Consultation documents

To receive draft monographs by email please contact Mrs Wendy Bonny (bonnyw@who.int), specifying that you wish to be added to the electronic mailing list.

The International Pharmacopoeia

Draft note for guidance on organic impurities in active pharmaceutical ingredients and finished pharmaceutical products

This is a draft proposal for The International Pharmacopoeia (Working document QAS/15.606, May 2015).

The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/.

Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, CH-1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidt@who.int.

[Note from the Secretariat. Considering current practices in use for The International Pharmacopoeia and available guidance on how to establish limits for impurities, the following note for guidance on organic impurities in active pharmaceutical substances and finished pharmaceutical products was drafted. It is intended to replace the text on Related substances in finished pharmaceutical product monographs in the folder Notes for guidance, Supplementary Information section with the following chapter.]

1. Scope

Impurities are critical quality attributes of active pharmaceutical ingredients (APIs) and finished pharmaceutical products (FPPs), which potentially affect their safety and efficacy. Therefore, all applicable monographs in The International Pharmacopoeia (Ph. Int.) shall contain requirements for the control of impurities.

Impurities in APIs and FPPs may include starting materials, by-products, intermediates, degradation products, reagents, ligands, catalysts and organic solvents. They can be classified as either organic or inorganic.

This note for guidance covers requirements for controlling organic process impurities and degradation products in APIs and FPPs, and provides guidance on how to assess compliance with Ph.Int. requirements.

Several statements in this document refer in particular to the future, i.e. they are applicable to monographs included in the Ph.Int. after the publication of this note of...
guidance. Compliance with previous monographs has to be evaluated using the replaced text "Related substances in finished pharmaceutical product monographs" or on a case-by-case basis.

Excluded from this note for guidance are biological/biotechnological products, peptides, oligonucleotides, radiopharmaceuticals, herbal products and crude products of animal and plant origin. These types of substances require specific considerations.

Further excluded are the following substances:

• extraneous contaminants that should not occur in APIs and FPPs and are more appropriately addressed as good manufacturing practices (GMP) issues;
• enantiomeric impurities;
• crystallographic modifications ("polymorphic forms");
• residual solvents resulting from API or FPP manufacture;
• impurities that arise from printing inks, container-closure systems or excipients (not excluded, however, are reaction products between excipients and APIs);
• organic impurities that are leached from container-closure systems.

2. Defining the purity of APIs and FPPs

To control relevant organic impurities specific monographs usually contain a discriminative, stability-indicating test entitled "Related substances". This test may be supplemented by a specific test where a given impurity is not adequately controlled by the related substances test or where there are particular reasons (for example, safety reasons) for requiring specific control.

Monographs on APIs shall include specifications for process-related impurities that result from the manufacturing process and degradation products observed during manufacture and stability studies, while monographs on FPPs shall include tests and limits for degradation products. If appropriate, tests for impurities in dosage forms may also limit impurities arising during the synthesis of APIs. This approach provides, in conjunction with the monograph on the API, the means for an independent control laboratory (e.g., a small regulatory laboratory) without access to manufacturer’s data, to establish whether or not an API of pharmacopoeial quality has been used to manufacture the FPP under examination. 3

Instruction for control of impurities may also be included in the manufacture section of a monograph, for example, where the only analytical method appropriate for the control of a given impurity is to be performed by the manufacturer since the method is too technically complex for general use. The production process (including the purification steps) needs to be validated to give sufficient control so that the product, if tested, would comply with the specified limits using a suitable analytical method.

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1 Since the publication of the Fourth Supplement of the Fourth Edition, the year of publication (together with a two digit number) is added below the title of each text (monograph, general chapter or text for the supplementary information).

2 Once this new note for guidance is adopted by the Expert Committee on Specifications for Pharmaceutical Substances, the replaced text can be found in The International Pharmacopoeia under "Omitted texts".

3 It is recognized that limits for degradation impurities given in FPP monographs may need to be higher than the limits for the same impurities that appear in the monograph for the corresponding API.
Under the section on “impurities” in the monographs for pharmaceutical substances and dosage forms, substances are listed (transparency list) that are known to be limited by the described test method(s). In dosage form monographs reference may also be made to the list in the monograph of the corresponding API. Whenever possible the impurities are identified as degradants and/or synthesis impurities.

Tests for related substances are intended to provide appropriate limitation of known potential or actual impurities rather than to protect against all possible impurities. The tests are not necessarily designed to detect any adventitious contaminants or adulteration. Material or products found to contain an impurity not detectable by means of the prescribed tests is not of pharmaceutical quality if the nature or amount of the impurity found is incompatible with good pharmaceutical practices (GPP) or applicable regulatory standards.

3. Setting acceptance criteria for organic impurities

Limits in the Ph.Int. are usually set based on:

- the evaluation of information, provided by manufacturers, concerning the nature of impurities, the reason for their presence, the concentrations that may be encountered in material prepared under conditions of good pharmaceutical manufacturing practices and the manner in which the API or FPP may change during storage and when subjected to stress conditions (e.g. light, heat, moisture, acid, base or oxygen), together with an indication of the toxicity of any impurity in relation to that of the substance itself;
- justified limits accepted by regulatory authorities or by the WHO Prequalification Team after a full consideration of the toxicity studies and clinical trials carried out before granting a marketing authorization or before inclusion of the product in the WHO list of prequalified medicinal products or the WHO list of prequalified APIs. The limits may be amended in the event that new safety data become available following regulatory evaluation;
- limits published by other pharmacopoeias applying good pharmacopoeial practices (GPhP);4
- principles published in current regulatory guidance documents, such as those published by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH).

