# Comments concerning texts published in Issue 12.2

*Brief descriptions of the modifications that have been made to new, revised and corrected texts adopted by the European Pharmacopoeia Commission at the March session and published in Issue 12.2 are provided below. Please note that these descriptions are not provided systematically for new and corrected texts, but are instead provided on a case-by-case basis. This information is reproduced in the Knowledge database under View history.*

*All revised, corrected or deleted parts of a text published in the European Pharmacopoeia are indicated by change marks in the form of triangles.*

## GENERAL CHAPTERS

### 2.5.25. Carbon monoxide in gases

***Method I***: this method has been deleted to avoid the use of chromium trioxide (REACH). Method I was only applied in the Reagents section of the Ph. Eur. for the determination of the carbon monoxide content in *argon R* (1008200). As a consequence, *argon R* is no longer required for the determination of carbon monoxide in argon, and the test for carbon monoxide has been deleted from the reagent description. The other Ph. Eur. texts describing the use of *argon R* are not impacted by the deletion of this method.

Headings included to better distinguish between the different sections.

### 2.6.7. Mycoplasmas

This general chapter has been revised extensively in order to reflect the current knowledge and expectations in the field.

***1. Introduction***. Important modifications have been made to this section to provide general information applicable to the rest of the chapter.

- The application of the chapter is not limited to the testing of the *Mycoplasma* genus, but covers the entire Mollicutes class (for which mycoplasmas is the trivial name).

- The culture and indicator cell culture methods described in general chapter *2.6.7* are adequate for detecting Mollicutes with a growth optimum of 35-38 °C, such as Mollicutes that could contaminate mammalian or avian cells. Testing for the presence of product-relevant mycoplasma species that are not indicated in the general chapter, or testing of other cell production systems such as insect, fish or plant cell lines, may require different conditions.

- The indication of which method(s) should be performed during which production stages has been deleted. It is now specified that the culture method and the indicator cell culture method (or, alternatively, an NAT method) should be used conjointly to ensure the detection of both “cultivable” and “non-cultivable” mycoplasmas, unless otherwise prescribed in a monograph, or unless otherwise justified by a risk assessment and authorised by the competent authority. The most suitable method(s) should be selected based on a risk assessment and depend on various parameters such as culture media, manufacturing process and potentially contaminating mycoplasma species.

- A sentence specifying that the samples should contain cells and supernatant, whenever possible, has been introduced.

***2. Culture method***

***2.1. Choice of culture media***:

- The recommendation to rotate the test micro-organisms used as positive controls in routine testing has been moved to section 2.5. Test for mycoplasmas in the product to be examined.

- The specific references to product types have been removed, as this general chapter focuses on methods and not product-specific requirements.

- The Collection de l'Institut Pasteur (CIP) culture collection references for strains other than *A. laidlawii* have been deleted as this information is missing in the CIP.

- The requirement for identification of strains after cloning has been removed as it is a GMP issue, which is outside the scope of the Ph. Eur.

***2.3. Nutritive properties***: the requirement for Petri dishes (i.e. 60 mm diameter, 9 mL of solid medium) has been removed to introduce some flexibility.

***2.5. Test for mycoplasmas in the product to be examined***:

- The use of a smaller sample volume for mycoplasmas testing in justified cases has been added.

- The possibility of including additional strains as positive controls other than those listed under section 2.1, based on a risk assessment of the type of product and manufacturing process characteristics, has been added.

***2.7. Recommended media for the culture method***: the requirement on the sera used in the media (i.e. unheated, inactivated at 56 °C for 30 min) has been removed to provide flexibility to users.

***3. Indicator cell culture method***

***3.1. Verification of the substrate***: the CIP reference for *M. orale* has been removed as this information is missing in the CIP.

***3.2. Inhibitory substances***: a new separate section has been introduced for inhibitory substances. The following information is of particular interest:

- Guidance on how to perform the test for inhibitory substances and how to interpret the results has been added.

- This section includes a reference to dilutions or neutralisations (already in the introduction of section 3. Indicator cell culture method of the previous version of chapter *2.6.7*) and now permits the use of alternative approaches such as testing of control cells, if justified and authorised.

***3.3. Test method***: for the description of the degree of confluence, in step 2, “about 50 per cent” is now stated, to allow some flexibility.

***4. Nucleic acid amplification techniques (NAT)***: this section has been extensively revised to adapt its content to the state of the art in science and technology.

