurities and the determination of radioactivity (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 to a particular analyte (complete discrimination). In this case, a combination of 2 or more analytical procedures is recommended to achieve the necessary level of discrimination.

2.2.2.1. *Identification*

Both the radionuclide and the chemical structure of the molecule or complex must be identified. In certain cases, e.g. potential mix up, the counter ion must be identified as well.

Radionuclides are identified by their physical characteristics, for example gamma-ray spectrum. The equipment shall be calibrated with traceable standards with respect to the energy of the radionuclides' emissions.

In cases where other (relevant) radionuclides have similar characteristics an additional test must be added to discriminate between radionuclides, for example a test for approximate half-life. For approximate half-life, the necessary time span for the measurement should be established.

In some cases it will be relevant to identify the main radionuclide after sufficient time for short-lived radionuclidic impurities to decay. This time has to be determined.

Validation of the identity confirmation of the chemical form in which the radionuclide is present mostly consists of 2 steps: demonstration that the radiochemical form in terms of chemical behaviour (e.g. separation behaviour in liquid chromatography or thin-layer chromatography) is similar to its non-radioactive homologue and demonstration that the non-radioactive homologue can be distinguished from closely related substances. So for this part the validation of a test for the chemical structures follows the general procedures for non-radioactive substances.

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 scientific judgement with a consideration of the interferences which could occur.

2.2.2.2. *Assays and impurity tests*

Radioactivity: If radionuclidic impurities have an impact on the determination of the content of the main radionuclide, the impact must be taken into account.

Radionuclidic purity: In preparations with long-lived impurities, it can be necessary to let the main radionuclide decay in order to be able to measure the radionuclidic impurities. Use of a preliminary test for the long-lived radionuclide should be investigated.

A chemical (or electrochemical) separation can be used to separate the main radionuclide from the radionuclidic impurity to be able to determine the content of the impurity.

Radiochemical purity: the retention time (in LC) or retardation factor (in TLC/PC) for the expected impurities should be determined.

A sample spiked with impurities shows the ability of the method to separate the impurities from the main compound. Where radiochemical impurities are not available as isolated compounds they can sometimes be generated (e.g. colloids, different complexes of the radionuclide in other oxidation state …) by stressing the preparation (by exposing it to heat, air, pH changes …). In such cases, the results of such stressed samples can contribute to the demonstration of specificity.

2.2.3. **Linearity**

Radioactivity. Due to the radioactive decay, it is important to evaluate the linearity of the radioactivity detector as the radioactivity is changing over time, for example when determining the total radioactivity or performing an identification test by approximate half-life determination.

Linearity of a detector's response is described in the general method *2.2.66 Detection and measurement of radioactivity.*

Radiochemical purity. For LC, TLC and PC linearity should be demonstrated under the chromatographic conditions for the actual method, e.g. flow rate, flow cell and detector settings.

The linearity of a radioactivity detector should be defined across the range of each analytical procedure. It is important to evaluate the linearity range of the main radioactive compound as well as the linearity range of the impurities obtained from the synthesis pathway. In certain justified cases, linearity may be evaluated for the main compound only, e.g. when the impurities are not available. Then the linearity exercise should cover both the range of the main compound and that of the impurities. In cases where it is shown that the analytical procedure has no impact on the radioactivity measurement results, demonstration of linearity of the detector only may be considered sufficient.

Different radioactive concentrations can be obtained by dilution of a radioactive sample or by using the natural radioactivity decrease between 2 measurements. The evaluation of 5 different concentrations around the concentration of the main compound and 5 different concentrations around the concentration set as the limit of the impurity is considered suitable. To define the linearity range of the impurities it may be necessary to spike the original sample with the radioactive impurity.

The radioactivity amount subjected to the measurement is calculated from the radioactive concentration at the time of calibration (decay-corrected, considering if relevant, the applied volumes and the dilutions). The areas of the signal peaks due to the radioactivity are plotted against the calculated radioactivity amount.

The correlation coefficient obtained from linear regression analysis of the graph should be ≥ 0.99 in cases of direct determination of the radioactivity of the main compound. When determining the radioactivity after a chemical operation, e.g. a chromatographic separation, or when determining an impurity, a less strict correlation coefficient can be acceptable. In justified cases, demonstration of the linearity of the complete analytical procedure can be skipped and demonstration of detector linearity is sufficient.

