EDQM & European Pharmacopoeia: State-of-the-art Science for Tomorrow's Medicines

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Workshop on Impurities

Moderator

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Impurity Control in the European Pharmacopoeia (Ph.Eur.)

EDQM and European Pharmacopoeia:
State-of-the-art Science for Tomorrow's Medicine
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Presentation Outline

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- Control of impurities in the Ph.Eur. | General considerations
- The way to an Individual Monograph
- The governing framework of the Ph.Eur.
- Organic impurities and Genotoxic (DNA reactive/mutagenic) impurities
  - Requirements & Type of impurities and tests
  - Description in the individual monographs
  - Establishment of a new test and related validation considerations
  - Revision of an existing test and related validation considerations
  - Calculation of impurity content and Limits.
- Inorganic- / Elemental impurities
- Residual Solvents
- The user perspective
- Q & A

Control of impurities in the Ph.Eur. General considerations

Control of impurities in the Ph.Eur.

- The current **general policy** of the Commission is to **include quantitative test(s) for impurities/degradants in monographs for** both **chemically defined active substances** (active pharmaceutical ingredient, **API**) and **finished product (FP)** monographs containing chemically defined active substances.
- The **impurities/degradants present** in those substances/products **have been evaluated by the competent authorities and** are **qualified with respect to safety at the maximum authorized content** (at the maximum daily dose) unless new safety data become available and justify lower limits.
- **The tests** included in the Ph.Eur. **are intended to cover for organic impurities** incl. **genotoxic impurities** (DNA reactive / mutagenic), **inorganic/elemental impurities** (as relevant) and **Residual Solvents.**
- The **test methods in the Monographs** and **General Chapters** have been **validated** according to current guidelines on analytical validation **and at least second laboratory** has **performed verification during the elaboration** of the monograph.

The way to an Individual Monograph

Control of impurities

The way to an Individual Monograph
Control of impurities

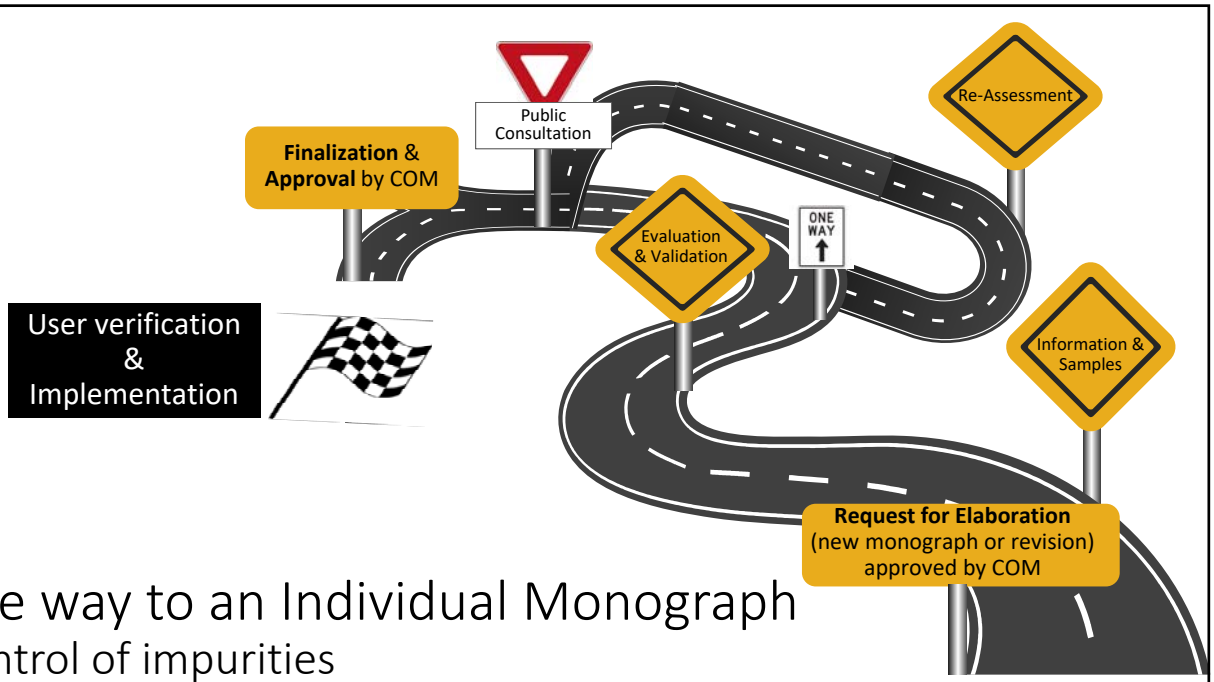
Approved control limits by competent authorities of Parties to the *European Pharmacopoeia Convention*

Validated, approved methods

Phe.Eur. and relevant ICH guidelines as governing framework

Representative batch data (release and stability)

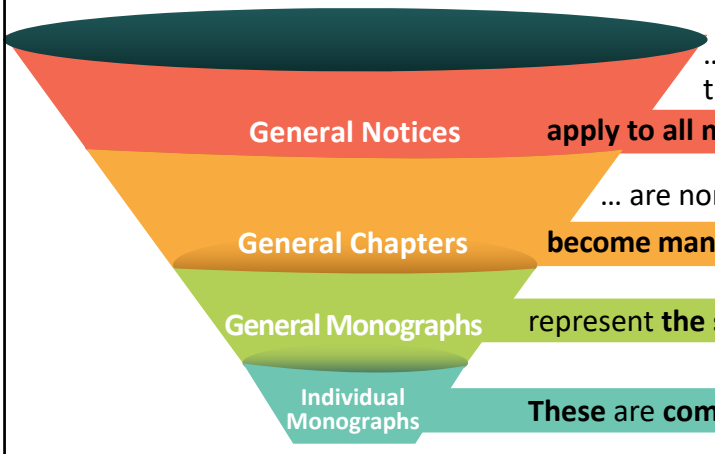
Evaluation/assessment (theoretical AND experimental) by the expert group



The way to an Individual Monograph
Control of impurities

The governing framework of the Ph.Eur.
For the elaboration & use of the texts

The governing framework of the Ph.Eur.
For the elaboration & use of the texts



…are the general rules for interpretation of the texts of the Ph.Eur.

apply to all monographs and other texts of the Ph.Eur.

… are normally given for information and

become mandatory when referred to in a monograph.

represent **the standards of the Ph.Eur.** together with

These are **complementary.**

General Monographs become applicable and **required to comply with when substances and preparations are subject of an Individual Monograph.** Cross-reference to the applicable General Monographs are normally NOT given in Individual Monographs.

The governing framework cont'd

Examples:
References made to General Notices and to General Chapters in General Monograph.

SUBSTANCES FOR PHARMACEUTICAL USE

Corpora ad usum pharmaceuticum

DEFINITION

Substances for pharmaceutical use are any organic or inorganic substances that are used as active substances or excipients for the production of medicinal products for human or veterinary use. They may be obtained from natural sources or produced by extraction from raw materials, fermentation or synthesis.

This general monograph does not apply to herbal drugs, herbal drugs for homoeopathic preparations, herbal drug preparations, herbal drug extracts, or mother tinctures for homoeopathic preparations, which are the subject of separate general monographs (*Herbal drugs (1433)*, *Herbal drugs for homoeopathic preparations (2045)*, *Herbal drug preparations (1434)*, *Herbal drug extracts (0765)*, *Mother tinctures for homoeopathic preparations (2029)*). It does not apply to raw materials for homoeopathic preparations, except where there is an individual monograph for the substance in the non-homoeopathic part of the Pharmacopoeia.

This monograph does not apply to chemical precursors for radiopharmaceutical preparations which are the subject of a separate monograph (*Chemical precursors for radiopharmaceutical preparations (2902)*).

Where a substance for pharmaceutical use not described in an individual monograph of the Pharmacopoeia is used in a medicinal product prepared for the special needs of individual

The manufacture of active substances must take place under conditions of good manufacturing practice.

The provisions of general chapter *5.10* apply to the control of impurities in substances for pharmaceutical use.

Whether or not it is specifically stated in the individual monograph that the substance for pharmaceutical use:

- is a recombinant protein or another substance obtained as a direct gene product based on genetic modification, where applicable, the substance also complies with the requirements of the general monograph *Products of recombinant DNA technology (0784)*;
- is obtained from animals susceptible to transmissible spongiform encephalopathies other than by experimental challenge, where applicable, the substance also complies with the requirements of the general monograph *Products with risk of transmitting agents of animal spongiform encephalopathies (1483)*;
- is a substance derived from a fermentation process, whether or not the micro-organisms involved are modified by traditional procedures or recombinant DNA (rDNA) technology, where applicable, the substance also complies with the requirements of the general monograph *Products of fermentation (1468)*.

If solvents are used during production, they are of suitable quality. In addition, their toxicity and their residual level are taken into consideration (*5.4*). If water is used during production, it is of suitable quality.

The identity of elemental impurities derived from intentionally added catalysts and reagents is known, and strategies for controlling them should be established using the principles of risk management.

General Notices (1) apply to all monographs and other texts

The governing framework
The Ph.Eur. context cont'd

Bambuterol hydrochloride EUROPEAN PHARMACOPOEIA 9.0

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: discs.

Comparison: *bambuterol hydrochloride CRS*.

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in a mixture of 1 volume of *water R* and 6 volumes of *acetone R*, cool in ice to precipitate and dry both precipitates *in vacuo* at 50 °C to constant weight. Record new spectra using the residues.

B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S. Dissolve 4.0 g in *carbon dioxide-free water R* and dilute to 20.0 mL with the same solvent.

Acidity or alkalinity. To 10 mL of solution S add 0.2 mL of *methyl red solution R* and 0.2 mL of *0.01 M hydrochloric acid*. The solution is red. Add 0.4 mL of *0.01 M sodium hydroxide*. The solution is yellow.

Optical rotation (*2.2.7*): − 0.10° to + 0.10°.

Dilute 1 mL of solution S to 10 mL with *carbon dioxide-free water R*.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 5.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a). Dissolve 1.0 mg of *formoterol fumarate dihydrate CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase. Mix 0.8 mL of this solution with 0.4 mL of the test solution and dilute to 100.0 mL with the mobile phase.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

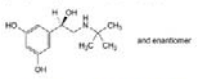
ASSAY

Dissolve 0.320 g in 50 mL of *ethanol (96 per cent) R* and add 5 mL of *0.01 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 40.39 mg of $C_{18}H_{29}ClN_4O_5$.

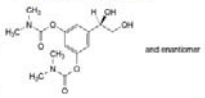
IMPURITIES

Specified impurities: A, B, C, D, E, F.



and enantiomer

A. (1*RS*)-1-(3,5-dihydroxyphenyl)-2-[(1,1-dimethylethyl)-amino]ethanol (terbutaline),



and enantiomer

R. 5-[(1*RS*)-1,2-dihydroxyethyl]-1,3-phenylene bis(dimethylcarbamate),

2.2.29. LIQUID CHROMATOGRAPHY

- a variety of chemically modified supports prepared from polymers, silica or porous graphite, used in normal-phase and reversed-phase LC (non-polar stationary phase and polar mobile phase), where the separation is based principally on partition of the molecules;
- resins or polymers with acidic or basic groups, used in ion-exchange chromatography, where separation is based on competition between the ions to be separated and those in the mobile phase;
- porous silica or polymers, used in size-exclusion chromatography (*2.2.30*), where separation is based on differences between the volumes of the molecules, corresponding to steric exclusion;
- specially modified stationary phases, e.g. cellulose or amylose derivatives, proteins or peptides, cyclodextrins etc., for the separation of enantiomers (chiral chromatography).