***4.1. Introduction***: the hybrid approach of media-based enrichment followed by NAT has been introduced and the definition of direct NAT has been clarified.

***4.3. Validation***:

- Detailed information has been introduced on the requirements for the preparation and characterisation of strains: when to harvest the Mollicutes, when and how to determine the

titre in CFU, what to consider during the determination of the nucleic acid copies (supernatant and cellular fraction) and the acceptance criterion for the GC/CFU ratio.

- For the acceptance criterion for the GC/CFU ratio, a limit “smaller than 10, unless otherwise justified” is now proposed. The GC/CFU ratio should be as low as possible, and the proposed limit takes into account the fact that for some strains it is difficult to obtain a low ratio.

- The list of species which can be used for the validation of the detection limit has been slightly modified (i.e. addition of *Mycoplasma salivarium*, *Spiroplasma citri* or another species of the *Spiroplasma* genus) and presented as a “proposed” list, instead of an “optimal” selection. The culture collection references have been adapted.

- A new paragraph on “generic assay” validations has been added to highlight the importance of selecting the strains based on a risk assessment.

- With regard to the reference standards, the possibility of using the recently established 1st WHO International Standard for mycoplasma DNA for nucleic acid amplification technique-based assays designed for generic mycoplasma detection has been introduced.

- The acceptance criteria for the comparability study for using NAT as an alternative to both the culture method and the indicator cell culture method have been added. For each of the three options, a corresponding GC/mL acceptance criterion has been introduced.

***4.4. Inhibitory substances***: this new section has been created to provide guidance to users on testing for inhibitory substances.

***Validation of nucleic acid amplification techniques (NAT) for the detection of mycoplasmas: guidelines***

This guidance document required a major revision to adapt its content to the state of the art in science and technology.

***2-2. Detection limit***:

- The expectation concerning the use of reference strains and mycoplasma DNA standards for the determination of the positive cut-off point has been clarified.

- The list of species that can be included in the validation of the detection limit has been slightly modified, and a paragraph has been introduced to describe a risk-based approach for the selection of suitable species. This list has been presented as a “proposed” list, instead of an “optimal” selection, which was considered more appropriate.

- A new paragraph has been introduced for the case when Mollicutes preparations are used for the determination of the positive cut-off point, to provide additional information on the preparation and characterisation of the strains.

- In the examples, testing “on different days” has been replaced by testing “in independent test sessions” to give greater flexibility to users.

***3. Comparability study***: the acceptance criteria for the comparability study for use of NAT as an alternative to both the culture method and the indicator cell culture method have been added. For each of the three options, a corresponding GC/mL acceptance criterion has been introduced.

***4. Test for inhibitory substances***: this new section has been created to provide guidance to users on testing for inhibitory substances. Two examples have been included, but other approaches could be acceptable if justified.

### 3.1.8. Silicone oil used as a lubricant

***Tests***: new related substances test by gas chromatography added to cover polysiloxanes D4, D5 and D6.

### 3.1.14. Materials based on plasticised poly(vinyl chloride) for containers for aqueous solutions for intravenous infusion

***Additives***: the explanatory note highlighting the ban on the use of plastic additive 01 (DEHP) in the manufacture of immediate packaging materials for medicinal products per the EU REACH regulation has been clarified.

### 5.32. Cell-based preparations for human use

This general chapter provides a framework of requirements for the production and control of cell-based preparations. These requirements are not necessarily comprehensive for any given case, and complementary or additional requirements may be necessary. The provisions of this general chapter do not exclude the use of alternative production and control methods that are acceptable to the competent authority.

The general chapter contains a Definition section, a section outlining general requirements common to all cell-based preparations and four detailed individual sections describing additional specific requirements for human haematopoietic stem/progenitor cells, human chondrocytes, human limbal stem cells and human mesenchymal stromal cells. This means that, for example, human chondrocyte preparations must meet both the general requirements and the additional requirements in the specific section on human chondrocytes.

Specific considerations related to genetic modifications and their associated requirements are described in the general monograph *Gene therapy medicinal products for human use (3186)*.

Cells already covered by the monograph *Human haematopoietic stem cells (2323)* are not in the scope of this general chapter.