Comments received during the public consultation of the draft monographs are evaluated and taken into consideration if relevant.

Acceptance criteria for impurities focus in particular on safety considerations. They should not be solely based on process capabilities. The historical safety record, the route of administration, the type of dosage form, the maximum daily dose, the duration of treatment, the need for and the availability of the medicine can also be taken into consideration when setting limits for impurities.

Highly toxic (e.g. genotoxic) impurities or degradation products are addressed using applicable guidance.

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4 At the time this note for guidance was drafted the draft proposal for good pharmacopoeial practices (GPhP) (QAS/13.526/Rev.5) was sent out for public consultation (see [http://www.who.int/medicines/areas/quality_safety/quality_assurance/GPhP-Rev5-QAS13-526.pdf?ua=1](http://www.who.int/medicines/areas/quality_safety/quality_assurance/GPhP-Rev5-QAS13-526.pdf?ua=1)). The statement made thus refers to the future, i.e. to a time when good pharmacopoeial practices have been implemented and put into practice by pharmacopoeias.
4. Compliance with the requirements

Where a monograph has no related substances test (or equivalent) or where the existing test does not comply with the requirements of the applicable regulatory standards the user of a monograph must nevertheless ensure that there is suitable control of organic impurities.

Where a pharmaceutical substance may contain impurities other than those mentioned in the Impurities section (for example, because it was manufactured using a new method of synthesis) it is necessary to verify that these impurities are detectable by the method(s) described in the monograph; otherwise a new method should be developed and a revision of the monograph should be requested.

Where a peak or a spot cannot be assigned unambiguously to a listed impurity using the means described in the monograph (retention times, relative retentions, Rf values or comparison to reference substances mentioned in the monograph) the user has to apply additional measures in order to identify the impurities conclusively. These means may include, for example, the analysis of reference substances of excipients, potential impurities not referred to in the monograph or the use of additional analytical techniques, e.g. so-called hyphenated analytical techniques, e.g. GC- or LC-mass spectroscopic methods.

Where an impurity other than those listed under the Impurities section is found in an API or in a dosage form it is the responsibility of the user of a monograph to check whether it has to be identified/qualified, depending on its content, nature and safety, on the maximum daily dose of the API and relevant identification/qualification thresholds for the impurity, etc., in accordance with the applicable regulatory standards and sound scientific principles to control impurities.

The general acceptance criterion for impurities (“any other impurity”, “other impurity”, “any impurity”) equivalent to a nominal content greater than the applicable identification threshold is valid only for those impurities identified in the transparency list, except those that have their own specific acceptance criterion in the monograph. It is thus the responsibility of the user to determine the validity of the acceptance criteria (i.e. to qualify the limit) for impurities not mentioned in the Impurity section.

See Figure 1 for guidance on how to assess compliance with the acceptance criteria of the tests for related substances in the Ph.Int.
**Glossary**

**degradation** product.
An impurity resulting from a chemical change in the active pharmaceutical ingredient (API) brought about during manufacture and/or storage of the API or the dosage form by the effect of, for example, light, oxygen, temperature, pH, water or by reaction with an excipient and/or the immediate container closure system.

**extraneous contaminant.**
An impurity arising from any source extraneous to the manufacturing process.

**identification threshold.**
A limit above (>) which an impurity should be identified, based on the applicable regulatory standards.

**identified impurity.**
An impurity for which a structural characterization has been achieved.
impurity (pharmaceutical substance). Any component of a pharmaceutical substance that is not the chemical entity defined as the pharmaceutical substance.

impurity (dosage form). Any component of the dosage form that is not the pharmaceutical substance or an excipient in the dosage form.

intermediate. A material produced during steps of the synthesis of an active pharmaceutical ingredient that undergoes further chemical transformation before it becomes an active pharmaceutical ingredient.

ligand. An agent with a strong affinity to a metal ion.

crystalline forms. Different crystalline forms of the active pharmaceutical ingredient. These can include solvation or hydration products (also known as pseudo-polymorphs) and amorphous forms.

qualification threshold. A limit above (>) which an impurity should be qualified.

specified impurity. An impurity that is individually listed and limited with a specific acceptance criterion in the monograph. A specific impurity can be either identified or unidentified.

starting material. A material used in the synthesis of an active pharmaceutical ingredient (API) that is incorporated as an element into the structure of an intermediate and/or of the API. Starting materials are normally commercially available and of defined chemical and physical properties and structure.

unidentified impurity. An impurity for which a structural characterization has not been achieved and that is defined solely by qualitative analytical properties (e.g. chromatographic retention time).

unspecified impurity. An impurity that is limited by a general acceptance criterion, but not individually listed with its own specific acceptance criterion (e.g. relative retention time).
Draft revision of the chapter on reference substances and reference spectra

This is a draft proposal for The International Pharmacopoeia (Working document QAS/15.607, May 2015).

The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/.
Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, CH-1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidth@who.int.