Most separations are based on reversed-phase LC utilising chemically modified silica as the stationary phase. The surface of the support, i.e. the silanol groups of silica, is reacted with various silane reagents to produce covalently bound silyl derivatives covering a varying number of active sites on the surface of the support. The nature of the bonded phase is an important parameter for determining the separation properties of the chromatographic system.

Unless otherwise stated by the manufacturer, silica-based reversed-phase columns are considered to be stable in mobile phases having an apparent pH in the range 2.0 to 8.0. Columns containing porous graphite or particles of polymeric materials such as styrene-divinylbenzene copolymer are stable over a wider pH range.

Analysis using normal-phase LC with unmodified silica or polar chemically modified silica (e.g. cyanopropyl or diol) as the stationary phase, with a non-polar mobile phase is applicable in certain cases.

For analytical separations, the particle size of the most commonly used stationary phases varies between 2 and 10 µm. The particles may be spherical or irregular, and of varying porosity and specific surface area. These properties contribute to the chromatographic behaviour of a particular stationary phase. In the case of reversed phases, the nature of the stationary phase, the extent of bonding, e.g. expressed as the carbon loading, and whether the stationary phase is end-capped (i.e. part of the residual silanol groups are silylated) are additional determining factors. Tailing of peaks, particularly of basic substances, can occur when residual silanol groups are present.

particles, and when special detectors, e.g. light scattering detectors, are used). Multicomponent mobile phases are prepared by measuring the required volumes (unless masses are specified) of the individual components, followed by mixing. Alternatively, the solvents may be delivered by individual pumps controlled by proportioning valves, by which mixing is performed according to the desired proportion. Solvents are normally degassed before pumping by sparging with helium, sonication and/or using on-line membrane/vacuum modules to avoid the creation of gas bubbles in the detector cell.

Solvents for the preparation of the mobile phase are normally free of stabilisers and, if an ultraviolet detector is employed, are transparent at the wavelength of detection. Solvents and other components employed are to be of appropriate quality. In particular, *water for chromatography R* is used for the preparation of mobile phases when water, or an aqueous solution, is 1 of the components. Any necessary adjustments of the pH are made to the aqueous component of the mobile phase and not the mixture. If buffer solutions or saline solutions are used, adequate rinsing of the system is carried out with a mixture of water and a small proportion of the organic part of the mobile phase (5 per cent *V/V*) to prevent crystallisation of salts after completion of the analysis.

Mobile phases may contain other components, for example a counter-ion for ion-pair chromatography or a chiral selector for chiral chromatography using an achiral stationary phase.

DETECTORS

Ultraviolet/visible (UV/Vis) spectrophotometers (including diode array detectors) (*2.2.25*), are the most commonly employed detectors. Fluorescence spectrophotometers, differential refractometers (RI), electrochemical detectors (ECD), light scattering detectors, charged aerosol detectors (CAD), mass spectrometers (MS) (*2.2.43*), radioactivity detectors, multi-angle light scattering (MALS) detectors or other detectors may be used.

PROCEDURE

Equilibrate the column with the prescribed mobile phase and flow rate, at room temperature or at the temperature specified in the monograph, until a stable baseline is achieved. Prepare the solution(s) of the substance to be examined and the reference solution(s) required. The solutions must be free from solid particles.

Criteria for assessing the suitability of the system are described in general chapter *2.2.46. Chromatographic separation techniques*. The extent to which adjustments of parameters of the chromatographic system can be made to satisfy the criteria of system suitability are also given in this chapter.

Control of impurities in the Ph.Eur
Organic Impurities

Control of impurities in the Ph.Eur
Organic Impurities

- **Essential part** of the control strategy and thus **of the Individual Monographs**.
- **Principles follow ICH Q3A for active substances** and **Q3B for finished products** and are described in:
  - General Monograph "**Substances for pharmaceutical use (2034)**".
  - General Monograph "**Pharmaceutical preparations (2619)**".
  - General Chapter **5.10.** "**Control if impurities in substances for pharmaceutical use**".
- The Ph.Eur. enforces **ICH M7** and **must be complied with for genotoxic impurities** (DNA reactive / mutagenic) **in actives substances** in case defined in the scope of the guideline.
- Unless otherwise prescribed or justified and authorized, **organic impurities/degradants in active substances/finished products are to be reported, identified** wherever possible **and qualified** (see requirements on next slides).

Control of impurities in the Ph.Eur
Organic Impurities cont'd

- Requirements for **active substances (excl. synthetic peptides)** 2034 and ICH Q3A

Use	Maximum daily dose	Reporting threshold	Identification threshold	Qualification threshold
Human or Human and Veterinary	≤ 2 g/day	> 0.05 per cent	> 0.10 per cent or a daily intake of >1.0 mg (whichever lower)	> 0.15 per cent or a daily intake of > 1.0 mg (whichever lower)
Human or Human and Veterinary	> 2 g/day	> 0.03 per cent	> 0.05 per cent	> 0.05 per cent
Veterinary only	Not applicable	> 0.10 per cent	> 0.20 per cent	> 0.50 per cent

- **Specific thresholds** may be applied **for impurities** known to be **unusually potent** or to produce **toxic** or **unexpected pharmacological effects.**

Control of impurities in the Ph.Eur
Organic Impurities cont'd

- Requirements for **pharmaceutical preparations (finished products)** ICH Q3B Attachment 1.

Maximum daily dose (amount of the active substance)	Reporting threshold	Maximum daily dose (amount of the active substance)	Identification Threshold (of the degradant)
≤ 1 g/day	> 0.1 per cent	< 1 mg 1 mg - 10 mg > 10 mg - 2 g > 2 g	1.0 per cent or 5 µg daily intake (whichever lower) 0.5 per cent or 20 µg daily intake (whichever lower) 0.2 per cent or 2 mg daily intake (whichever lower) 0.10%
> 1 g/day	> 0.05 per cent	< 10 mg 10 mg - 100 mg > 100 mg -2 g > 2 g	1.0 per cent or 50 µg daily intake (whichever lower) 0.5 per cent or 200 µg daily intake (whichever lower) 0.2 per cent or 3 mg daily intake (whichever lower) 0.15%

- **Specific thresholds** may be applied **for degradation products** known to be **unusually potent** or to produce **toxic** or **unexpected pharmacological effects.**

Control of impurities in the Ph.Eur
Organic Impurities cont'd

- **Specified impurities (degradants)** are **individually listed** and limited **with a specific acceptance criterion** in a Monograph.
  - **Identified specified** impurities (degradants) have **structural characterization**.
  - **Unidentified specified** impurities (degradants) have **NO structural characterization**.
- **Unspecified impurities (degradants)** are impurities **limited by a general acceptance criterion** and **not individually listed** with their own acceptance criterion.
- **Other detectable impurities (degradants)** are potential impurities with a defined structure and are known to be **detected by the tests** in the monograph **but not** know to be normally **present above the identification threshold**. These are **unspecified impurities (degradants)** and thus are limited by a general acceptance criterion.

Control of impurities in the Ph.Eur
Organic Impurities cont'd

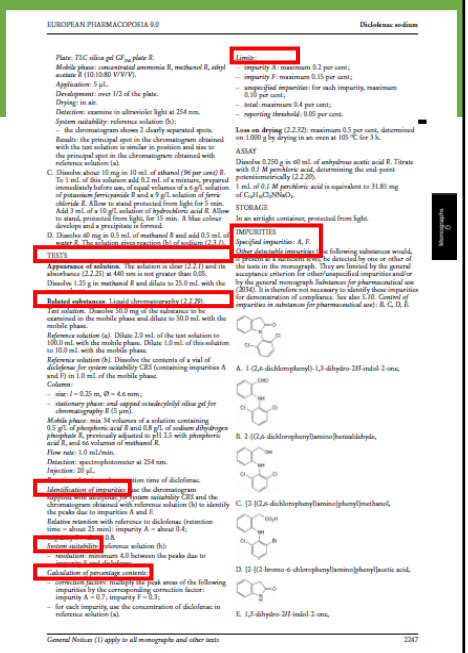
- Most often **separation techniques** (LC, GC, TLC, CE etc.) in combination **with different detection techniques** (UV/VIS, RI, MS, ELSD, CAD, FID etc.) are being **used for the determination of organic impurities**.
  For special intended use other analytical techniques e.g. UV absorption spectrophotometry (e.g.: riboflavin) or titration (e.g: free acids in testosterone esters) are also an option.
- For **pharmaceutical preparations (finished products) only degradation products are in scope**.

Control of impurities in the Ph.Eur
Organic Impurities cont'd

- … **in the Individual Monographs** is described under the **TESTS | Related Substances section**. Instructions may be included in the **PRODUCTION section** of a monograph.
- Procedures for **Identification** of the relevant peaks, **System Suitability** and **Calculation for percentage content** are described.
- **Limits** are defined for
  - **Specified** impurities.
  - **Unspecified** impurities.
  - **Total** (of impuritites).
  - **Reporting Threshold** (disregard limit).
- The **IMPURITIES section** at the end of each Individual Monograph includes the **impurities (structure and name, wherever possible)** that are known to be **detected by the tests** described in the Individual Monograph.
  Specified impurities and as applicable and indicated **other detectable impurities**, the latter for information only, are listed.

Control of impurities in the Ph.Eur
Organic Impurities cont'd

Example: Diclofenac Sodium (1002)



EUROPEAN PHARMACOPOEIA 9.0 Diclofenac sodium

Limits:
- *impurity A:* maximum 0.2 per cent;
- *impurity F:* maximum 0.15 per cent;
- *unspecified impurities:* for each impurity, maximum 0.10 per cent;
- *total:* maximum 0.4 per cent;
- *reporting threshold:* 0.05 per cent.

Loss on drying (2.2.32): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

ASSAY

Dissolve 0.250 g in 60 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.2.20).

1 ml of *0.1 M perchloric acid* is equivalent to 31.81 mg of $C_{14}H_{10}Cl_2NNaO_2$.

STORAGE

In an airtight container, protected from light.

IMPURITIES

Specified impurities: A, F.

Other detectable impurities: [illegible] the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use* (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. *Control of impurities in substances for pharmaceutical use*): B, C, D, E.

A. 1-(2,6-dichlorophenyl)-1,3-dihydro-2*H*-indol-2-one,

B. 2-[(2,6-dichlorophenyl)amino]benzaldehyde,

C. [2-[(2,6-dichlorophenyl)amino]phenyl]methanol,

D. [2-[(2-bromo-6-chlorophenyl)amino]phenyl]acetic acid,

E. 1,3-dihydro-2*H*-indol-2-one,

TESTS

Appearance of solution. The solution is clear (2.2.1) and its absorbance (2.2.25) at 440 nm is not greater than 0.05. Dissolve 1.20 g in *methanol R* and dilute to 25.0 ml. with the

Related substances. Liquid chromatography (2.2.29).

Test solution. Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml. with the mobile phase.