## GENERAL MONOGRAPHS

### Vaccines for human use (0153)

The monograph has been revised to 1) take into account new vaccine classes and technologies that emerged in recent years, in particular mRNA vaccines and viral-vectored vaccines, 2) extend to the final bulk the possibility to replace the sterility test with a test for bioburden with a stringent limit, and 3) introduce a cross-reference to new general chapter *2.6.40. Monocyte-activation test for vaccines containing inherently pyrogenic components*.

***Definition***. The section has been updated to cover nucleic acid-based vaccines and viral-vectored vaccines, and reorganised in sub-categories (bacterial vaccines, viral vaccines, viral-vectored vaccines, recombinant vaccines, synthetic antigen vaccines, and nucleic acid-based vaccines). Information related to the appearance and presentation of the final vaccine has been gathered in a dedicated paragraph.

***General provisions***. The paragraph on the preparation of seed lots has been updated to improve clarity and reflect current approaches. Demonstration of safety has evolved since the original elaboration of the general monograph: the focus is on ensuring that the product remains within its established safety profile (rather than making the seed lot ‘harmless’).

In the paragraph on pyrogenicity, a cross-reference to new general chapter *2.6.40* has also been introduced to guide users to an appropriate method for vaccines containing inherently pyrogenic components.

***Substrates for propagation.*** A requirement to use a cell-bank system for vaccines produced using human diploid cells or continuous cell lines has been introduced.

***Test for sterility of intermediates.*** The paragraph allowing for the replacement of the sterility test with a test for bioburden with a stringent limit in agreement with the competent authority has been revised to extend this possibility to the final bulk.

***Final bulk***. The paragraph has been revised to allow, for vaccines that undergo final sterile filtration, that the final bulk may be prepared by blending the ingredients of the vaccine under controlled, low-bioburden conditions before final sterile filtration, as an alternative to blending the ingredients of the vaccine under aseptic conditions.

***Stability of intermediates.*** Carrier proteins and intermediates for mRNA vaccines and recombinant viral-vectored vaccines have been added. The sentence “For final bulk vaccine, stability studies may be carried out on representative samples in conditions equivalent to those intended to be used for storage” has been amended to delete the phrase “for final bulk vaccine” as the requirements is considered to apply to other intermediates and is not specific to the final bulk.

***Identification, Assay.*** New sections Identification and Assay have been introduced.

***Tests***. Under Pyrogenicity, a cross-reference to new general chapter *2.6.40* has been introduced.

***Storage***. The section has been reorganised and updated to include general recommendations on storage temperatures and storage conditions, and take into account the case of nucleic acid-based vaccines and viral-vectored vaccines, which may require lower storage temperatures.

***Labelling***. The section has been updated.

### Vaccines for veterinary use (0062)

***BATCH TESTS: Mycoplasmas (section 3-11)***. “culture method” has been removed following the revision of general chapter *2.6.7*.

It should also be noted that, according to the revised general chapter *2.6.7* (published in the same issue of the European Pharmacopoeia), users are expected to carry out both the culture method and the indicator cell culture method (or alternatively, an NAT method), unless otherwise prescribed in a monograph, or unless justified by a risk assessment and authorised by the competent authority.

## VACCINES FOR HUMAN USE

### Hepatitis A vaccine (inactivated, adsorbed) (1107)

***VIRUS PROPAGATION AND HARVEST. Mycoplasmas.*** The volume to be used for the mycoplasmas test has been deleted because it is already given in general chapter *2.6.7. Mycoplasmas*, which is referenced in the monograph.

It should also be noted that, according to the revised general chapter *2.6.7* (published in the same issue of the European Pharmacopoeia), users are expected to carry out both the culture method and the indicator cell culture method (or alternatively, an NAT method), unless

otherwise prescribed in a monograph, or unless justified by a risk assessment and authorised by the competent authority.

## Influenza vaccine (split virion, inactivated) (0158)

***VIRUS SEED LOT. Mycoplasmas.*** The volume to be used for the mycoplasmas test has been deleted because it is already given in general chapter *2.6.7. Mycoplasmas*, which is referenced in the monograph.

It should also be noted that, according to the revised general chapter *2.6.7* (published in the same issue of the European Pharmacopoeia), users are expected to carry out both the culture method and the indicator cell culture method (or alternatively, an NAT method), unless otherwise prescribed in a monograph, or unless justified by a risk assessment and authorised by the competent authority.