2.2.4. **Range**

Radionuclidic identity. For determination of approximate half-life the measurements shall be within the linear range of the detector.

Radioactivity. For determination of total radioactivity and radioactive concentration the measurements shall be within the linear range of the detector.

Radionuclidic purity. The counting time shall be established, in order to be able to control the presence of potential radionuclidic impurities above the stated specification limit. In case the range to be covered is not in line with the linear range of the detector, giving 2 ranges is an option, e.g. one range with an extended counting time for the determination of radionuclidic impurities and another range for the determination of the overall radioactivity, with a shorter counting time or on a more diluted preparation.

Radiochemical purity. The range should be established in relation to the intended use and in relation to the specification limit. It should be ensured that it is possible to determine the radiochemical impurities at a given amount within the linear range and sufficient counting time.

The range should cover at least the radioactivity between the quantification limit of the impurities and up to 120 per cent of the maximum injected/applied amount of radioactivity.

2.2.5. **Accuracy**

2.2.5.1. *Radioactivity*

Accuracy should be established by comparison with calibrated (traceable) standards or by use of a calibrated instrument.

2.2.5.2. *Radionuclidic impurities*

Accuracy should be assessed on samples spiked with known amounts of potential radionuclidic impurities. In case the radionuclidic impurities are not available, a calibrated instrument can be used. The necessary counting time shall be stated in order to have a good statistic on the counting (and the results).

2.2.5.3. *Radiochemical impurities*

The accuracy should take into account the overall procedure including for example sample preparation, separation procedure, recovery and radioactivity measurement.

2.2.5.4. *Recommended data*

Accuracy should be assessed using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range (e.g. 3 concentrations with 3 replicates each). In case the half-life is very short compared to the time of analysis, another set up may be chosen, e.g. a series of replicate measurements in which the results are corrected for the decay.

Accuracy should be reported as per cent recovery of the known added amount of radioactivity in the sample or as the difference between the mean and the accepted true value together with the confidence intervals.

2.2.6. **Precision**

Radioactivity and radionuclidic impurities: the necessary counting time and detector settings shall be established in order to obtain a sufficient precision.

2.2.6.1. *Repeatability*

Radiochemical purity: repeated tests (e.g. 6 applications/injections) under the same conditions are performed.

In those cases where the preparation is too unstable for repeated injections, the repeatability, intermediate precision and robustness can also be established by assessing these characteristics in the validation of the chemical purity test of the preparation. In doing this, the sample handling and chromatography are validated. It can be accepted that the repeatability, intermediate precision and robustness for the radio-detection have been established. If no chemical purity test is used, these characteristics can be established by different labellings.

2.2.6.2. *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 effects of random events on the precision of the analytical procedure should be evaluated. Typical variations to be studied include days, analysts, equipment, etc. It is not necessary to study these effects individually. The use of experimental design (matrix) is encouraged.

2.2.6.3. *Reproducibility*

Reproducibility is assessed by means of an inter-laboratory trial. Reproducibility should be considered when standardising the analytical procedure. Analysis of 2 subsamples in 2 different laboratories of the same site (e.g. quality control laboratory and method development laboratory) is an option, e.g. in cases where it is not possible to send a sample out to a more distant laboratory.

2.2.6.4. *Recommended data*

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

2.2.7. **Detection limit**

2.2.7.1. *Based on qualified software*

If qualified software is used for the detection and quantification of radioisotopes, this software can also be used to determine the detection limit (DL) (also referred to as 'limit of detection, LOD').

In cases where qualified software is not available, several approaches for determining the detection limit are possible, depending on whether the procedure is non-instrumental or instrumental. Approaches other than those listed below may be applicable.

2.2.7.2. *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.

2.2.7.3. *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.[1]

$$DL = X_b + 3S_b,$$

Where:

X_b is the signal of the blank, and

S_b is the standard deviation of the signal of the blank.

2.2.7.4. *Based on signal-to-noise*

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 3:1 and 2:1 is generally acceptable.

2.2.7.5. *Recommended data*

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

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1   This is a simplification of the true statistical analysis as given, e.g in James N. Miller & Jane C. Miller. *Statistics and Chemometrics for Analytical Chemistry*, Chapter/Section 5.7 *Limits of detection*; Pearson Education Limited, Sixth Edition, 2010.

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.