Reference solution (a). Dilute 2.0 ml. of the test solution to 100.0 ml. with the mobile phase. Dilute 1.0 ml. of this solution to 10.0 ml. with the mobile phase.

Reference solution (b). Dissolve the contents of a vial of *diclofenac for system suitability CRS* (containing impurities A and F) in 1.0 ml. of the mobile phase.

Column:
- *size:* $l = 0.25$ m, $\varnothing = 4.6$ mm;
- *stationary phase: end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: mix 34 volumes of a solution containing 0.5 g/l of *phosphoric acid R* and 0.8 g/l of *sodium dihydrogen phosphate R*, previously adjusted to pH 2.5 with *phosphoric acid R*, and 66 volumes of *methanol R*.

Flow rate: 1.0 ml/min.

Detection: spectrophotometer at 254 nm.

Injection: 20 µl.

[illegible] tion time of diclofenac.

Identification of impurities: use the chromatogram supplied with *diclofenac for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A and F.

Relative retention with reference to diclofenac (retention time = about 25 min): impurity A = about 0.4;

[illegible] 1.8.

System suitability: reference solution (b):
- resolution: minimum 4.0 between the peaks due to [illegible] and diclofenac.

Calculation of percentage contents:
- correction factors: multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.7, impurity F = 0.3;
- for each impurity, use the concentration of diclofenac in reference solution (a).

General Notices (1) apply to all monographs and other texts 2247

Control of impurities in the Ph.Eur
Organic Impurities (genotoxic, DNA reactive / mutagenic) cont'd

Example: Valsartan (2423)

DRAFT MONOGRAPH

PRODUCTION

As *N*-nitrosodimethylamine (NDMA) and *N*-nitrosodiethylamine (NDEA) are classified as probable human carcinogens, manufacturers must ensure that their manufacturing process does not generate such impurities. To allow manufacturers to make the necessary changes to their process, a transition period has been agreed by Competent Authorities and strict temporary limits on levels of these impurities introduced in the Test section.

CHARACTERS

Appearance: white or almost white, hygroscopic powder.

Solubility: practically insoluble in water, freely soluble in anhydrous ethanol, sparingly soluble in methylene chloride.

IDENTIFICATION

Carry out either tests A, B or tests A, C.

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *valsartan CRS*.

B. Enantiomeric purity (see Tests).

C. Specific optical rotation (*2.2.7*): – 69.0 to – 64.0 (anhydrous substance).
Dissolve 0.200 g in *methanol R* and dilute to 20.0 mL with the same solvent.

TESTS

Nitrosamines. Carry out the test by a suitable method[(4)].

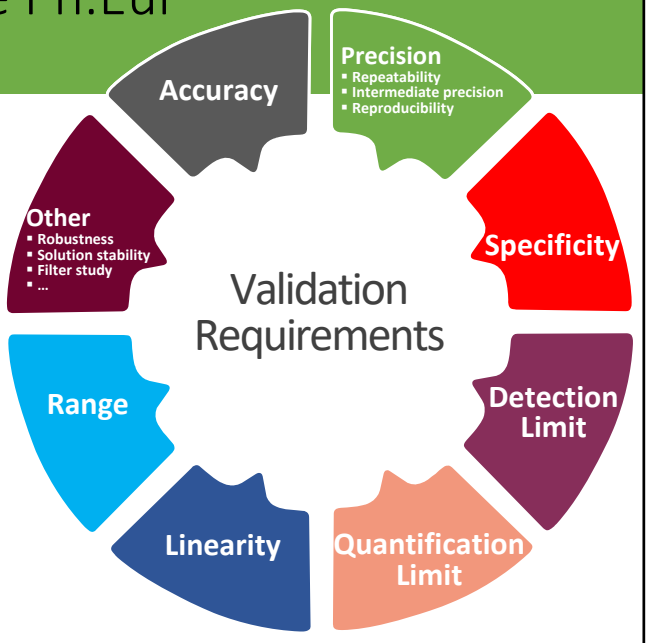
The substance to be examined does not contain either NDMA or NDEA above the limits provided below or both impurities at whatever level:

– *N-nitrosodimethylamine (NDMA)*: maximum 0.300 ppm;

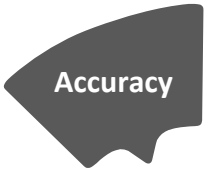
– *N-nitrosodiethylamine (NDEA)*: maximum 0.082 ppm.

Control of impurities in the Ph.Eur
Organic Impurities cont'd

- As per Technical Guide
- In line with ICH Q2

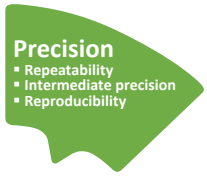


Organic impurities
Validation requirements cont'd



- … 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.
- … should be established **across the specified range** of the analytical procedure, using a **minimum of 9 determinations over a minimum of 3 concentration** levels covering the specified range (e.g. 3 concentrations/3 replicates each).
- … should be assessed **on samples spiked with known amounts of impurities**. In cases where it is impossible to obtain samples of certain impurities and/or degradation products, it is acceptable to compare results obtained by an independent procedure. The response factor of the drug substance can be used.

Organic impurities
Validation requirements cont'd



- … 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.
  - **Repeatability**: expresses the precision under the same operating conditions over a short interval of time. A minimum of 9 determinations covering the specified range for the procedure (e.g. 3 concentrations/3 replicates each) or a minimum of 6 determinations at 100 % of the test concentration.

⚠ Requirements apply for SST as per **General Chapter "Chromatographic separation techniques " 2.2.46.**

  - **Intermediate precision**: expresses variations within laboratories: different days, different analysts, different equipment, etc.. In case reproducibility has been performed, this is not needed.
  - **Reproducibility**: expresses the precision between laboratories (collaborative studies, usually applied to standardisation of methodology).

Organic impurities

Validation requirements cont'd

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.
- If an analytical procedure is not specific for a particular analyte, a combination of 2 or more analytical procedures is needed.
- This definition has the following implications:
  - **Identification:** to ensure the identity of an analyte, described in the **"Identification of impurities" paragraph** typically by the **use of Chemical Reference Substance (CRS) for system suitability**. **Retention times and relative retentions times in a monograph are given for information only!**
  - **Purity tests**: to ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc.
  - **Assay** (content or potency): to provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample.

Organic impurities

Validation requirements cont'd

Specificity

- **…** results **in combination with** the **requirements** described **in the General Chapter "Chromatographic separation techniques "** 2.2.46. **important SST criteria are derived, e.g:**
- **Symmetry Factor (A_s):**
  - is 0.8 to 1.5 (unless otherwise prescribed) for a peak in the reference solution used for quantification.
- **Resolution (R_s):**
  - The resolution calculated by using the half-height of the peaks.
- **Peak-to-valley (*p/v*) ratio:**
  - when complete separation between 2 adjacent peaks cannot be achieved, i.e. when the resolution factor is less than 1.5.
  - The peak-to-valley ratio should not be less than 1.5. Often even better separations are necessary to ensure a meaningful integration of impurity peaks.

Organic impurities

Combination of more analytical procedures is needed…

Specificity

Example: Rosuvastatin Calcium (2631)

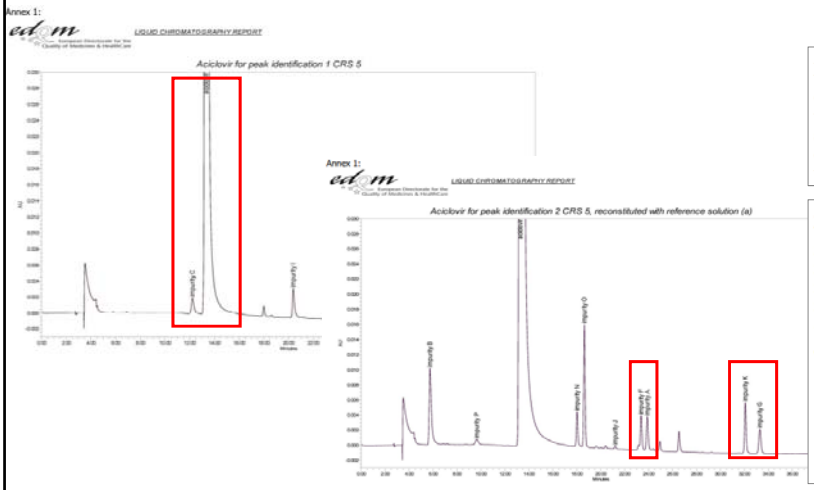
- **Enantiomeric purity (Impurity G)**
- **Impurity L**
- **Related substances**

Organic impurities

Identification and other SST criteria derived from…

Specificity

Example: Aciclovir (0968)



Identification of impurities: use the chromatogram supplied with *aciclovir for peak identification 1 CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities C and I; use the chromatogram supplied with *aciclovir for peak identification 2 CRS* and the chromatogram obtained with reference solution (d) to identify the peaks due to impurities A, B, F, G, J, K, N, O and P.

Relative retention with reference to aciclovir (retention time = about 13 min): impurity B = about 0.4; impurity P = about 0.7; impurity C = about 0.9; impurity N = about 1.37; impurities O and Q = about 1.42; impurity I = about 1.57; impurity J = about 1.62; impurity F = about 1.7; impurity A = about 1.8; impurities K and R = about 2.5; impurity G = about 2.6.

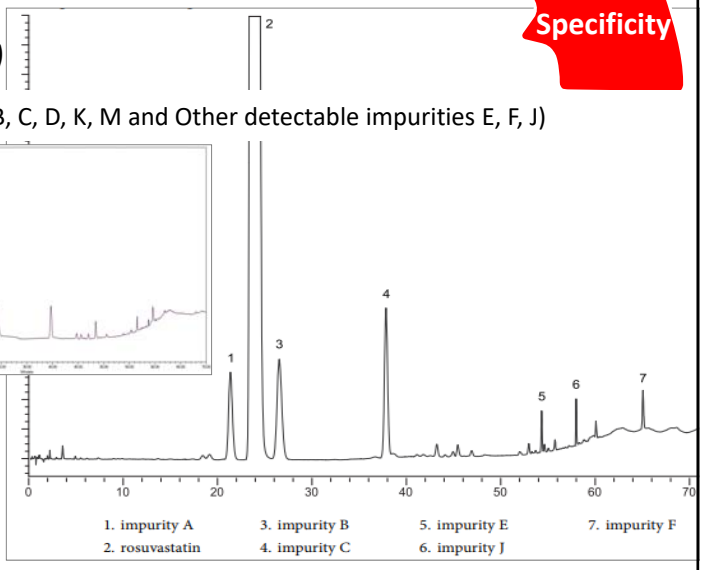
System suitability:

- *resolution*: minimum 1.5 between the peaks due to impurity C and aciclovir in the chromatogram obtained with reference solution (c); minimum 1.5 between the peaks due to impurities F and A and minimum 1.5 between the peaks due to impurities K and G in the chromatogram obtained with reference solution (d).