## Influenza vaccine (surface antigen, inactivated) (0869)

***VIRUS SEED LOT. Mycoplasmas***. The volume to be used for the mycoplasmas test has been deleted because it is already given in general chapter *2.6.7. Mycoplasmas*, which is referenced in the monograph.

It should also be noted that, according to the revised general chapter *2.6.7* (published in the same issue of the European Pharmacopoeia), users are expected to carry out both the culture method and the indicator cell culture method (or alternatively, an NAT method), unless otherwise prescribed in a monograph, or unless justified by a risk assessment and authorised by the competent authority.

## Influenza vaccine (surface antigen, inactivated, prepared in cell cultures) (2149)

***PROPAGATION AND SINGLE HARVEST. Mycoplasmas***. The volume to be used for the mycoplasmas test has been deleted because it is already given in general chapter *2.6.7. Mycoplasmas*, which is referenced in the monograph.

It should also be noted that, according to the revised general chapter *2.6.7* (published in the same issue of the European Pharmacopoeia), users are expected to carry out both the culture method and the indicator cell culture method (or alternatively, an NAT method), unless otherwise prescribed in a monograph, or unless justified by a risk assessment and authorised by the competent authority.

## Influenza vaccine (surface antigen, inactivated, virosome) (2053)

***VIRUS SEED LOT. Mycoplasmas***. The volume to be used for the mycoplasmas test has been deleted because it is already given in general chapter *2.6.7. Mycoplasmas*, which is referenced in the monograph.

It should also be noted that, according to the revised general chapter *2.6.7* (published in the same issue of the European Pharmacopoeia), users are expected to carry out both the culture method and the indicator cell culture method (or alternatively, an NAT method), unless otherwise prescribed in a monograph, or unless justified by a risk assessment and authorised by the competent authority.

## Influenza vaccine (whole virion, inactivated) (0159)

***VIRUS SEED LOT. Mycoplasmas***. The volume to be used for the mycoplasmas test has been deleted because it is already given in general chapter *2.6.7. Mycoplasmas*, which is referenced in the monograph.

It should also be noted that, according to the revised general chapter *2.6.7* (published in the same issue of the European Pharmacopoeia), users are expected to carry out both the culture method and the indicator cell culture method (or alternatively, an NAT method), unless otherwise prescribed in a monograph, or unless justified by a risk assessment and authorised by the competent authority.

## Poliomyelitis vaccine (inactivated) (0214)

***VIRUS PROPAGATION AND HARVEST: Mycoplasmas***. The volume to be used for the mycoplasmas test has been deleted because it is already given in general chapter *2.6.7. Mycoplasmas*, which is referenced in the monograph.

It should also be noted that, according to the revised general chapter *2.6.7* (published in the same issue of the European Pharmacopoeia), users are expected to carry out both the culture method and the indicator cell culture method (or alternatively, an NAT method), unless otherwise prescribed in a monograph, or unless justified by a risk assessment and authorised by the competent authority.

## Smallpox vaccine (live) (0164)

***POOLED HARVEST. Mycoplasmas***:

- The volume to be used for the mycoplasmas test has been deleted because it is already given in general chapter *2.6.7. Mycoplasmas*, which is referenced in the monograph.

- The text now reflects that the test for mycoplasmas is performed on the pooled harvest and not on the final bulk, for vaccines produced in eggs because it is considered that mycoplasma contamination is more likely to be detected at this stage. For vaccines produced in cell cultures, the test carried out on the single harvest may be performed on the pooled harvest instead, where justified and authorised. A statement has been introduced to this effect.

It should also be noted that, according to the revised general chapter *2.6.7* (published in the same issue of the European Pharmacopoeia), users are expected to carry out both the culture method and the indicator cell culture method (or alternatively, an NAT method), unless otherwise prescribed in a monograph, or unless justified by a risk assessment and authorised by the competent authority.

***Bacterial and fungal contamination***: correction to move the requirement on the bacterial and fungal contamination of the final bulk to its correct location (i.e. under Final bulk vaccine).

***FINAL LOT. TESTS. Ovalbumin***: “within the limits” has been corrected to “not more than the limit” to align with the wording of other vaccine monographs and to reflect that only an upper limit applies for this impurity.

## Tick-borne encephalitis vaccine (inactivated) (1375)

***VIRUS PROPAGATION AND HARVEST: Mycoplasmas***. The volume to be used for the mycoplasmas test has been deleted because it is already given in general chapter *2.6.7. Mycoplasmas*, which is referenced in the monograph.