[Note from the Secretariat. Following up on a recommendation of the forty-ninth meeting of the Expert Committee on Specifications for Pharmaceutical Preparations to use in The International Pharmacopoeia, where appropriate, ultraviolet (UV) absorptivity values for assays and other quantification purposes with a view to limit reference to International Chemical Reference Substances (ICRS), it is proposed to revise the chapter on reference substances and reference spectra. Additional changes are proposed to reflect recent discussions within the ICRS Board.

[Note from the editor. In accordance with WHO editorial policy the text reproduced below does not include tracked changes. Changes from the current monograph are indicated by insert and delete in the working document available at the above-mentioned web address.]

1. International Chemical Reference Substances

1.1 Introduction

International Chemical Reference Substances (ICRS) are primary chemical reference substances for use in physical and chemical tests and assays described in The International Pharmacopoeia or in other World Health Organization (WHO) quality assurance documents adopted by the WHO Expert Committee on Specifications for Pharmaceutical Preparations. ICRS are used to identify, determine the purity or assay of pharmaceutical substances and preparations or to verify the performance of test methods.

This chapter describes principles to be applied during the establishment and use of ICRS, which guarantee that the reference substances are suitable for their intended purpose.

This chapter is not applicable to WHO International Biological Reference Preparations.

1.2 Terminology

Chemical reference substance

The term chemical reference substance, as used in this text, refers to an authenticated, uniform material that is intended for use in specified chemical and physical tests, in which its properties are compared with those of the product under examination and which possesses a degree of purity adequate for its intended use.

Primary chemical reference substance

A designated primary chemical reference substance is one that is widely acknowledged to have the appropriate qualities within a specified context and whose assigned content
when used as an assay standard is accepted without requiring comparison with another chemical substance.

**Secondary chemical reference substance**
A secondary chemical reference substance is a substance whose characteristics are assigned and/or calibrated by comparison with a primary chemical reference substance.

### 1.3 Purpose of ICRS

The purpose of establishing ICRS is to provide users of *The International Pharmacopoeia* or other WHO quality assurance documents adopted by the WHO Expert Committee on Specifications for Pharmaceutical Preparations with authenticated substances for reference. Many analytical tests and assays are based on comparison of physical or chemical attributes of a sample with those of the reference substance. ICRS serve as such reference substances and thus enable the analyst to achieve accurate and traceable results. Furthermore ICRS may be used to assess system suitability during analyses and to calibrate analytical instruments.

ICRS may also be employed to establish secondary reference substances for routine analysis according to the WHO *General guidelines for the establishment, maintenance and distribution of chemical reference substances*.¹ In cases of doubtful results or dispute, however, the tests performed using ICRS are the only authoritative ones.

### 1.4 Production of ICRS

All operations related to the establishment and distribution of ICRS should be carried out according to the relevant guidelines. Among these, the WHO *General guidelines for the establishment, maintenance and distribution of chemical reference substances*¹ and International Organization for Standardization (ISO) Guide 34 – *General requirements for the competence of reference material producers* (including related guides) take precedence.

**Manufacture**

WHO encourages pharmaceutical manufacturers to donate suitable candidate materials and thus to contribute to the availability of ICRS.

Candidate material for the establishment of ICRS may be synthesized and purified for this purpose or may be selected from the pharmaceutical production provided that the purity and homogeneity are suitable. In some cases, for example, in order to improve the stability of the reference substance it may be useful to process the reference substance (e.g. by freeze drying) or to select an alternative salt (or salt vs base), solvate or hydrate. The content assigned to the standard takes into account which substance is selected.

Compliance with the relevant tests of the corresponding monograph as published in *The International Pharmacopoeia* is required where applicable.

Reference substances are dispensed into suitable containers under appropriate filling and closure conditions, to ensure the integrity of the reference material. The containers employed are preferably single-use in order to minimize the risk of decomposition, contamination and moisture uptake. Where multiple-use containers are employed

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appropriate use and handling controls should be implemented by the user to assure their suitability.

**Analytical characterization**

The candidate material should be tested with suitable analytical techniques aiming to characterize all relevant quality attributes. The identity is confirmed and the purity is determined, usually based on results obtained with the validated methods of the respective monographs. However, the use of further analytical techniques may be appropriate in order to fully characterize the candidate material. Absolute methods (for example, volumetric titrations, differential scanning calorimetry) should be employed to complement and verify the results of relative methods where the properties of a sample are compared with those of a reference substance (for example, chromatographic methods). The extent of testing and the number of laboratories involved in characterizing the material depends on the intended use of the reference substance to be established. If required, assay standards are characterized in interlaboratory trials to increase the accuracy of the assigned value.

A thorough purity investigation of the candidate material is performed to verify the identity of all relevant components (i.e. main component, organic and inorganic impurities, water and residual solvents) and to quantify them. The cumulative percentage of all components should yield 100% (mass balance approach).

The purity of a candidate material is calculated on the “as is” basis, so that the analyst can use the substance without pretreatment, for example, drying.