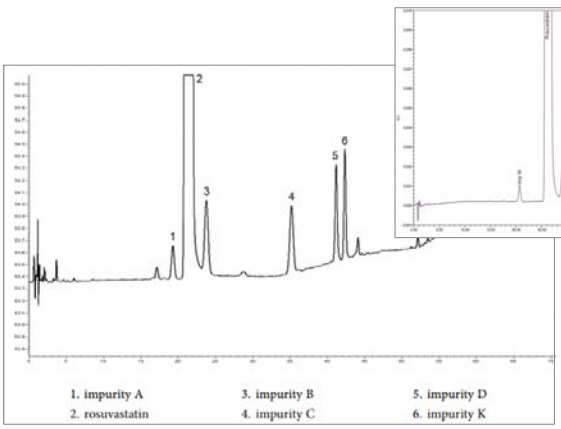
Organic impurities
Identification…

Specificity

Example: Rosuvastatin Calcium (2631)



Related substances (Specified impurities A, B, C, D, K, M and Other detectable impurities E, F, J)

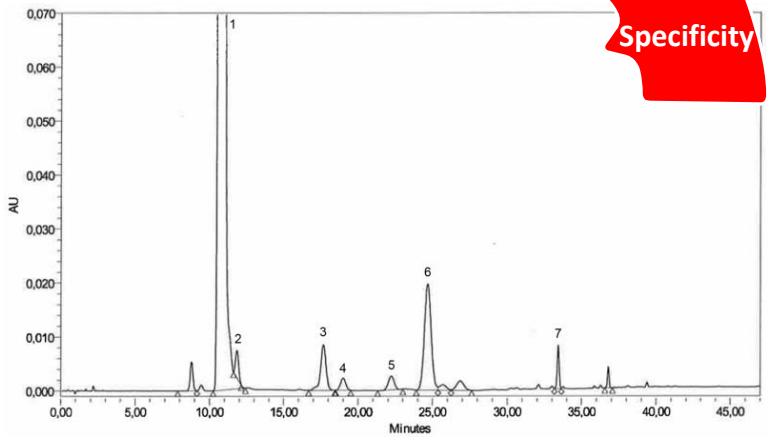


Organic impurities
Identification…

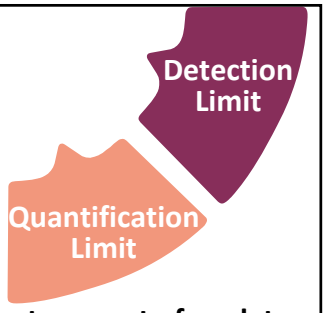
Specificity

Example:
Rosuvastatin Tablets (3008)

Related substances
Specified (impurities) degradants C, D, FP-A



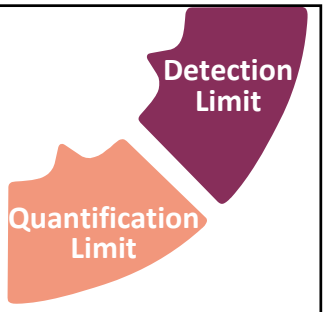
Organic impurities
Validation requirements cont'd



- The **detection limit** of an individual analytical procedure is **the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value**.
- 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**.
- Several approaches are possible:
  - visual evaluation,
  - signal-to-noise (S/N ratio),
  - standard deviation of the response and the slope of the calibration curve.
- **Quantification Limit must be ≤ than the reporting threshold (disregard limit).**
- **S/N ratio ≥ 10** at the reporting threshold (disregard limit) apply for SST as per **General Chapter "Chromatographic separation techniques " 2.2.46.**
  Additional sensitivity criterion may be necessary, especially in case of low responding impurities.

Organic impurities
SST criteria derived from…

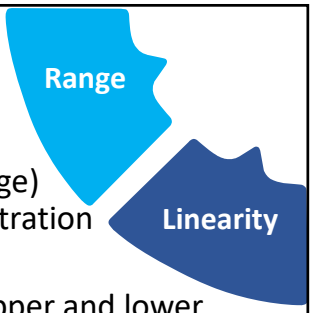
Example:
Ascorbic acid (0253)



System suitability:
- *resolution*: minimum 3.0 between the peaks due to ascorbic acid and impurity C in the chromatogram obtained with reference solution (c);
- *signal-to-noise ratio*: minimum 20 for the peak due to impurity C in the chromatogram obtained with reference solution (b).

Limits:
- *impurities C, D*: for each impurity, not more than 1.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the peak due to ascorbic acid in the chromatogram obtained with reference solution (b) (0.10 per cent);
- *sum of impurities other than C and D*: not more than twice the area of the peak due to ascorbic acid in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *disregard limit*: 0.5 times the area of the peak due to ascorbic acid in the chromatogram obtained with reference solution (b) (0.05 per cent).

Organic impurities
Validation requirements cont'd



- **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.
- 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.
- A minimum of **5 concentrations** is recommended to be included. **For the determination of an impurity/degradant: from QL or from 50 % of the specification of each impurity/degradant, whichever is greater, to 120 % of the specification.**
- It is also essential to demonstrate the similarity of **response of the substance** and **known impurities, to establish Response- and Correction Factors for the Calculation of impurity content**.

Organic impurities
Validation requirements cont'd

- … shows the reliability of an analysis with respect to deliberate variations in method parameters. The evaluation depends on the type of procedure under study and is preferably considered during the development phase.
- In the case of LC, typical variations are: influence of variations of pH in a mobile phase; -mobile phase composition; different columns (different lots and/or suppliers); temperature; flow rate; stability of solutions.
- If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled and/or a precautionary statement should be included in the procedure.
- A series of system suitability parameters is to be established to ensure that the validity of the analytical procedure is maintained whenever used.

Organic impurities
Validation requirements cont'd

Other
- Robustness
- Solution stability
- Filter study
- …

Example: Rosuvastatin Tablets (3008)

- **Special instructions are given in the individual monograph due to**
  - stability of solutions

TESTS

Related substances. Liquid chromatography (*2.2.29*). *Carry out the test protected from light. Prepare the solutions immediately before use.*

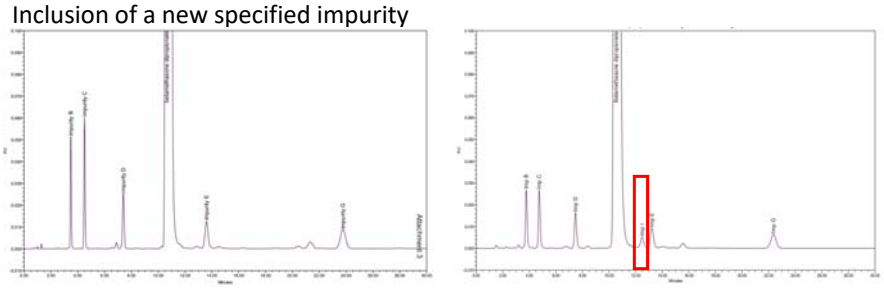
  - critical filter parameter

Dissolution (*2.9.3, Apparatus 2*).

Test solution. The samples withdrawn from the dissolution vessel and filtered, using a polyvinylidene fluoride filter[4].

Organic impurities
Validation requirements cont'd

- Revision of Related substance test:
  - Inclusion of a new specified impurity



- What (re)validation is required?
  - If the original validated method is maintained -> partial revalidation regarding only the new impurity (specificity, sensitivity, linearity, response factor, precision, robustness).
  - If the original validated method needs to be modified or a specific new method is needed -> full validation will be required.

Organic impurities

Calculation of impurity content and Limits

- **If the response factor of an individual impurity is < 0.8 or > 1.2 correction factor (CF)** (reciprocal value of the response factor) **or individual impurity as an external standard (if the CF value to be applied is >5) must be applied when the proposed limit is 0.1 % or greater.**
- For the response factor determination the **purity of the substances and the salt forms must be considered.**
- **Limits are based on**:
  - **normal analytical errors and acceptable variations in manufacturing, compounding,**
  - **deterioration to an acceptable extent (stability considerations),**
  - **qualified/approved specification levels, which might become more stringent if NOT supported by actual batch data.**
- **Calculation Option 1: External calibration**
  - **Dilution of the test solution -> the preferred methodology by the Ph.Eur.,**
  - Using an **impurity standard.**
- **Calculation Option 2: Peak area normalization**

Organic impurities

Calculation of impurity content | Option 1: Dilution of test solution

Example: Diclofenac Sodium (1002)

Test solution. Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a). Dilute 2.0 mL of the test solution to 100.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Reference solution (b). Dissolve the contents of a vial of *diclofenac for system suitability CRS* (containing impurities A and F) in 1.0 mL of the mobile phase.

Calculation of percentage contents:

- *correction factors*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.7; impurity F = 0.3;
- for each impurity, use the concentration of diclofenac in reference solution (a).

Limits:

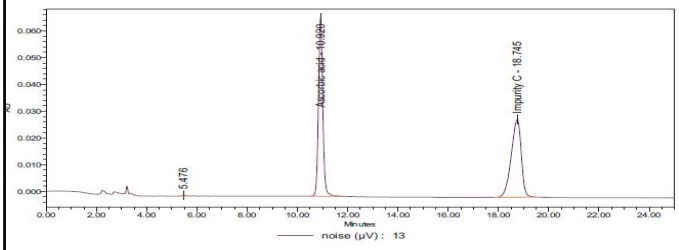
- *impurity A*: maximum 0.2 per cent;
- *impurity F*: maximum 0.15 per cent;
- *unspecified impurities*: for each impurity, maximum 0.10 per cent;
- *total*: maximum 0.4 per cent;
- *reporting threshold*: 0.05 per cent.

Organic impurities

Calculation of impurity content | Option 1: Impurity standard

Example: Ascorbic acid (0253)

Solution containing 8x more Impurity C than ascorbic acid



Peak Results

	Name	RT	RT Ratio	Area	% Area	Height	Resolution	S/N	Int Type
1	Ascorbic acid	10.920		805026	49.89	69675	21.42	10258	BB
2	Impurity C	18.745	1.72	805706	49.93	29006	15.09	4463	BB

Test solution. Dissolve 0.500 g of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (a). Dissolve 10.0 mg of *ascorbic acid impurity C CRS* in the mobile phase and dilute to 5.0 mL with the mobile phase.

Reference solution (b). Dissolve 5.0 mg of *ascorbic acid impurity D CRS* and 5.0 mg of *ascorbic acid CRS* in the mobile phase, add 2.5 mL of reference solution (a) and dilute to 100.0 mL with the mobile phase.

Reference solution (c). Dilute 1.0 mL of the test solution to 200.0 mL with the mobile phase. Mix 1.0 mL of this solution with 1.0 mL of reference solution (a).

Organic impurities

Calculation of impurity content | Option 2: Peak area normalization

Example: Aciclovir (0968)

Test solution. Dissolve 25 mg of the substance to be examined in 5.0 mL of *dimethyl sulfoxide R* and dilute to 25.0 mL with *water R*.

Reference solution (a). Dissolve 5 mg of *aciclovir for system suitability CRS* (containing impurities A, B, J, K, N, O and P) in 1 mL of *dimethyl sulfoxide R* and dilute to 5.0 mL with *water R*.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (c). Dissolve the contents of a vial of *aciclovir for peak identification 1 CRS* (containing impurities C and I) in 200 µL of *dimethyl sulfoxide R* and dilute to 1.0 mL with *water R*.

Reference solution (d). Dissolve the contents of a vial of *aciclovir for peak identification 2 CRS* (containing impurities F and G) in 1.0 mL of reference solution (a).