It should also be noted that, according to the revised general chapter *2.6.7* (published in the same issue of the European Pharmacopoeia), users are expected to carry out both the culture method and the indicator cell culture method (or alternatively, an NAT method), unless

otherwise prescribed in a monograph, or unless justified by a risk assessment and authorised by the competent authority.

### Yellow fever vaccine (live) (0537)

***PROPAGATION AND HARVEST. Mycoplasmas***:

- The volume to be used for the mycoplasmas test has been deleted because it is already given in general chapter *2.6.7. Mycoplasmas*, which is referenced in the monograph.

- The text now reflects that the test is preferably carried out on the single harvest because it is considered that mycoplasma contamination is more likely to be detected at this stage. Nevertheless, with the agreement of the competent authority, the test may be carried out on the pool of single harvests instead.

It should also be noted that, according to the revised general chapter *2.6.7* (published in the same issue of the European Pharmacopoeia), users are expected to carry out both the culture method and the indicator cell culture method (or alternatively, an NAT method), unless otherwise prescribed in a monograph, or unless justified by a risk assessment and authorised by the competent authority.

## HERBAL DRUGS AND HERBAL DRUG PREPARATIONS

### Clary sage oil (1850)

***Identification A***: TLC replaced by HPTLC in accordance with chapter *2.8.25*.

***Chromatographic profile***:

- new analytical procedure including an optimised temperature programme to assure elution of sclareol;

- requirements for geraniol, geranyl acetate and β-caryophyllene added as important for the characterisation of the oil;

- α- and β-thujone deleted from the chromatographic profile as not natural components of clary sage oil.

### Clove (0376)

***Identification C***: TLC replaced by HPTLC in accordance with general chapter *2.8.25*.

### Clove oil (1091)

***Identification A***: TLC replaced by HPTLC in accordance with general chapter *2.8.25*.

***Chromatographic profile***:

- hexane replaced by heptane due to toxicity;

- temperature profile prolonged to ensure elution of late eluting peaks;

- minimum resolution increased to provide an appropriate requirement;

- injection port temperature reduced to 230 °C and detector temperature reduced to 250 °C to protect the column;

- upper and lower limits for β-caryophyllene lowered as higher amounts indicate degradation;

- lower limit for acetyleugenol raised to ensure good quality;

- reporting threshold and corresponding reference solution added.

## Ispaghula husk (1334)

***Definition***: modified to indicate that the herbal drug consists predominantly of the episperm.

***Identification***: in test A, description of fragments from the endosperm and embryo of the seed included; in test C, TLC replaced by HPTLC in accordance with general chapter *2.8.25*, preparation of the test solution optimised, reagent used to treat the plate modified, fourth reference substance added.

## Ispaghula seed (1333)

***Definition***: synonym name updated according to current taxonomy.

***Identification***: in test A (macroscopic identification), ranges for seed width and thickness updated based on the most recent data available; in test B (microscopic identification), illustration of powdered herbal drug added and identification updated to include cross-references from text to illustration; in test C, TLC replaced by HPTLC in accordance with general chapter *2.8.25*, preparation of the test solution optimised, reagent used to treat the plate modified, fourth reference substance added.

## Ligusticum root and rhizome (2431)

***Definition***: botanical name updated according to the Medicinal Plant Names Services (MPNS).

## Liquorice dry extract for flavouring purposes (2378)

***Identification***: the preparation of the test solution has been updated to avoid decomposition of ethyl acetate.

***Assay***: column dimensions and stationary phase updated. The monograph *Liquorice root (0277)* has been aligned accordingly.

## Liquorice root (0277)

***Identification B***: illustration of powdered herbal drug introduced and its legend integrated into text of identification B.

***Assay***: updated and aligned with monograph *Liquorice dry extract for flavouring purposes (2378)*:

- three-point calibration replaced with single-point calibration;

- column dimensions and stationary phase updated;

- system suitability requirements added.

## Psyllium seed (0858)

***Identification***: HPTLC test introduced in accordance with general chapter *2.8.25*.

### Szechwan lovage rhizome (2634)

***Definition***: botanical name updated according to the Medicinal Plant Names Services (MPNS); clarification added that the herbal drug is a cultivar of the one described in the monograph on *Ligusticum root and rhizome (2431)*.