Provided that all components themselves are expressed as a percentage of the weight of sample taken the “as is” content can be calculated as follows:

\[
\text{Purity} = 100 \text{ – organic impurities [%] – inorganic impurities [%] – water [%] – residual solvents [%]}
\]

**Formula 1.** Formula to calculate the purity of ICRS on an “as is” basis.

When chromatographic methods are used to test for related substances impurity concentrations are often determined in relation to the principal compound. The “as is” content of organic impurities, to be substituted in formula 1, can be calculated as follows:

\[
\text{Organic impurities} = \frac{\text{chromatographic result} \times (100 \text{ [%]} \text{ – water [%]} \text{ – residual solvents [%]} \text{ – inorganic impurities [%])}}{100}
\]

**Formula 2.** Formula to calculate the percentage of organic impurities, determined by a chromatographic method, on an “as is” basis.

The content assigned to a quantitative ICRS depends on the purity of the candidate material and is specific to the method for which the substance will serve as a reference. If the reference substance is intended to be used with a method that has the same selectivity as the method used to determine its purity the calculated purity will be assigned as the content of the ICRS. However, if the intended method is less discriminative it may be necessary to add to the purity the content of impurities that cannot be discriminated from the response of the parent compound. The following example illustrates this:
A candidate material is analysed with different analytical methods to identify and quantify all relevant components. The results reveal that, besides the labelled substance, the following components are present: 2.0% water (analysed by Karl Fischer titration, calculated on an “as is” basis); 1.0% enantiomer of the labelled substance (analysed by chiral high-performance liquid chromatography (HPLC), calculated in relation to the sum of the peak areas of both enantiomers); and two organic impurities, each 0.75% (analysed by an achiral HPLC method, calculated in relation to the sum of the peak area of all peaks, ignoring solvent and injection peaks). The purity of the standard is calculated to 95.55% (purity = 100% – (2.5% x 0.98) – 2%). The candidate material is intended to be used as a reference in an assay test, which stipulates the use of the same HPLC method as already applied to determine the organic impurities in the characterization of the candidate material. A content of 96.53% is assigned to the reference substance (assigned content = 100% – (1.5% x 0.98) – 2%). The concentration of the enantiomer is not taken into consideration as the method, for which the reference substance is intended, is not selective for the enantiomer.

Labelling

The labelling should provide all the necessary information to use the reference substance as intended, i.e. the name of the reference substance, the batch number, storage conditions, etc. If intended for quantification the assigned content or potency (for microbiological assays) is also given. The accompanying leaflet is considered to be part of the labelling.

Release and adoption

ICRS are established and released under the authority of the WHO Expert Committee on Specifications for Pharmaceutical Preparations. The Committee adopts new ICRS and new lots as being suitable for use as described in *The International Pharmacopoeia* or in other WHO quality assurance documents.

Stability monitoring and distribution

At the WHO custodian centre for ICRS the established reference substances are stored and distributed under conditions suitable to ensure their stability.

The fitness-for-purpose of ICRS is monitored by regular re-examinations. Their frequency and extent is based on:

- the stability of the ICRS;
- the container and closure systems;
- the storage conditions;
- the hygroscopicity;
- the physical form;
- the intended use.

The analytical methods employed to verify the stability are chosen among those used during the establishment of the reference standard. The maximum permitted deviation from the assigned value should be predefined and, if exceeded, the batch should be re-established or replaced.
1.5 Use and storage of ICRS by the user

The letters RS after the name of a substance in a test or assay described in *The International Pharmacopoeia* or in other WHO quality assurance documents adopted by the WHO Expert Committee on Specifications for Pharmaceutical Preparations indicate the use of the respective ICRS.

ICRS are suitable for the analytical purpose described in *The International Pharmacopoeia* or other WHO quality assurance documents adopted by the WHO Expert Committee on Specifications for Pharmaceutical Preparations. The analytical specifications and test methods in these documents are being revised to stay abreast of advances in analytical science and regulatory. Along with these changes the intended use of already established ICRS often needs to be adjusted, for example, because an ICRS previously used for identification only shall newly also be employed in quantitative tests. Information on the actually established intended uses of an ICRS can be found in the leaflet enclosed with the substance when distributed or accessible via the ICRS online database (see http://www.edqm.eu). The information found in the current leaflets is applicable to all standards of the respective batch number.

If used for other purposes the responsibility of assessing the suitability rests with the user or the authority that prescribes or authorizes this use. If reference substances other than ICRS are used for purposes described in *The International Pharmacopoeia* or in other WHO quality assurance documents adopted by the WHO Expert Committee on Specifications for Pharmaceutical Preparations the suitability of these substances has to be demonstrated by the user.

The user has to apply an assigned content in assay determinations or when it is indicated in the method description.

ICRS are supplied in adequate quantities for immediate use after opening of the container. Users should purchase only sufficient units for short-term use.

It is generally recommended that the user stores ICRS protected from light and moisture and preferably at a temperature of about 5 ± 3 °C. When special storage conditions are required this is stated on the label or in the accompanying leaflet.

If an unopened container is stored under the recommended conditions it remains suitable for use as long as the respective batch is valid. Information on current batch numbers is provided on the website of the WHO custodian centre for ICRS (see under Ordering information).

Reference standards that are normally stored at 5 ± 3 °C are dispatched at ambient temperature since short-term excursions from the storage recommendations are not considered to be deleterious to the reference substance. Reference substances stored at -20 °C are packed on ice or dry ice and dispatched by courier. Reference substances stored at -80 °C or stored under liquid nitrogen are packed on dry ice and dispatched by courier.