Limits:

- *correction factor*: for the calculation of content, multiply the peak area of impurity I by 1.5;
- *impurity B*: not more than 7 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.7 per cent);
- *sum of impurities O and Q*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);
- *sum of impurities K and R*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *impurities A, G, J, N, P*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
- *impurities C, F, I*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent);
- *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent);
- *total*: not more than 15 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent);
- *disregard limit*: 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.03 per cent).

Control of impurities in the Ph.Eur
Inorganic-/Elemental Impurities

Control of impurities in the Ph.Eur
Inorganic-/Elemental Impurities

- **Inorganic impurities** (inorganic salts, residues of processing aids/reagents, heavy metals) and other residual **elemental impurities** (metal catalysts or -reagents) are controlled by the following tests:
  - **Sulfated ash** (General Chapter **2.4.14.**).
  - **Heavy metals** (General Chapter **2.4.8.**, for substances and products for veterinary use only).
  - **Specific tests** (AAS, AES, ICP-AES, ICP-MS) **for elemental impurities.**
- **Principles are aligned with ICH Q3D** and apply to human medicinal products and thus **Individual Monographs of substances for pharmaceutical use (with the exception of substances for veterinary use) do NOT contain the requirement for elemental impurities, unless otherwise specified.**
- The requirements are given in:
  - General Monograph "**Substances for pharmaceutical use (2034)**"
  - General Monograph "**Pharmaceutical preparations (2619)**"
  - General Chapter **5.20.** "**Elemental impurities**"
  - General Chapter **2.4.20.** "**Determination of elemental impurities**"

Control of impurities in the Ph.Eur
Residual Solvents

Control of impurities in the Ph.Eur
Residual Solvents

- **Principles are aligned with ICH Q3C** and the requirements are given in:
  - General Monograph "**Substances for pharmaceutical use (2034)**".
  - General Monograph "**Pharmaceutical preparations (2619)**".
  - General Chapter **5.4.** "**Residual solvents**".
  - General Chapter **2.4.24.** "**Identification and control of residual solvents**".

- **All active substances, excipients and medicinal products are subject to test for residual solvents, if a solvent is used during its manufacture, even when the Individual Monograph does NOT specify this test.** 

Control of impurities in the Ph.Eur
Residual Solvents cont'd

- **Only Class 3 solvents** used **AND limit ≤ 0.5%** → test for **Loss on drying**.
- **Only Class 3 solvents** used **AND limit > 0.5%** or when **Class 2 or 1 solvents** are used → **specific test** is needed, preferably as per **General Chapters 2.4.24.** "**Identification and control of residual solvents**" by **gas chromatography with static head-space injection (2.2.28)** or other suitable validated method.
- When a quantitative determination of a residual solvent is performed and Loss on drying is not tested, the result is taken into account for the calculation of the assay content of the substance, the specific optical rotation and the specific absorbance.

Control of impurities in the Ph.Eur.
The user perspective

Control of impurities in the Ph.Eur.
The user perspective ⚠

- **Compliance with all applicable texts** (General Notices, -Chapters, -Monographs) **of the Ph.Eur.** is required.
- **Compliance with the individual monograph** by the user may be established **by carrying out only the tests relevant to the known impurity/degradant profile for the source of the substance/product**.
- **If** the **Individual Monograph does not provide suitable control for a new impurity**, a **suitable test** for control **must be developed/validated and included in the specification** of the substance/product **by the manufacturer**.
- **Unless otherwise stated**, **validation** of the test methods **by the user is NOT required**, **only verification**.

THANK YOU!

Q & A

19-20 June 2019, Strasbourg

Challenges linked to Control of Antibiotics – Industry View

Dr. Jan W.H. Smeets

Director Regulatory Affairs

CENTRIENT Pharmaceuticals

About Centrient Pharmaceuticals

- We are a leading manufacturer of beta-lactam antibiotics, and a provider of next generation statins and anti-fungals
- We produce and sell intermediates, active pharmaceutical ingredients (APIs) and finished dosage forms (FDFs)
- Quality, Reliability and Sustainability shape how we do things as a company
- Our world-leading proprietary enzymatic technology ensures an unmatched eco-friendly production process for high-quality products
- Our backward-integrated global manufacturing footprint ensures security of supply

Content of presentation

1. Antibiotics, a diverse product group
2. Applicable Impurity guidelines for antibiotics
3. Challenges of expert group 7 to elaborate / update antibiotic Ph. Eur. monographs
4. Challenges for industry
5. Suggestions for improvements
6. Conclusions

Antibiotics, a diverse product group

Definition:
A drug used to treat bacterial infections.

Manufactured via different processes:

- *Fermentation*: benzyl penicillin, gentamicin, tobramycin, erythromycin, clarithromycin, kanamycin
- *Semi-synthesis*: amoxicillin, flucloxacillin, cefalexin, amikacin
- *Synthetic*: sulfonamides, quinolones, oxazolidinones, chloramphenicol

Vary from pure compounds till mixtures of structurally related substances:

- *Mono compounds*: e.g. most penicillins and cefalosporins. Assay normally expressed in %.
- *Mixtures*: e.g. gentamycin sulfate (aminoglycoside), tyrothricin/gramicidin (polypeptides). Assay normally expressed in IU/ mg.

Example: Gentamicin sulfate

Gentamicin sulfate:
(mixture with 5 main components produced by fermentation,
Micromonospora purpurea, Content in IU/mg)

A *composition method* defines the crude composition: C1 (25%-45%), C1a (10%-30%), and C2+C2a+C2b (35%-55%)

Next to composition method a *related substance method* is included:

Impurities A,B	NMT 3.0%
any other impurity	NMT 3.0%
total	NMT 10.0%
disregard limit	0.5%

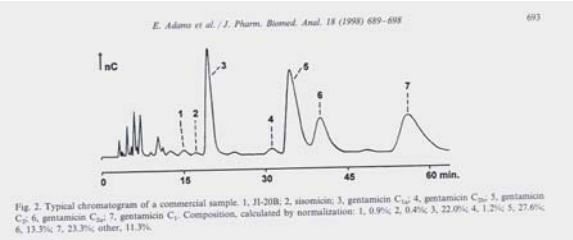


Fig. 2. Typical chromatogram of a commercial sample. 1, JI-20B; 2, sisomicin; 3, gentamicin C_{1a}; 4, gentamicin C_{2b}; 5, gentamicin C_2; 6, gentamicin C_{2a}; 7, gentamicin C_1. Composition, calculated by normalization: 1, 0.9%; 2, 0.4%; 3, 22.0%; 4, 1.2%; 5, 27.6%; 6, 13.3%; 7, 23.3%; other, 11.3%.

Which impurity guidelines apply for antibiotics?

Organic impurities:

- *ICH Q3A. Impurities in new drug substances.*
  Only antibiotics manufactured by chemical synthesis are within scope.
  Antibiotics produced by fermentation and semi-synthesis are out of scope.
- *Ph. Eur. Monograph: Substances for Pharmaceutical Use.*
  Enlarges the scope of ICH Q3A to existing substances, but semi-synthesis and fermentation are still out of scope.
- *USA: ANDAs: Impurities in drug substances.*
  Also increases ICH Q3A scope to existing drug substances; semi-synthesis and fermentation out of scope.
- *EMA Guideline on setting specifications for related impurities in antibiotics.*
  Applies to fermentation and semi-synthesis antibiotics APIs/products. New APIs and new sources of existing APIs.

EMA Guideline on setting specifications for related impurities in antibiotics

- This guideline has come into effect 30 June 2013.
- Scope: new active substances and new sources of existing substances

API thresholds human	Semi-synthetic*	Fermentation single	Fermentation family	Peptides
Reporting	0.05%/0.03%	0.10%	0.10%	0.1%
Identification	0.10%/0.05%	0.15%	0.15%	0.5%
Qualification	0.15%/0.05%	0.15%	0.50%**/0.2%	1.0%

* If the structure consists of a family of compounds, then thresholds for fermentation, family may be necessary

** Structurally closely related impurity according to definition

Which impurity guidelines apply for antibiotics (continued)?

Residual solvents:
ICH Q3C Guideline for residual solvents. Originally only for new API/products. Nowadays applicable also for existing API/Products. Applicable for antibiotics.

Mutagenic impurities:
M7 Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk. Fermentation APIs/products excluded.

Elemental impurities:
ICH Q3D on elemental impurities
Applicable for new and existing products. So fully applicable for antibiotics.

Impurity description for antibiotics in Ph. Eur. API monographs

- Many antibiotic monographs are currently under revision by Expert group 7. So the Ph. Eur. includes a mix of updated monographs according to current standards and some older style monographs.

- The impurity section of each **modern antibiotic monograph** consist of:
  a) Following *organic impurity specifications*:
  - *each specified impurity*
  - *any unspecified impurity*[*]
  - *total impurities*

  [*] *If impossible to reach guideline identification threshold we establish a higher threshold and name it "any other impurity".*

  b) *identification of each specified impurity*[#] by using:
  - relative retention times of each specified impurity (identified and unidentified)
  - impurity reference standards which consist preferably of all specified impurities. This can be separate impurities, mix of impurities, and/or "dirty batch". In latter case correction factors are needed for quantitation purposes.
  - *In situ* degradation
  - LC-MS techniques
  -"fingerprint chromatogram" for very complex impurity profiles

  [#]*combination of techniques is possible*

Impurity description for antibiotics in Ph. Eur. API monographs

- **Some remarks:**
  - Impurity CRS should be available before revised/new monograph will come into force.
  - Goal is to have all impurities which are present in approved sources of the EU market in one impurity method.

- **Old monographs exist in different style (or combinations of below):**
  - Sometimes only one spec for all impurities. For instance "all impurities 1%"
  - no relative retention times available
  - no spec for "any other impurities"
  - no total impurities
  - no impurity CRS or other mean to identify impurities available
  - no correction factors

Challenges for the antibiotic expert group 7 of the Ph. Eur. to modernize the antibiotic monographs

- To develop one impurity method which covers impurities of all manufacturers. Multiple manufacturers with different processes result in multiple process impurities, which might coelute. Method might be difficult to handle if the number of impurities becomes (too) high.
- It is very complicated to establish a limited number impurity CRSs if impurities are coming from multiple manufacturers. Consequence might be multiple impurity standards for one method.
- Synthesis or isolation of impurities can be difficult or impossible, especially when multiple isomers can be formed. Determination of correction factors might not be possible.
- Difficulty to meet thresholds of the EMA antibiotic impurities guideline as they are very strict. Moreover the low LOD (should be below reporting threshold) requested by this guideline is not always manageable (for instance for methods using electrochemical detection).

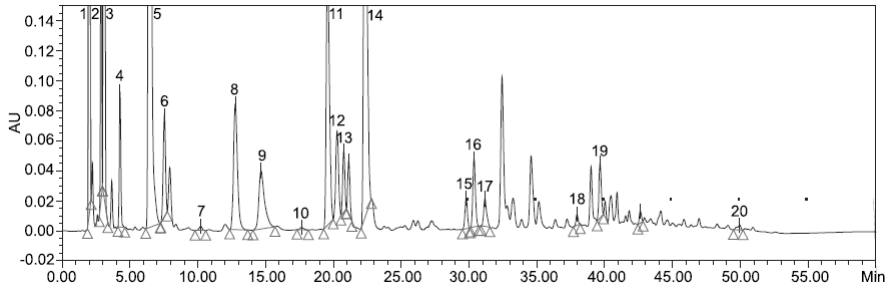
Amoxicillin as an example to demonstrate the challenges of Expert group 7.