2.2.8. Quantification limit

2.2.8.1. *Based on qualified software*

If qualified software is used for the detection and quantification of radioisotopes, this software can also be used to determine the quantification limit (also referred to as 'limit of quantification, LOQ').

In cases where qualified software is not available, several approaches for determining the quantification limit are possible, depending on whether the procedure is non-instrumental or instrumental. Approaches other than those listed below may be applicable.

2.2.8.2. *Based on visual evaluation*

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

The quantification 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 quantified.

2.2.8.3. *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.

$$QL = X_b + 10S_b,$$

Where:

X_b is the signal of the blank, and

S_b is the standard deviation of the signal of the blank.

2.2.8.4. *Based on signal-to-noise*

This approach can be applied only 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 quantified. A typical signal-to-noise ratio is 10:1.

2.2.8.5. *Recommended data*

The quantification limit and the method used for determining the quantification 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 quantification limit.

2.2.9. 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.

Radioactivity and radionuclidic impurities. Factors which potentially influence the result are investigated, e.g. geometry, counting time, matrix effects and shielding.

Radiochemical purity. Factors influencing the results are investigated, e.g. column material, flow rate of the mobile phase, purity of mobile phase, mixture proportion of the mobile phases, plates, activation of plates or not, pH, temperature, applied volume, drying (or not) of applied spots and development length. Stability of the solvents has to be considered.

2.2.10. **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 also general method *2.2.46. Chromatographic separation techniques*.

2.3. **Specific application to methods used in the Ph. Eur.**

The following sections describe a number of points that are important for the validation of methods employing specific analytical techniques. These guidelines are to be used in conjunction with the general methods of the Ph. Eur. and the validation requirements given previously in this guide.

2.3.1. **pH determination**

This test is used to verify that the pH of the solution is compatible with the desired chemical form of the radionuclide and that it falls within physiological limits. Either pH strips (Ph. Eur. method 2.2.3) or a pH meter (Ph. Eur. method 2.2.4) can be used for the determination. It is essential that:
  • The precision and the accuracy of the result are in line with that of the required limit.
  • The chosen method is suitable for the radiopharmaceutical preparation in question and that there are no interferences, e.g. by solvents or high radioactivity in case of determination using electrodes, or by colour of the solutions or colloids, when using pH strips.

2.3.2. **Gamma-ray spectrometry**

Gamma-ray spectrometry is used for determination of radionuclidic identity, radioactivity and radionuclidic purity.

The use of gamma-ray spectrometry requires performance of suitable energy and efficiency calibrations of the equipment and variables affecting spectrometry results include the type and size of the detector used, sample geometry, sample composition and source-to-detector geometry. The calibration of equipment for gamma-ray spectrometry is described in general method *2.2.66. Detection and measurement of radioactivity*. Below are special aspects to be aware of beside the calibration of the equipment. It is assumed that the detector settings and counting conditions are fixed.

Radionuclidic identity. The identification of the radionuclide is usually done by comparing an energy spectrum of the sample of interest with a standard reference spectrum for a standard source of the radionuclide or with the tabulated values (general chapter *5.7 Table of physical characteristics of radionuclides*) for the main radionuclide in the preparation.

• Specificity. It shall be investigated if there is a potential risk for misidentification due to the presence of other radionuclides with the same or similar energy.

Radioactivity. The determination of the radioactivity is usually done by comparing the radioactivity of the sample of interest with the certified radioactivity of a standard, or by using an instrument calibrated with the aid of such a standard.

• Accuracy. It should be evaluated by a comparison with traceable reference standards or with samples standardised by measurements in calibrated equipment.

• Precision. It should be evaluated by repeated measurements of a sample containing the radionuclide of interest in the declared activity range, under the specified counting conditions.

• Specificity. It should be investigated if radionuclidic impurities in the preparation can have an impact on the result in order to decide if it is necessary to correct for the impurities.

• Linearity. It should be demonstrated that the response of the detector is linear within the range.

• Range. The range should cover at least the radioactivity between the limit of quantification and up to 120 per cent of maximum detected radioactivity. The range is set on the basis of the accuracy, precision and linearity.

• Robustness. The influence of variations in source and detector geometry, matrix self-attenuation, dead time, background and coincidence summing should be investigated and discussed.