1.6 Rational use of ICRS

Specifications and test procedures of *The International Pharmacopoeia* are intended to be applicable in all WHO Member States wishing to implement them. Procuring reference substances may, however, be difficult in certain areas of the world due to delays in their delivery and the cost of purchase. *The International Pharmacopoeia* therefore endeavours to reduce the number of reference substances required to
perform the included tests and assays. For this purpose the following strategies and practices may be applied during the elaboration of monographs:

- in situ preparation of impurities for identification purposes;
- quantification of impurities by comparing their responses with the response of the parent compound in a diluted sample solution along with the establishment of correction factors to compensate for differences in the responses of the impurity and the parent compound;
- provision of International Infrared Reference Spectra (IIRS) for use in identification tests;
- provision of assay methods not requiring reference substances, like titrations and UV spectrophotometry using absorptivity values. These methods shall be provided as alternatives in particular to chromatographic assays in monographs for pharmaceutical substances.

These strategies, however, shall only be applied when, during the elaboration of the methods, evidence could be obtained that the intended measures do not compromise the quality of the analytical results and are equally satisfying to conclusively demonstrate conformance to the applicable standards.

1.7 Analytical data provided in the leaflet of the ICRS

The leaflets of the ICRS may provide analytical information, including, but not limited to:

- the IR spectrum of the substance (together with a description of the sample preparation);
- additional analytical information at the time of establishment;
- the assigned content.

The section "Additional analytical information at the time of establishment" provides data about the purity of the reference substance and the methods used to determine it. The information was valid at the time of the establishment of the standard and will not be monitored or adjusted. The information may help the user to understand the calculation of the content that has been assigned to a standard for quantification. It may further be of value to assess risks or uncertainties associated with an unintended use of an ICRS. This information, however, is not given to authorize such an unintended use. As laid down under section 1.5, ICRS are adopted by the WHO Expert Committee on Specifications for Pharmaceutical Preparations for their intended uses only; the responsibility for an unintended use of an ICRS rests with the user or the authority that prescribes or authorizes this use.

1.8 Ordering information

Since April 2010 the European Directorate for the Quality of Medicines & HealthCare (EDQM), Council of Europe, is responsible for the establishment, preparation, storage and distribution of ICRS for The International Pharmacopoeia. A list of currently available ICRS can be found on its website (see http://www.edqm.eu).
Orders for ICRS should be sent to:
European Directorate for the Quality of Medicines & HealthCare
7 allée Kastner
CS 30026
F-67081 Strasbourg, France
Fax: +33 (0)3 88 41 27 71 - to the attention of EDQM Sales Section
Email: orders@edqm.eu

The current price for ICRS per package, as well as the cost for the delivery is available on the above-mentioned website.

2. International Infrared Reference Spectra

International infrared reference spectra are provided for use in identification tests as described in monographs of The International Pharmacopoeia or other WHO quality assurance documents adopted by the WHO Expert Committee on Specifications for Pharmaceutical Preparations.

The reference spectra are produced from authenticated material using an appropriate sample preparation technique. They are recorded with a Fourier transform infrared spectrophotometer (FTIR). Instructions for the preparation of spectra are given in 1.7 Spectrophotometry in the infrared region; Identification by reference spectrum.

A spectrum of the test substance is considered to be concordant with a reference spectrum if the transmission minima (absorption maxima) of the principal bands in the test spectrum correspond in position, relative intensities and shape to those in the reference spectrum.

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Levonorgestrelum
Levonorgestrel

This is a draft proposal for The International Pharmacopoeia (Working document QAS/15.614, May 2015).

The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/.

Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, CH-1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidth@who.int.

[Note from the Secretariat. It is proposed to revise the monograph on Levonorgestrel. Comments are particularly sought on whether the monograph should include a limit test for dextronorgestrel.]

[Note from the editor. In accordance with WHO editorial policy the text reproduced below does not include tracked changes. Changes from the current monograph are indicated by insert and delete in the working document available at the above-mentioned web address.]

Molecular formula. C_{21}H_{28}O_{2}
Relative molecular mass. 312.5

Graphic formula

Chemical name. (-)-13-Ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one; CAS Reg. No. 797-63-7.

Description. A white or almost white, crystalline powder.

Solubility. Practically insoluble in water; sparingly soluble in dichloromethane R, slightly soluble in ethanol (~750 g/L) TS and ether R.

Category. Contraceptive.

Storage. Levonorgestrel should be kept in a well-closed container, protected from light.

Requirements

Definition. Levonorgestrel contains not less than 98.0% and not more than 102.0% of C_{21}H_{28}O_{2}, calculated with reference to the dried substance.

Identity tests

• Either tests A and C or tests B and C may be applied.
A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from levonorgestrel RS or with the reference spectrum of levonorgestrel.

B. Carry out the examination as described under 1.14.4 High-performance liquid chromatography using the conditions described under “Related substances”, Method A. Prepare the following solutions. For solution (1) dissolve 10 mg of the test substance in 7 mL of acetonitrile R using sonication and dilute to 10 mL with water R. Dilute 1 volume to 100 volumes with a solvent mixture consisting of 30 volumes of water R and 70 volumes of acetonitrile R. For solution (2) use a solution containing 0.01 mg levonorgestrel RS per mL of the same solvent mixture. Inject 50 µL of solution (1) and (2). The retention time of the principal peak in the chromatogram obtained from solution (1) is similar to the principal peak in the chromatogram obtained from solution (2).