- Current Amoxicillin monograph has one general limit for all impurities of 1%, no RRTs, no correction factors no impurity CRS
- Many manufacturers with different manufacturing processes (chemical/enzymatic)
- EMA thresholds(>2g/day):

reporting threshold	0.03%
identification threshold	0.05%
qualification threshold	0.05%

- Group 7 published a method in Pharmeuropa 26.2 (2014) which had a any other impurity spec of 0.15 %. This method consisted of 20 specified impurities and 24 peaks (one unknown, some coeluting). It appeared unworkable to prepare an impurity CRS. In November 2016 the Ph. Eur commission adopted a method in which the spec for any other impurities raised to 0.3%. This reduced the number of specified impurities to 8 (11 peaks). Preparation of CRS is still in progress.

Amoxicillin as an example to demonstrate the challenges of the expert group.



How does USP handle impurity methods in API monographs?

- USP published sometimes multiple impurity methods in one monograph and advises users to choose that method which has the impurity profile that correlates to relevant manufacturing process.
  For instance ampicillin monograph consist of 3 different impurity methods with 4 different specifications (one method has different specs for human and veterinary application).

- The disadvantage of such an approach is that:
  - sometimes same impurities have different specifications in different methods
  - many drug manufacturers request testing multiple methods as they want to be sure that impurities in other method(s) are not in the API and request to use both methods
  - manufacturers might have different manufacturing processes which can lead to an impurity profile which contains impurities of multiple USP monograph methods.

Challenges for industry

- Even though Ph. Eur. expert group 7 aims to include impurity specs in antibiotic monographs as close as possible to the EMA guideline requirements they sometimes are (slightly) more relaxed. This in fact leads to **2 sets of specifications.**
  Regulatory authorities often request the strictest of the 2 which is not always possible.

- Final dosage form manufacturers using the API and some Regulatory authorities **request impurity standards especially those which are not available from Pharmacopoeias.** This means that API manufacturers need to synthesize/isolate large amounts of impurities which is often very difficult and costly or not possible at all.
  Moreover monographs include impurities coming from different processes and some of them are not formed in a some processes. Despite of this, API manufacturers often are requested to supply these impurities and demonstrate absence in API.

Challenges for industry

- Different CRSs for same API in different countries can lead in certain cases to **different potencies** for the same API.
  Example nystatin: Because of different reference standards for Nystatin in Ph. Eur. and USP my company had for some time a remarkable difference in potency for nystatin API in EU and USA.

- In old monographs sometimes **wording is not clear** leading to confusion:
  Example ampicillin or amoxicillin:
  Any impurity: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1 per cent).
  Some impurities consist of multiple diastereomers (like penicilloic acids or penilloic acids). Question: is each peak (each diastereomer) 1 per cent or the sum of each diastereomer is 1 per cent?
  In new updated monographs the wording “the sum of” is used to prevent misunderstandings.

Challenges for industry

- In past years expert group 7 had **replaced the microbiological assay (IU/mg) by HPLC (%)** in some monographs.
  In some cases industry had big problems with this switch, because of **dosing of the Dosage Form in International Units.** The correlation between units and % was not always easy to make.
  For polymyxin monograph (mixture of polypeptides) the microbial assay has recently re-instated by expert group 7 because of this problem. The former assay method was converted to a test for composition.
  In future group 7 will only switch to HPLC assay when purity >90% and if there is a known correlation between IU and %.
  Examples of complex antibiotics which kept microbiologic assay are bacitracin, colistin (both mixtures of polypeptides) and tylosin (mixture of macrolides).
  For those complex antibiotic mixtures which often can not meet thresholds of impurity guideline, following analytical methods are included in monograph:
  - microbial assay
  - composition (normalization procedure)
  - related substance method

Challenges for industry

- General (also valid for non-antibiotic APIs) impurity challenge:
  Increase of number of manufacturing steps in registration files as a result of starting material definitions has resulted in extensive impurity carry-over discussions. Challenge for API industry is to **receive all detailed confidential process info from suppliers of starting materials and intermediates** which are purchased.

- **Non-harmonization:**
  - Pharmacopoeias are not harmonized on API monograph level resulting in:
  a) different or additional analytical methods and acceptance criteria for different markets or
  b) extensive cross-validation efforts required
  - Guideline for antibiotic impurities only exists in Europe, however other countries request compliance to this guideline (e.g. China).

Some suggestions for improvements

- Instead of trying to include all process related impurities from all processes in one related substance method we could try for complicated and multi source APIs to only include the decomposition impurities of the API which are equal for all processes. All specified process impurities should be identified and qualified by each manufacturer. This could keep the analytical method easier to handle and avoids demonstrating absence of impurities which cannot be formed by the specific process and makes it easier to prepare impurity CRSs.
- Increased harmonization of API monographs between major pharmacopoeias. This would avoid many cross-validations and or using different analytical methods for different markets.
- Harmonise reference standards for APIs and impurities between the major pharmacopoeias. This would save a lot of money and testing efforts. Moreover the possibility of having different assays for different markets would be diminished.

Conclusions

- Antibiotics form a diverse group of compounds with regard to purity and way of manufacturing.
- Expert group 7 of the Ph. Eur. is aiming to constantly improve the quality of the antibiotic monographs and align the content with the approved quality currently on the market.
- Full compliance with the thresholds of the EMA impurity guideline for antibiotics is not always possible.
- Current challenges for both developers of the monographs and industry that has to use these monographs have been discussed and some suggestions for future improvements were given.

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Challenges linked to the control of antibiotics: the EU Antibiotics Guideline and its impact on dossiers and assessments

Dr. Uwe Lipke

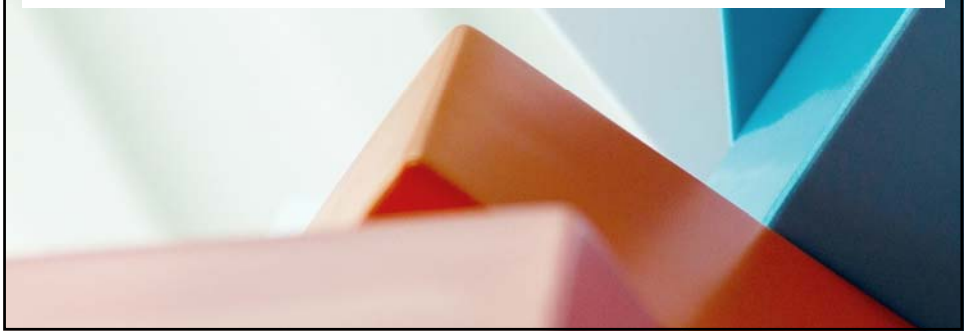

Table of Content

Guideline on setting specifications for related impurities in antibiotics (EMA/CHMP/CVMP/QWP/199250/2009 corr) Situation before implementation

- peptides, fermentation products, and semi-synthetic substances were not covered by ICH Q3A or by the EP monograph "substances for pharmaceutical use" regarding control of related substances
- consequences:
  - no thresholds for impurities (identification, qualification)
  - case-by-case evaluation and assessment
  - different acceptance criteria for the same API / same product possible
- several monographs had acceptance criteria for „any other impurity" of e.g. 2.0 % or higher
- qualification of impurities is not possible based on the limit for "any other impurity": unknown means unknown

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Challenges for control of related impurities in antibiotics

- borderline between active component and impurity not well defined – some „impurities" may show relevant therapeutic activity
- complex impurity profiles lead to challenges for the analytical procedures to control them
- fermentation processes may lead to variable impurity profiles from batch to batch due to the inherent variability of biological systems
- there is a wide variety of impurity profiles – from clear defined like for chemical substances to nearly chaotic meaning that the ICH Q3A concept of reporting, identification, and qualification thresholds is almost not applicable
- impurity profiles of multi-source antibiotics may differ significantly making establishment of a monograph a very difficult task

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Intentions of the guideline

- Guidance on setting specifications for related impurities in antibiotics that are fermentation products or semi-synthetic substances derived from fermentation products
- Guidance on content and qualification of related impurities in both active substances and in medicinal products
- Defining thresholds for reporting, identification, and qualification of impurities dependent on classification of antibiotics as:
  - manufactured by semi-synthesis
  - manufactured by fermentation, single compound
  - manufactured by fermentation, family of compounds
  - peptides manufactured by fermentation or semi-synthesis (only peptides without further functional groups)
  - veterinary use only
  - special cases with very complex impurity profiles
- Guidance on special cases using descriptive specifications (section 5.6; annex 3)
- Establish best practice and initiate revisions of relevant EP monographs

Overview over Thresholds of the Guideline (API only)

Production	Single/ family	Reporting threshold %	Identification threshold %	Qualification threshold %	Remark
Semi-synthetic	Single	0.05/0.03	0.10/0.05	0.15/0.05	same as ICH Q3A; 2nd value for maximum daily dose ≥ 2g/day
	Family	0.10	0.15	0.50/0.2	0.50 % is for structurally closely related impurities only
Fermen-tation	Single	0.10	0.15	0.15	
	Family	0.10	0.15	0.50/0.2	0.50 % is for structurally closely related impurities only
Peptides	n/a	0.1	0.5	1.0	only for peptides without any additional functional group
Veteri-nary only	n/a	0.10	0.20	0.50	if manufactured by fermentation and consisting of a family of compounds, threshold is assessed case-by-case
Special cases		case-by-case			descriptive specifications for very complex impurity profiles

Special cases for very complex impurity profiles: „fingerprint chromatogram“ approach

Very complex impurity profiles for which identification of individual peaks is impossible should at least be characterised by a descriptive specification based on a sufficient high number of manufactured batches. A descriptive specification may consist of the following parameters:

- Limitation of the number of peaks (if applicable: as a range) and their corresponding contents (as a sum) occurring within a predefined, narrow RRT-window.
- Relative specification limits in a predefined RRT-window (e.g. at least one peak between RRT x and y with content between A and B %).
- Limitation of the number of peaks occurring above a threshold in a predefined RRT-window (e.g. any individual peak between RRT w and z not more than C %, but not more than one peak above D % whereby C is greater than D, e.g. C = 2.0 %, D = 1.5 % or similar)

Characterisation necessary for differentiation of qualified from non-qualified impurity profiles

Examples: monographs for vancomycin or teicoplanin

Specifications before implementation of the guideline

e.g. cefaclor (first generation cephalo-sporine; semi-synthetic; single compound)

Related substances (% w/w)	
Highest individual unknown related substance	NMT 0.2
Highest individual known related substance	NMT 0.5
Total related substances	NMT 2.0

results cefaclor

RELATED SUBSTANCES		
- Maximum individual related substance	0.11 % w/w	NMT 0.5 % w/w
- Total related substances	0.11 % w/w	NMT 2.0 % w/w

e.g. vancomycin (glycopeptide; fermentation product; family of compounds)

Vancomycin B	Not less than 93.0%
Largest Individual Related Substance	Not more than 3.0%
Total Related Substances	Not more than 7.0%

results vancomycin

Chromatographic Purity		
Vancomycin B (NLT 93.0%)	93.2	93.3
Largest Individual Related Substance (NMT 3.0%)	0.9	1.0
Total Related Substances (NMT 7.0%)	6.8	6.7