Radionuclidic purity:

• Accuracy. In cases where suitable radionuclidic impurity standards are not available, calibration with a multipeak source is possible (see general method *2.2.66. Detection and measurement of radioactivity*), provided the equipment is qualified for the relevant range of radioactivity. The response versus the energies should be plotted.

• Precision. As for radioactivity.

• Specificity. It should be investigated if it is possible to detect all potential impurities under the specified counting conditions. Decide if it is necessary to measure after the main radionuclide is decayed to an amount where it will be possible to detect the potential impurities.

• Detection limit (DL)/quantification limit (QL) or minimum detectable activity (MDA). They have to be determined under the specified method conditions.

• Linearity. As for radioactivity.

• Range. The individual range should cover minimum from 50 per cent to 120 per cent of the specified limit for the radionuclidic impurity.

• Robustness. As for Radioactivity.

2.3.3. Total beta-particle counting and beta-particle spectrometry

Total beta-particle counting and beta-particle spectrometry are used for determination of pure beta-particle emitters activity and radionuclidic purity.

Given the continuous shape of the beta-particle spectrum, a radionuclide identification based only on spectrometric considerations is not necessarily accurate. Nevertheless, radioactivity determination can be carried out using suitable radionuclide separation followed by total beta-particle

counting. Some information can also be obtained by examination of the mean and/or maximum energy of the beta-particle spectrum and analysis of beta-particle count rate variation with time.

The use of total beta-particle counting and beta-particle spectrometry requires a proper source preparation, determination of the chemical separation yield and efficiency calibration of the equipment. Variables affecting spectrometry results include: type and size of the detector used, sample geometry and source-to-detector geometry. The calibration of equipment for beta-particle counting and spectrometry is described in general method *2.2.66. Detection and measurement of radioactivity*. Below are special aspects to be aware of besides the calibration of the equipment. It is assumed that the detector settings and counting conditions are fixed.

Radionuclidic identity. The radioactivity determination of the radionuclide of interest should be carried out with the following steps:
  • If applicable, chemical separation of the radionuclide of interest;
  • preparation of suitable sources for beta-particle counting (i.e. solid sources in 2π geometry, liquid scintillation sources);
  • total beta-particle counting or beta-particle spectrometry.

Specificity. The risk for mix up with other radionuclides with the same or similar maximum beta-particle energy has to be taken in consideration. Supporting information is obtained from gamma-ray spectrometry or approximate half-life determination.

Radioactivity. The same considerations apply as for gamma-ray spectrometry.

Radionuclidic purity. The same considerations apply as for gamma-ray spectrometry.

2.3.4. **Alpha particle spectrometry**

The use of alpha-particle spectrometry requires a proper source preparation, determination of the chemical separation yield and efficiency calibration of the equipment. The alpha particles have high energies, but a short range. Although precautions are undertaken to facilitate the alpha particle detection, it may be difficult to detect alpha particle energies corresponding to the literature values.

The calibration of equipment for alpha-counting and spectrometry is described in general method *2.2.66. Detection and measurement of radioactivity*. Below are special aspects to be aware of besides the calibration of the equipment. It is assumed that the detector settings and counting conditions are fixed.

When reference samples are measured to estimate the calibration factor, it must be ensured that the reference sample resembles the unknown sample. The samples must be dry and with a minimum of solid residues to minimise matrix effects. Differences in these parameters may influence the alpha particle attenuation and energy absorption in the matrix, and thus decrease the accuracy of the alpha-particle energy spectrum.

Detection and quantification limit:

In alpha-particle spectrometry, the limits of detection and quantification depend on the detector, the sample position used and the time given to perform the analysis. The alpha detector software system may, on a calibrated detector system, use the detected alpha-particle energies to estimate the most probable radionuclide(s) in the sample and their amount of radioactivity (in becquerels). However, due to matrix effects, the reliability of such software estimates should be reviewed and interpreted. For complex energy spectra with several alpha emitters with multiple alpha-particle energies, the results must be analysed by experienced analysts. The actual quantification limit will

depend on the complexity of the spectrum obtained and needs to be determined in each individual case.

Radionuclidic identity. The radioactivity determination of the radionuclide of interest should be carried out with the following steps:
- if applicable, chemical separation of the radionuclide of interest;
- preparation of suitable sources for alpha-particle counting;
- alpha-particle spectrometry.

Radioactivity. The same considerations apply as for gamma-ray spectrometry.