C. Determine the specific optical rotation (1.4) using a 10 mg per mL solution of the test substance in dichloromethane R. Calculate with reference to the anhydrous substance; the specific optical rotation is between -35° to -30°.

Sulfated ash (2.3). Not more than 1.0 mg/g, determined on 1.0 g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.

Related substances.
• Perform test A and B.

A. Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (25 cm × 4.6 mm) packed with base-deactivated particles of silica gel, the surface of which has been modified with chemically-bonded octylsilyl gel groups (5 µm). The material contains embedded polar groups.¹

Use the following conditions for gradient elution:

Mobile phase A: Mix 400 volumes of acetonitrile R with 600 volumes of water R.

Mobile phase B: Use acetonitrile R.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (%v/v)</th>
<th>Mobile phase B (%v/v)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–50</td>
<td>100 to 20</td>
<td>0 to 80</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>50–51</td>
<td>20 to 100</td>
<td>80 to 0</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>51–65</td>
<td>100</td>
<td>0</td>
<td>Re-equilibration</td>
</tr>
</tbody>
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Operate with a flow of 0.7 mL/min. As a detector use an ultraviolet spectrophotometer set at a wavelength of 215 nm and, for impurity O, at 200 nm. Maintain the column at 30°C.

Prepare as a solvent solution a mixture of 30 volumes of water R and 70 volumes of acetonitrile R.

Prepare the following solutions. For solution (1) dissolve about 10 mg of the test substance in 7 mL of acetonitrile R using sonication and dilute to 10 mL with water R.

¹ A Symmetry Shield RP8 column was found suitable.
For solution (2) dilute 1 volume of solution (1) to 1000 volumes with the solvent solution. For solution (3) dissolve 5.0 mg of norethisterone RS in 35 mL of acetonitrile R and dilute to 50.0 mL with water R. Dilute 1.0 mL of this solution to 100 mL with solution (2).

Inject solution 50 µL of solution (3). The assay is not valid unless the resolution factor between the two principal peaks due to levonorgestrel (retention time about 20 minutes) and the peak due to norethisterone (impurity U) (with a relative retention of about 0.8) is at least 3.0.

Inject alternately 50 µL each of solutions (1) and (2). The chromatogram obtained with solution (1) may show the following impurities at the following relative retention with reference to levonorgestrel (retention time about 20 minutes): impurity H: about 0.5; impurity U: about 0.8; impurity K: about 0.85; impurity A: about 0.91; impurity M: about 0.95; impurity O: about 1.16; impurity B: about 1.26; impurity S: about 1.9. Use also the chromatogram obtained with solution (3) to identify impurity U.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity A, when multiplied by a correction factor of 0.4, is not greater than 3 times the area of the principal peak obtained with solution (2) (0.3%);
- the area of any peak corresponding to either impurity B or K is not greater than 3 times the area of the principal peak obtained with solution (2) (0.3%);
- the area of any peak corresponding to impurity M, when multiplied by a correction factor of 3.1, is not greater than 2 times the area of the principal peak obtained with solution (2) (0.2%);
- the area of any peak corresponding to impurity O (recorded at 200 nm), when multiplied by a correction factor of 2.6, is not greater than 3 times the area of the principal peak obtained with solution (2) (0.3%);
- the area of any peak corresponding to either impurity S or U is not greater than 2 times the area of the principal peak obtained with solution (2) (0.3%);
- the area of any peak corresponding to impurity H is not greater than 1.5 times the area of the principal peak obtained with solution (2) (0.2%);
- the area of any other peak, other than the principal peak due to levonorgestrel, is not greater than the area of the principal peak obtained with solution (2) (0.15%);
- the area of any other peak, other than the principal peak due to levonorgestrel, is not greater than 10 times the area of the principal peak obtained with solution (2) (1.0%). Disregard any peak with an area less than 0.05 times the area of the principal peak obtained with solution (2) (0.05%).

B. Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (15 cm × 4.6 mm) packed with base-deactivated particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl gel groups (3 µm).² Use the following conditions for gradient elution:

Mobile phase A: Mix 400 volumes of acetonitrile R with 600 volumes of water R.

Mobile phase B: Mix 100 volumes of water R with 900 volumes of acetonitrile R.

² A Pack ODS-AQ (YMC) column was found suitable.
<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (%v/v)</th>
<th>Mobile phase B (%v/v)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–1</td>
<td>92</td>
<td>8</td>
<td>Isocratic</td>
</tr>
<tr>
<td>1–3</td>
<td>92 to 82</td>
<td>8 to 18</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>3–6</td>
<td>82</td>
<td>18</td>
<td>Isocratic</td>
</tr>
<tr>
<td>6–16</td>
<td>82 to 60</td>
<td>18 to 40</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>16–21</td>
<td>60 to 0</td>
<td>40 to 100</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>21–32</td>
<td>0</td>
<td>100</td>
<td>Isocratic</td>
</tr>
<tr>
<td>32–33</td>
<td>0 to 92</td>
<td>100 to 8</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>33–50</td>
<td>92</td>
<td>8</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

Operate with a flow of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 200 nm.