Specifications after implementation of the guideline (1)

e.g. cefaclor

- 3-Cl-7-ACCA	NMT 0.5
- Delta-3-Cefaclor	NMT 0.5
- Any known impurity (EP impurities)	NMT 0.5
- Any unspecified impurity	NMT 0.1
- Total related substances	NMT 2.0

Specifications after implementation of the guideline (2)

e.g. vancomycin

Specification			Results
Name	**rRT**	**Limit**	
Peaks ahead of vancomycin B (rRT with reference to Vancomycin B)			
Impurity vb1	0.17-0.20 (VanB)	≤ 1.5 %	0.10%
Impurity vb2	0.27-0.31 (VanB)	≤ 1.5 %	0.08%
Impurity vb3	0.43-0.47 (VanB)	≤ 1.5 %	0.31%
Impurity vb4	0.51-0.55 (VanB)	≤ 1.5 %	0.09%
Impurity vb5	0.56-0.60 (VanB)	≤ 1.5 %	1.11%
Impurity vb6	0.66-0.68 (VanB)	≤ 1.5 %	n.d.
Impurity vb7	0.69-0.71 (VanB)	≤ 1.5 %	n.d.
Impurity vb8	0.73-0.76 (VanB)	≤ 1.5 %	0.42%
Impurity A N-demethylvancomycin B	0.78-0.80 (VanB)	≤ 4.0 %	0.26%
Impurity vb9	0.89-0.94 (VanB)	≤ 1.5 %	n.d.
Peaks after Vancomycin B (rRT with reference to Desamidovancomycin B)			
Impurity da1	0.63-0.65 (Desam.)	≤ 1.5 %	n.d.
Impurity da2	0.75-0.82 (Desam.)	≤ 1.5 %	0.15%
Impurity da3	0.83-0.87 (Desam.)	≤ 1.5 %	n.d.
Impurity D Desvancosaminylvancomycin B	0.95-0.98 (Desam.)	≤ 4.0 %	1.36%
Impurity B Desamidovancomycin B	approx. 1.00 (Desam.)	≤ 4.0 %	0.26%
Impurity da4	1.06-1.09 (Desam.)	≤ 1.5 %	n.d.
Impurity C Aglucovancomycin B	1.10-1.12 (Desam.)	≤ 4.0 %	n.d.
Impurity da5	1.22-1.24 (Desam.)	≤ 1.5 %	0.08%
Unspecified impurities, single		≤ 0.5 %	n.d.
Total impurities		≤ 7.0 %	4.41%

However, what we really get to see in a dossier depends mainly on the relevant EP monograph in force at the time of submission.

Revision of EP Monographs: Cefaclor vs. Vancomycin

CEFACLOR

Limits:

- *any impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

VANCOMYCIN HYDROCHLORIDE 07/2019

Limits:

- *vancomycin B*: minimum 91.0 per cent;
- *impurities A, H*: for each impurity, maximum 3.0 per cent;
- *sum of impurities B and E*: maximum 2.0 per cent;
- *impurity J*: maximum 1.6 per cent;
- *impurities D, F, M*: for each impurity, maximum 1.5 per cent;
- *impurities G, I, K*: for each impurity, maximum 1.2 per cent;
- *impurity C*: maximum 1.0 per cent;
- *any other impurity eluting before vancomycin B*: for each impurity, maximum 0.8 per cent, and not more than 5 such impurities exceed 0.30 per cent;
- *any other impurity eluting after vancomycin B*: for each impurity, maximum 0.8 per cent, and not more than 3 such impurities exceed 0.30 per cent;
- *total of impurities*: maximum 9.0 per cent;
- *reporting threshold*: 0.10 per cent.

before (until EP 9.7)

Limits:

- *any impurity*: for each impurity, maximum 4.0 per cent;
- *total*: maximum 7.0 per cent;
- *disregard limit*: the area of the principal peak in the chromatogram obtained with test solution (c) (0.1 per cent).

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Impact on monographs and CRS strategy

Challenges for smooth revisions of monographs: antibiotic reference substances

- many antibiotics are susceptible to humidity leading to increased degradation
- almost all antibiotics are multi-source substances with distinct but different impurity profiles depending on the actual manufacturer
- monographs for multi-source substances must identify all specified impurities in the chromatogram of the related substance test
- in order to get suitable CRSs identifying all specified impurities, samples of the API of different origin and/or individual impurity standards have to be mixed
- since dry mixing does not result in homogeneous mixtures, different samples and substances are usually dissolved, mixed, and evaporated/dried to produce CRS for the system suitability test – however, antibiotic reference standards may degrade to an unpredictable extent during this procedure

⇨ it may be necessary to have more than one CRS for the system suitability test in order to identify all specified impurities

⇨ difficult logistics, high costs for routine analysis, and life-cycle of CRS not assured

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EP monograph for amoxicillin trihydrate

Current specification:

- *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1 %)
- *Total impurities:* not specified
- *reporting threshold:* not defined

Proposed specification (more than 5 years ago):

- *impurity E (sum of isomers E1, E2)*: maximum 1.5 %
- *impurities C (sum of isomers C1, C2), D (sum of isomers D1, D2), G, I, J*: for each impurity, maximum 1.0 %
- *impurity L:* maximum 0.5 %
- *impurity N:* maximum 0.4 %
- *impurities A, B, H, K, M, O, R, S, T:* for each impurity, maximum 0.3 %
- *sum of impurities F, P, and Q:* for each impurity, maximum 0.3 %
- *any other impurity:* maximum 0.3 % or 0.15 %
- *total:* maximum 3.5 %
- *reporting threshold:* 0.05 %

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CRS for amoxicillin – current challenges

- Impurity profile for each source is not very complex and separation is achievable with normal RP-HPLC column; even a limit of NMT 0.10 % for any unspecified impurity would be achievable and is given in CEPs; typical batch results are between below 0.05 % and 0.08 %
- Amoxicillin is a multi-source antibiotic and highly susceptible to degradation by humidity – dissolution, mixing, and evaporation for getting only one suitable CRS for the SST is not feasible
- Taking into account all sources, about 20 impurities need to be specified if a limit of NMT 0.15 % would be applied as several manufacturers claim presence of their impurities up to 0.3 % in amoxicillin
- Several of these impurities are specific for the actual synthesis performed (e.g. esters from solvents used in the synthesis)
- Acceptance criterion for any other impurity (NMT 0.15 % or NMT 0.3 %) influences the number of required CRS (6 or 4, respectively) as nine additional impurities have to be identified in the chromatogram

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Monograph for amoxicillin – Quality Assessors proposal

1. To prepare a monograph which reflects the actual purity of amoxicillin on the market.
2. To specify impurities which are at a level above 0.3 %.
3. To provide CRSs for the specified impurities (>0.3 %) including for those needed for the system suitability test.
4. To add a production section with the following wording:

   There might be impurities at a level between 0.15 and 0.3 % potentially present in the material of some suppliers but not necessarily listed under specified impurities. It will then be the responsibility of the manufacturer (applicant) to identify these impurities (for instance from the list "Any other impurities" and to propose a limit; it will be then considered as a specified impurity for this particular supplier but not specified in the monograph.

However, this proposal was not accepted by the EDQM Presidium: monograph should be usable independent of reference to a manufacturer

Why do we need differentiated specifications for antibiotics?

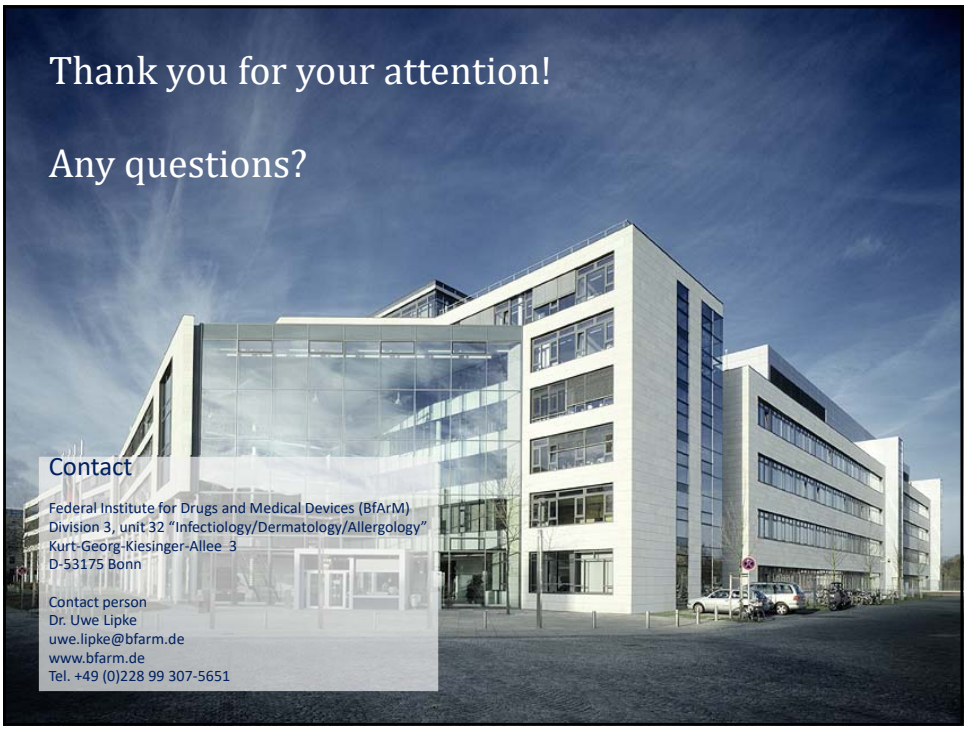
- Global market situation calls for cheaper APIs.
- More and more antibiotics are manufactured in China and/or India only, sometimes with different fermentation and syntheses processes than in Europe.
- Traditional fermentation or synthesis processes are „improved" focussing on price only (see Valsartan case).
- This may lead to unexpected changes in the impurity profiles even for well-known antibiotics marketed for decades.
- It is not comprehensible why for antibiotics manufactured by fermentation or semi-synthesis a lower safety level shall apply as for chemically synthesised substances.

⇨ Therefore, it is necessary to effectively control the impurity profile acknowledged as being qualified-by-use.

- High limits for any other impurity would simplify routine control of antibiotics but jeopardise effective control of the actual impurity profile of a certain batch.
- For this reasons, descriptive specifications are recommended in the Antibiotics guideline for complex impurity profiles where individual limits are not feasible.

Thank you for your attention!

Any questions?