## HOMOEOPATHIC PREPARATIONS

### Cadmium sulfuricum for homoeopathic preparations (2143)

***Arsenic***: in line with the Ph. Eur. implementation strategy for the ICH Q3D guideline on elemental impurities (please see Press release), the test has been deleted.

### Cuprum metallicum for homoeopathic preparations (1610)

***Lead***: in line with the Ph. Eur. Implementation strategy for the ICH Q3D guideline on elemental impurities (please see Press release), the test has been deleted.

### Ferrum metallicum for homoeopathic preparations (2026)

***Arsenic, Copper, Lead***: in line with the Ph. Eur. implementation strategy for the ICH Q3D guideline on elemental impurities (please see (please see Press release), the tests have been deleted.

### Magnesium phosphoricum for homoeopathic preparations (2505)

***Arsenic***: in line with the Ph. Eur. implementation strategy for the ICH Q3D guideline on elemental impurities (please see (please see Press release), the test has been deleted.

## MONOGRAPHS

### Amphotericin B (1292)

***Characters***: the solubility in propylene glycol has been revised.

### Carboprost trometamol (1712)

***Related substances***: preparation of reference solution (b) amended; procedure for degradation of carboprost trometamol to impurity B proposed instead of the use of a reagent; *carboprost for system suitability CRS* now used for identification of specified impurities A and B and for system suitability; “Prepare the solutions immediately before use” added to avoid degradation in solutions.

### Cellulose acetate butyrate (1406)

***Functionality-related characteristics***: a section has been added to introduce characteristics that may be considered critical and useful material attributes when cellulose

acetate butyrate is used as a film former in modified-release tablets and capsules and as a matrix former in modified-release tablets and capsules:

- for cellulose acetate butyrate used as film former in modified-release tablets and capsules, the 2 characteristics added are degree of substitution and solubility of a film;

- for cellulose acetate butyrate used as matrix former in modified-release tablets and capsules, the 2 characteristics added are particle-size distribution and degree of substitution.

***Assay***: grades of solvents amended in accordance with the Technical Guide (2022).

## Cysteine hydrochloride monohydrate (0895)

***Ninhydrin-positive substances***: reference solution (b) modified to replace *dilute hydrochloric acid R1* by a 10.3 g/L solution of *hydrochloric acid R* to allow complete dissolution of *L-cystine R* (impurity A); calculation of dilution adjusted.

## Erlotinib hydrochloride (3094)

***Related substances***: in view of feedback from users and additional experimental work, it was confirmed that column temperature and pH were critical parameters; therefore, a specific pH range (± 0.1) and a column temperature of 20 °C have been introduced.

## Flunitrazepam (0717)

***Related substances***: recent experimental work showed that impurity C levels were overestimated because the value of the correction factor (CF) for impurity C had to be lowered to 2 (previously 2.44). This has no impact on the current limit of 0.10 per cent, since the levels of impurity C determined with the new CF are lower than with the old CF.

## Folic acid hydrate (0067)

***Related substances***: “Protect the solutions from light” added to prevent formation of impurity J, which interferes with impurity C.

***Impurities***: impurity J added based on data from a manufacturer.

## Gabapentin (2173)

***Related substances***: following further investigation and feedback from users, a disregard limit statement has been introduced in test A to avoid the quantification of any peak with relative retention of 0.7, which is due to the solvents.

## Glycerol monostearate 40-55 (0495)

***Functionality-related characteristics***: a new use has been added to include characteristics that may be considered critical and useful material attributes when glycerol monostearate 40-55 is used as a lubricant in tablets and capsules and as an antiadhesive in coated tablets and capsules. The 3 characteristics added are composition of fatty acids, particle-size distribution and thermal analysis.

The expression "matrix former in prolonged-release oral solid dosage forms" has been changed to “matrix former in modified-release solid dosage forms” to allow for the inclusion of non-oral dosage forms. Moreover, the expression “prolonged-release” was changed to “modified-release” in order to cover delayed- and prolonged-release dosage forms (see *Glossary (1502)*).

## Golimumab concentrated solution (3103)

***Charged variants***: the two-step capillary isoelectric focusing procedure described in general chapter *2.5.44, Procedure A*, has been added as a suitable method in addition to the example imaged capillary isoelectric focusing procedure; solution B has been renamed “Marker solution” in line with the monograph on *Golimumab injection (3187)*.