Prepare as a solvent solution a mixture of 30 volumes of water R and 70 volumes of acetonitrile R.

Prepare the following solutions. For solution (1) dissolve 10.0 mg of the test substance in 7 mL of acetonitrile R using sonication and dilute to 10.0 mL with water R. For solution (2) dissolve 5.0 mg of ethinylestradiol RS in 35 mL of acetonitrile R using sonication and dilute to 50.0 mL with water R. Dilute 3.0 mL of the solution to 100.0 mL with the solvent mixture. For solution (3) dilute 1.0 mL of solution (1) to 100.0 mL with solution (2).

Inject solution 50 µL of solution (3). The assay is not valid unless the resolution factor between the two principal peaks due to levonorgestrel (retention time about 12 minutes) and the peak due to ethinylestradiol (with a relative retention of about x) is at least x.x.

[Note from the Secretariat. The missing figures will be added at a later stage.]

Inject alternately 50 µL each of solutions (1) and (2). The chromatogram obtained with solution (1) may show the following impurities at the following relative retention with reference to levonorgestrel (retention time about 12 minutes): impurity W: about 0.9; impurity V: about 1.9.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity W is not greater than the area of the principal peak obtained with solution (2) (0.3%);
- the area of any peak corresponding to impurity V is not greater than 0.5 times the area of the principal peak obtained with solution (2) (0.15%).

**Assay.** Dissolve 0.200 g in 45 mL of tetrahydrofuran R. Add 10 mL of silver nitrate (100 g/L) TS. After 1 minute titrate with sodium hydroxide (0.1 mol/L) VS, determining the end-point potentiometrically. Carry out a blank titration. 1 mL of sodium hydroxide (0.1 mol/L) VS, is equivalent to 31.25 mg of C₂₁H₂₉O₂.
Impurities

A. 13-ethyl-17-hydroxy-18,19-dinor-17α-pregna-4,8(14)-dien-20-yn-3-one,

B. 13-ethyl-17-hydroxy-18,19-dinor-17α-pregn-5(10)-en-20-yn-3-one,

C. 13-ethyl-3-ethynyl-18,19-dinor-17α-pregna-3,5-dien-20-yn-17-ol,

D. 13-ethyl-18,19-dinor-17α-pregn-4-en-20-yn-17-ol (3-deoxolevonorgestrel),

G. 13-ethyl-6α,17-dihydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one
   (6α-hydroxylevonorgestrel),

H. 13-ethyl-6β,17-dihydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one
   (6β-hydroxylevonorgestrel),
I. 13-ethyl-10,17-dihydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one (10-hydroxylevonorgestrel),

J. 13-ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3,6-dione (6-oxolevonorgestrel),

K. 13-ethyl-17β-hydroxygon-4-en-3-one (18-methylnandrolone),

L. 13-ethylgon-4-ene-3,17-dione (levodione),

M. 13-ethyl-17-hydroxy-18,19-dinor-17α-pregna-4,6-dien-20-yn-3-one (Δ6-levonorgestrel),

N. 13-ethylgon-5(10)-ene-3,17-dione (Δ5(10)-levodione),
O. 13-ethyl-17-hydroxy-5α-methoxy-18,19-dinor-17α-pregn-20-yn-3-one (4,5-dihydro-5α-methoxylevonorgestrel),

P. 13-ethyl-17-hydroxy-18,19-dinor-17α-pregn-5-en-20-yn-3-one (Δ5-levonorgestrel),

Q. 13-ethyl-3-methoxygon-2,5(10)-dien-17β-ol,

R. 13-ethyl-3-methoxygon-2,5(10)-dien-17-one,

S. 13-ethyl-3-methoxy-18,19-dinor-17α-pregna-3,5-dien-20-yn-17-ol,

T. 13-ethyl-3-methoxy-18,19-dinor-17α-pregna-2,5(10)-dien-20-yn-17-ol,
U. 17-hydroxy-19-nor-17α-pregn-4-en-20-yn-3-one (norethisterone),

V. 13-ethyl-3-methoxy-18,19-dinor-17α-pregna-1,3,5(10)-trien-20-yn-17-ol,

W. 13-ethyl-17-hydroxy-18,19-dinor-17α-pregna-5,7,9-trien-20-yn-3-one.
**Estradioli cypionas**

Estradiol cypionate

This is a draft proposal for *The International Pharmacopoeia* (Working document QAS/15.618, May 2015).

The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/.

Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, CH-1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidt@who.int.

**Molecular formula.** $C_{26}H_{36}O_3$

**Relative molecular mass.** 396.56

**Graphic formula**

![Graphic formula of Estradiol cypionate]


**Description.** A white to almost white, crystalline powder.

**Solubility.** Soluble in alcohol, acetone and dioxane; sparingly soluble in vegetable oils; insoluble in water.

**Category.** Contraceptive.

**Storage.** Estradiol cypionate should be kept in tightly closed containers, protected from light.

**Requirements**

**Definition.** Estradiol cypionate contains not less than 97.0% and not more than 102.0% (“Assay”, Method A) or not less than 98.0% and not more than 102.0% (“Assay”, Method B) of $C_{26}H_{36}O_3$, calculated with reference to the dried substance.