Radionuclidic purity. The same considerations apply as for gamma-ray spectrometry.

Most alpha-particle emitters have gamma-ray emitting daughters. Due to the complexity of alpha-particle spectrometry it is recommended to use gamma-ray spectrometry and use indirect measurements both for radionuclide identity, radionuclide purity and radioactivity. Methods must be validated accordingly.

2.3.5. **Separation techniques**

The different chromatographic procedures (TLC, PC and LC) may be employed in the identification and quantification of impurities. The methods are to be validated according to the principles already described but there are aspects of the different chromatographic techniques that should be considered during protocol preparation.

Radiopharmaceutical aspects to be considered:

Complete elution: LC versus TLC/PC

For the development/validation of a radiochemical purity test it must be shown that, when using a LC method, all radiochemical compounds elute from the column.

This can be achieved by measuring the activity on the column after the chromatographic procedure has been completed (in case of gamma-ray emitters) or by comparing the amount of radioactivity injected and eluted (by calculation) in cases where the radiation does not penetrate the column wall. With TLC testing, this is not necessary, as the radioactivity remains on the plate and the radioactivity on the complete plate is determined. However, consideration should be given to the potential for the generation of volatile radioactive impurities based on the knowledge of the synthetic pathway.

Separation

In contrast to most classical TLC applications, where the plates are inspected visually, the distribution of the radioactivity on the TLC plate is determined using a radioactivity detector. The resulting graphical presentation is similar to that of an LC chromatogram. In TLC and LC chromatograms, peaks should preferably have a baseline separation. A system suitability test, defining the resolution, should be developed and tested during the validation. If baseline separation cannot be achieved, a system suitability test with a peak-to-valley ratio can be set as a criterion. For TLC tests that are used only for identification of radiopharmaceuticals, system suitability tests are not required in the monograph. However, it should have been proven during the development of the test that the method is indeed capable of distinguishing substances that are related (e.g. starting material and final compound).

Quantification by integration

Although the majority of the radiochemical purity tests are limit tests (as they relate to another peak area) and the peaks need to be integrated to compare the peak area with the peak area of a reference peak, aspects of the validation of quantitative impurity tests apply, such as the detection/quantification limits and linearity.

The amount of radioactivity used to determine the radiochemical purity should be chosen carefully not to exceed the linearity of the detector at high levels of radioactivity and to limit the influence of the background at very low levels of radioactivity (signal-to-noise ratio).

The integration parameters/methods should be described, especially where there is no baseline separation of the peaks to ensure that the correct counts are assigned to the correct peak (for instance a peak on the tail end of another peak). The integration parameters can be validated by having the same chromatogram interpreted/integrated independently by multiple analysts.

Chromatograms from validated analytical methods should be assessed for irregularities before integrating as the actual performance of the test can be influenced by variables in the test.

TLC chromatograms should preferably be scanned and the peak areas determined by integration. The cutting of TLC strips is considered obsolete and is to be avoided.

Using the appropriate detector

During the development of the radiochemical purity test method, a suitable detector should be chosen. For different radionuclides different detector types may be necessary.

For each system the linearity of detectors, as well as the LOD/LOQ will need to be determined (see general method *2.2.66. Detection and measurement of radioactivity*).

Background

It should be ensured that during the actual testing of the radiopharmaceutical preparation the conditions of the environment are similar to the conditions during the validation of the test. For instance, a difference in the background signal can alter the sensitivity of the method.

2.3.5.1. *Thin-layer chromatography*

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 equipment 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. Specificity may not be attainable for a limit test, in which case (an)other test(s) must be described to control the unseparated impurities. Discrimination power is to be demonstrated.
- *Stationary phase*. It is to be demonstrated that the test can be performed successfully using plates of the same type but of different origin. Separations that can be achieved only on one particular type of plate are to be avoided, if possible.
- *Treatment of materials*. The test procedure should clearly describe how material (especially TLC plates) needs to be stored/prepared (e.g. activation of the plates by pre-heating) as this can

influence the performance of the test. The degree of variability permissible in the storage/preparation of the TLC plates should be investigated in the validation of the method.

- *Performance test (system suitability test).* Such a test is generally performed to verify the separation of 2 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 radiochemical impurities.