***Potency***: the preparation of the reference solution has been amended, and the concentration is now expressed in IU/mL.

## Human albumin solution (0255)

***Protein composition***: the test procedure has been updated to take into account current practice:

- removal of the cellulose acetate gel electrophoresis procedure, which is phasing out;

- addition of a reference to using capillary zone electrophoresis as an alternative procedure in order to address the increasing use of this state-of-the art technology in the field.

Editorial changes have been made throughout the text.

## Hydroxyzine hydrochloride (0916)

***Related substances***: more robust UHPLC method is introduced to control additional impurities; impurity specifications updated in view of recent batch and stability data from manufacturers on the European market.

***Impurities***: transparency list has been updated.

## Metronidazole benzoate (0934)

***Identification***: former test B (UV) deleted as the necessary equipment to carry out this test is not available in most pharmacy laboratories, the remaining tests are considered sufficient for the purpose of the second identification; IR reference spectrum replaced by a CRS in test B (IR, formerly C).

***Related substances***: liquid chromatography procedure updated as a quantitative test where all impurities are quantified with reference to a 0.05 per cent diluted test solution; impurities A, B and C listed as unspecified impurities; limit for unspecified impurities introduced thus the limit for any impurity other than A, B and C also tightened to maximum 0.05 per cent; reporting threshold widened to 0.03 per cent, since the maximum daily dose of metronidazole benzoate is more than 2 g; concentration of the active substance in reference solution (a) decreased in accordance with the limit for unspecified impurities; in the preparation of reference solution (b), volume and mass expressed using fewer significant figures due to the qualitative use of this solution; reagent used to describe stationary phase modified; grade of acetonitrile in the mobile phase amended in accordance with the Technical Guide (2022).

***Impurities***: section modified in accordance with the new limits proposed in the test for related substances.

## Midazolam (0936)

***Second identification***: tests D and E deleted as considered unnecessary for the purpose of the second identification. In addition, users have reported via the HelpDesk that the reagent ‘*zirconyl nitrate solution R*' used in test D is difficult to obtain and will probably be discontinued from the market in Europe.

***Related substances***: in the preparation of reference solution (b), volume expressed using fewer significant figures due to the qualitative use of this solution.

## Nifedipine (0627)

***Characters***: the statement regarding the preparation of solutions and protection from light has been moved to the Tests and Assay sections, since it is essential information for users, it is a mandatory requirement and it applies to all tests in those sections.

***Related substances***: liquid chromatography procedure updated as a quantitative test where specified impurities A and B are quantified with external reference standards and other impurities are quantified with respect to the concentration of nifedipine; impurity C listed as unspecified impurity; description of stationary phase aligned to the one used during validation, grades of solvents amended in accordance with the Technical Guide (2022).

## Oleyl alcohol (2073)

***Composition of fatty alcohols***: α-linolenyl alcohol replaced by γ-linolenyl alcohol as the initial specification was for γ-linolenyl alcohol; relative retention times corrected.

## Procaine hydrochloride (0050)

***Definition***: content limit widened in accordance with the replacement of the titrimetric assay procedure by liquid chromatography.

***Characters***: solubility in heptane added.

***Identification***: former tests C (using fuming nitric acid) and F (colour reaction for primary aromatic amines, *2.3.1*) deleted, as tests A (melting point), C (colorimetric test with potassium permanganate, formerly test D) and D (reaction of chlorides, formerly test E) are considered sufficient for the purpose of the second identification.

***Related substances***: thin-layer chromatography replaced by high-performance liquid chromatography (HPLC) covering nine new impurities; specifications and limits for unspecified impurities and total impurities added; reporting threshold included.

***Assay***: titrimetric procedure replaced by a more robust HPLC procedure based on the same method proposed for the test for related substances.

***Impurities***: section added in accordance with the proposed test for related substances.

## Sorafenib tosilate tablets (3022)

***Dissolution***: in the preparation of the reference solution, the volume of *methanol R* has been increased to 5 mL to ensure complete dissolution of *sorafenib tosilate CRS*.

## Testosterone enantate (1048)

***Impurity A***: colour-indicator test deleted as impurity A is now covered by the new high-performance liquid chromatography (HPLC) procedure.

***Impurity H***: thin-layer chromatography procedure deleted as impurity H is now covered by the new HPLC procedure.

***Related substances B***: new HPLC procedure introduced to control impurities A, H and additional non-polar impurities.