**Identity tests**

- Either test A alone or tests B and C may be applied.
  
  **A.** Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from estradiol cypionate RS or with the reference spectrum of estradiol cypionate.

  **B.** Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given under “Assay”, Method A. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time of the peak due to estradiol in the chromatogram obtained with solution (2).

  **C.** Melting range, 149°C–153°C.
Specific optical rotation (1.4). Use a 20 mg/mL solution in dioxane R, $[\alpha]_D^{20^\circ} = +39^\circ$ to $+44^\circ$.

Loss on drying. Dry at 105°C for 4 hours; it loses not more than 10 mg/g.

Sulfated ash (2.3). Not more than 1.0 mg/g.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 10 μg/g.

Related substances. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using the conditions given below under “Assay”, Method A.

Prepare the following solutions in acetonitrile R. For solution (1) transfer 50 mg of the test substance to a 50 mL volumetric flask and dilute to volume. For solution (2) dilute 1.0 mL of solution (1) to 100.0 mL. For solution (3) use a solution containing 0.5 mg of estradiol cypionate RS and 0.5 mg of estradiol cypionate impurity B RS per mL.

Inject 25 μL of solution (3). The test is not valid unless the resolution between the peak due to impurity B (with a relative retention of about 0.91) and the peak due to estradiol cypionate (retention time about 15 minutes) is at least 1.5.

Inject alternately 25 μL each of solution (1) and (2).

In the chromatogram obtained with solution (1) the following impurities, if present, are eluted at the following relative retention with reference to estradiol cypionate: impurity A about 0.17; impurity E about 0.76; impurity B about 0.91; impurity C about 1.44; and impurity D about 2.22.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity A, impurity C or impurity D is not greater than 0.15 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15%);
- the area of any peak corresponding to impurity B, when multiplied by a correction factor of 0.4, is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);
- the area of any peak corresponding to impurity E is not greater than 0.15 times the area of the principal peak in the chromatogram obtained with solution (2) (0.15%);
- the area of any other peak, other than the principal peak, is not greater than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.10%);
- the sum of the area of any peak corresponding to impurity A, C, D, E, the corrected area of any peak corresponding to impurity B and the areas of all other peaks, other than the principal peak, is not greater than the area of the principal peak obtained with solution (2) (1.0%). Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Assay

- Either test A or test B may be applied.
  A. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (25 cm x 4.6 mm) packed with base-deactivated
particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 µm).1

Use the following conditions for gradient elution:

Mobile phase A: Water R
Mobile phase B: Acetonitrile R

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (% v/v)</th>
<th>Mobile phase B (% v/v)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–25</td>
<td>20</td>
<td>80</td>
<td>Isocratic</td>
</tr>
<tr>
<td>25–30</td>
<td>20 to 0</td>
<td>80 to 100</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>30–40</td>
<td>0</td>
<td>100</td>
<td>Isocratic</td>
</tr>
<tr>
<td>40–41</td>
<td>0 to 20</td>
<td>100 to 80</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>41–50</td>
<td>20</td>
<td>80</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

Operate with a flow rate of 1.2 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 280 nm.

Prepare the following solutions in acetonitrile R. For solution (1) use 1.0 mg solution of the test substance per mL. For solution (2) use 1.0 mg of estradiol cypionate RS per mL. For solution (3) use a solution containing 0.5 mg of estradiol cypionate RS and 0.5 mg of estradiol cypionate impurity B RS per mL.

Inject 25 µL of solution (3). The test is not valid unless the resolution between the peak due to impurity B (with a relative retention of about 0.91) and the peak due to estradiol cypionate (retention time about 15 minutes) is at least 1.5.

Inject alternately 25 µL each of solution (1) and (2). Measure the areas of the peaks corresponding to estradiol cypionate obtained in the chromatograms, and calculate the percentage content of estradiol cypionate (C\textsubscript{26}H\textsubscript{36}O\textsubscript{3}) in estradiol cypionate RS.

B. Dissolve about 0.05 g, accurately weighed, in sufficient methanol R to produce 100 mL; dilute 2.0 mL of this solution to 100 mL with the same solvent. Measure the absorbance (1.6) of a 1 cm layer of the diluted solution at the maximum at about 280 nm and calculate the percentage content of estradiol cypionate (C\textsubscript{26}H\textsubscript{36}O\textsubscript{3}) using the absorptivity value of estradiol cypionate.

[Note from the Secretariat. It is intended to determine the absorptivity value of estradiol cypionate during the establishment of estradiol cypionate RS. The value will then be included in the test description.]

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1 An Agilent ZORBAX SB-C\textsubscript{18} column has been found suitable.
Impurities

A. Estradiol

B. 3–Hydroxyestra–1,3,5(10),9(11)-tetraene-17β–yl cyclopentanepropanoate

C. 3–Hydroxy–4-methylestra–1,3,5(10)-tri en–17β–yl cyclopentanepropanoate; 4-Methylestradiol cypionate

D. Estra–1,3,5(10)-tri en–3,17 β–diyl di(cyclopentanepropanoate); Estradiol dicypionate

E. Estra–1,3,5(10)–triene–3,17–diol,(17β)–,17-cyclopentaneacetate

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