Additional aspects that require further documentation when TLC is applied to test for radiochemical impurities:

- Treatment of spots: immediate development, drying in a current of warm air, drying in air.
- Detection limit: when applying a quantitative instrumental procedure, one of the described methods for the calculation of the detection limit applies. When a visual method is applied, it is to be demonstrated that the quantity corresponding to the specified limit is detectable.
- Quantification limit, linearity, range and repeatability: data are also required when an instrumental quantitative TLC procedure is applied.

2.3.5.2. *Liquid chromatography*

LC is usually performed to identify the component of interest in the radiopharmaceutical preparation and to determine the content of impurities. Attention is to be paid to a number of aspects peculiar to LC. Some of these aspects are related to the examination of non-radioactive compounds only (e.g. response factors).

Identification

This is usually done by comparing the retention time of the radioactive compound with the retention time of the non-radioactive analogue.

- *Specificity.* 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 of the impurities and of the substance itself being reported. Such information is to be supplied for stationary phases of similar type and different brands.

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. The retention times or relative retentions of the impurities and the substance itself must be reported. Such information is to be supplied for stationary phases of a similar type and different brands.
  - *Discrimination power of the detection system*: the choice of the detector and the detector conditions employed must be justified.
- *Detection and quantification 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 quantification limits are to be determined on this impurity. One of the methods for calculation of both the detection limit and the quantification limit 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.
- *System suitability test*. As described for TLC. The use of the S/N ratio is required only when the detection limit and the specified limit are similar.

Limit tests must be validated as described above for linearity, repeatability and reproducibility.

2.3.6. **Radioactivity**

Radiation emitted by a radionuclide occurs independently of the chemical form in which the radionuclide is present and, in most cases, independently of the presence of other chemical substances in the preparation. For this reason the validation of the radioactivity measurement of a preparation is not specific to the individual preparation being measured but specific to the concerned radionuclide when measured using the proposed radioactivity measuring assembly (sample preparation, instrument, instrument settings, geometry, counting time, etc.). According to this assumption, the validation of the measurement of radioactivity does not need to be done for each individual preparation but for each radionuclide when measured as proposed. The only exception to this principle is the need to validate the proposed measurement assembly for the individual preparation considering the eventual impact of potential radionuclidic impurities that could interfere with the measurement because the potential radionuclidic impurities will depend on the production method of the intended radionuclide. In some cases, the potential interference of other substances present in the preparation could be specific to the preparation and needs also to be considered (e.g. the presence of quenching substances when using liquid scintillation detectors).

Radioactivity measurement is done in practice by using traceable reference standards or by using measurement instruments calibrated using suitable standards for the radionuclide(s) to be measured. As radioactivity changes with time, all measurements should be corrected for decay time.

When using a traceable reference standard in the measurement, its suitability for the intended radionuclide and radioactivity levels should be justified.

When using an instrument calibrated by its manufacturer, the user has the responsibility to know the relevant information on calibration procedures and results so as to decide that the instrument is suitable for the intended measurement(s).

If calibration is conducted in-house by the user, the suitability of the calibration procedure should be justified and the calibration results should be provided.

When using an ionisation chamber, the response time necessary to provide a stable reading over the established range of radioactivity should be taken into account.

Radioactivity measurements using solid state detectors are particularly sensitive to counting geometry. Most solid state counters are calibrated for energy to allow the user to select an energy window suitable for the intended radionuclide. Counting times necessary to obtain reliable readings should be established for the intended radionuclide across the established radioactivity range.

Accuracy. It should be established across the specified range of the assay method. If the instrument is not calibrated for the radionuclide of interest, the accuracy should be established by the use of traceable reference standards.

Precision:

- *Repeatability (intra-assay precision)*. It should be established by the repeated measurement of samples under the same operational conditions over a short period of time in relation to the half-life of the radionuclide.

- *Intermediate precision (within laboratory precision)*. It can be done by the measurement of a sample in different instruments (if available), by different analysts and over a period of time sufficiently long to allow random variations but sufficiently short considering the half-life of the radionuclide.

Specificity. The reliability of the radioactivity measurement requires that the radionuclidic purity tests have ruled out the presence of relevant quantities of any potential radionuclidic impurities, unless the contribution of these radionuclidic impurities is known.

Linearity. Linearity can be demonstrated as suggested in general method *2.2.66. Detection and measurement of radioactivity*.

Range. Range is established based on the results of linearity, accuracy and precision.