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THE EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES & HEALTHCARE (EDQM)

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Experience of implementation of ICH Q3D within the Certification Procedure (CEPs)

Mr Cristian Sampaolesi
Certification of Substances Department, EDQM

Elemental impurities - ICH Q3D

ICH Q3D effective in the European Union from June 2016 for new marketing authorisation applications and from December 2017 for authorised medicinal products

EDQM (in the context of the Certification Procedure) does not make a decision on compliance with ICH Q3D

The CEP provides transparency, to be considered by the manufacturer of medicinal product in the context of a MAA

Elemental impurities - PA/PH/CEP (16) 23, 1R

PA/PH/CEP (16) 23, 1R published in July 2018

- Publicly available on the EDQM website
- Applicable to substances used in products within the scope of Q3D (e.g. not implemented for vet. only, herbals, etc.)
- Guidance on how to implement ICH Q3D in the procedure for "Certification of Suitability to the monographs of the European Pharmacopoeia" (CEP). It has been revised based on experience gained by EDQM since the initial implementation of the policy

Elemental impurities - PA/PH/CEP (16) 23, 1R

- Serve the Component Approach as per Q3D: provide necessary information to MAH for their risk assessment on the Drug Product;
- Be useful for substances manufacturers and MAH and keep the benefits of the centralized assessment foreseen by the CEP Procedure

Elemental impurities - PA/PH/CEP (16) 23, 1R

2 possible options in a CEP dossier:

1. The substance manufacturer can submit a risk management summary (RMS) for elemental impurities (component approach). This helps the DP manufacturer's risk assessment and it is evaluated by assessors
2. No RMS given by the substance manufacturer.

The EDQM encourages the submission of a RMS in the CEP Dossier.

Applicants are also reminded that it is a requirement to submit the synthesis of the API in the Dossier including information on metal catalysts or reagents used.

If the RMS is included in the Dossier…

- It should be apparent that this approach is followed
- The RMS should provide the reasons why certain impurities are considered and the justification of the chosen control strategy
- The RMS table is intended to carry necessary information about the level of contamination of the substance source, in order to implement the ICH Q3D component approach in the finished medicinal product.
- A screening alone is not a risk management summary. Screening results may support but do not replace a RMS
- Where insufficient data is given to support this option, the application is considered as if no RMS is provided.

How to build the RMS

- The RMS should consider all potential sources of contamination; including elemental impurities intentionally introduced into the process after the introduction of the starting material(s), contributions from materials (starting materials, reagents, solvents, catalysts, process aids, water, etc.), equipment and packaging
- The intended route of administration / use of the substance should be indicated. This forms the basis of the risk management discussion
- The RMS should take into consideration all 24 elemental impurities mentioned in ICH Q3D

How to define the control strategy

- The control strategy should focus on absence/presence of elemental impurities (using preferably option 1 of the ICH Q3D guideline)
- An elemental impurity is absent when purged to levels consistently below 30% of the calculated concentration limit based on the indicated route of administration and on the option 1 daily intake, in a minimum of 3 consecutive commercial or 6 consecutive pilot batches of final substance. Other approaches may be considered, if scientifically justified
- When applicable, a justified specification for elemental impurities in the final substance should be introduced. For elemental impurities intentionally introduced into the last synthetic step, specifications in the final substance are normally expected unless levels below 30% of ICH Q3D option 1 limit.

How to define the control strategy

With regard to the analytical methods used:

- **For screening purposes:** The analytical methodology used should be mentioned along with minimum validation information such as indication of the specificity and sensitivity of the method (LOD/LOQ)
- **Control included in the specification of the final substance:** A detailed description of the analytical method used should be provided which is suitable to be annexed to the CEP. The analytical method should be validated in accordance with the requirements of ICH Q2

RMS table

Intended route of administration / Use of the substance:				
Element	**Class**	**Intentionally added?**	**Considered in risk management?**	**Conclusion**
Cd	1	*	Yes	**
Pb	1	*	Yes	**
As	1	*	Yes	**
Hg	1	*	Yes	**
Co	2A	*	Yes	**
V	2A	*	Yes	**
Ni	2A	*	Yes	**
Tl	2B	*	*	**
Au	2B	*	*	**
Pd	2B	*	*	**
Ir	2B	*	*	**
Os	2B	*	*	**
Rh	2B	*	*	**
Ru	2B	*	*	**
Se	2B	*	*	**
Ag	2B	*	*	**
Pt	2B	*	*	**
Li	3	*	*	**
Sb	3	*	*	**
Ba	3	*	*	**
Mo	3	*	*	**
Cu	3	*	*	**
Sn	3	*	*	**
Cr	3	*	*	**

*** Yes / No**

Does not restrict the use of the CEP!

"Yes" for all which have been discussed

RMS table

** The following statements may be used as explained under 3.1:

-**"Absent"** with its meaning definition (e.g. "less than 30% of ICH Q3D option 1 limit", or "less than X ppm"),

- **or "< X ppm",**

- **or "No risk identified"**

Individual batch results should not be included in the table

Batch results for an EI	To be reported in the table as conclusion
0.2 ppm / 0.1 ppm / 0.4 ppm	< 0.5 ppm or < 1 ppm or < 5 ppm
1.5 ppm / 0.9 ppm / 1.8 ppm	< 2 ppm or < 5 ppm or < 10 ppm

If the RMS is included in the Dossier…

Information on the CEP…

A risk management summary for elemental impurities has been provided.	(Annex 2)

… and if applicable…

A risk management summary for elemental impurities has been provided.		(Annex 2)
– Tests for elemental impurities by ICP-MS		
Palladium	not more than 3 ppm	(Annex 3)
Nickel	not more than 6 ppm	(Annex 4)

If the RMS is NOT provided…

The following points should be addressed in the CEP application:

- Any elemental impurities (whatever the class) intentionally introduced should be declared; data showing their level in the final substance should be provided
- For any elemental impurity intentionally introduced into the last synthetic step, a specification in the final substance is normally expected unless levels below 30% of ICH Q3D option 1 limit
- The limits applied to control elemental impurities in the final substance should reflect the process capabilities. The PDE of ICH Q3D may be used as reference
- The method used to control elemental impurities in the final substance should be described in detail (in a format to be annexed to the CEP) and validation data according to ICH Q2 should be submitted

If the RMS is NOT provided…

Information on the CEP…

> No elemental impurity classified in ICH Q3D is intentionally introduced in the manufacture of the substance.

… or all elemental impurities intentionally added after the introduction of the starting material(s) are listed on the CEP, regardless of the levels found in the final substance…

> The following elemental impurity classified in ICH Q3D is intentionally introduced in the manufacture of the substance: Lithium.

… and if applicable…

> The following elemental impurity classified in ICH Q3D is intentionally introduced in the manufacture of the substance: Palladium.
>
> – Test for elemental impurities by atomic absorption spectrometry (Annex 3)
> Palladium not more than 10 ppm

Revisions and renewals of CEPs

- Introduction or revision of a RMS: *minor*. This request for revision may be submitted at any time during the lifecycle of the dossier, except during an on-going procedure

4.II.2.1 Change in the specification parameters and/or limits of the final substance	Conditions	Specific documentation	Type of change
h) Introduction or revision (non-editorial changes) of a RMS (Risk management summary) regarding elemental impurities	8	6	MIN

Conditions	
8.	The route of synthesis of the final substance remains unchanged.
Documentation	
6.	Risk management discussion and summary for elemental impurities.

- If changes to the manufacturing process have an impact on elemental impurities, CEP holders are given the possibility to submit a RMS. If a RMS has already been introduced, its validity should be verified and discussed.

Revisions and renewals of CEPs

- Changes to the control strategy: to be classified according to the applicable guideline on revisions/renewals of CEPs.
- The renewal application is also an opportunity for CEP holders to submit a RMS in their application

Messages to take back home and conclusions

- The Certification Procedure can serve the component approach as per ICH Q3D;
- The EDQM does not make a decision on compliance with ICH Q3D;
- The EDQM encourages the submission of a RMS in the CEP dossier. All needed information is publicly available;
- A screening alone is not risk management summary. Details on the risk assessment performed should be provided.
- 852 valid chemical CEP's out of 4670 have a RMS appended (information extracted on April 17th, 2019).

Thank you for your attention

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Control of impurities

Challenges linked to the establishment of reference standards

Dr Jochen Pauwels
Laboratory Department,
EDQM, Council of Europe

OUTLINE

- Role of reference standards
- Establishment of reference standards
- Challenges
- Example
- Final remarks

CONTROL OF IMPURITIES

What is the role of reference standards?

ROLE OF REFERENCE STANDARDS

Technical guide for the elaboration of monographs (7th edition – 2015)

II.5.8. RELATED SUBSTANCES

Monographs should provide a reliable means of locating all specified impurities on the chromatogram. Identification of unspecified impurities is necessary if a correction factor is to be applied. Peaks may be located using:

- a **reference standard** for each impurity;
- a **reference standard** of the substance to be examined containing some or all of the specified impurities, provided with a chromatogram.

Location by relative retention is not generally considered sufficient for pharmacopoeial purposes, notably for gradient elution.

→ Peak identification

ROLE OF REFERENCE STANDARDS

Technical guide for the elaboration of monographs (7th edition – 2015)

A large difference in the detector response of an impurity necessitates the use of a specific external standard, which may be:

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

→ Quantification

→ System suitability

*(In-situ degradation … offers an alternative approach to define the suitability of the system … to produce decomposition products, the peaks of which can be used to determine a resolution or a peak-to-valley ratio. This may be a useful approach to avoid the use of impurity **reference standards**.)*

CONTROL OF IMPURITIES

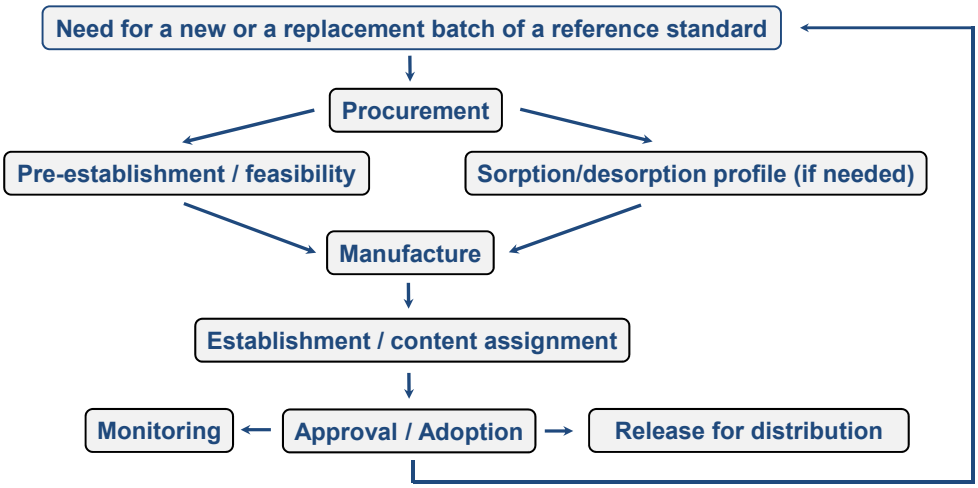
How are reference standards established?

PH.EUR. CHAPTER 5.12. REFERENCE STANDARDS

- **Terminology**
- **Use of Ph.Eur. Reference Standards**
- **Establishment of Reference Standards**
  - Primary Standards
  - Ph.Eur CRS
  - Ph.Eur HRS
  - Ph.Eur. BRP
- **Manufacturing, Labelling, Storage and Distribution of Ph.Eur. Reference Standards**
- **Re-Test Programme of Ph.Eur. Standards**

⇨ Intended purpose !!!

REFERENCE STANDARDS



CONTROL OF IMPURITIES

What are the challenges linked to the establishment of reference standards?

PEAK IDENTIFICATION

Reference standard strategy

- Individual impurity or mixture
- Commercial reagent or *in situ* degradation can be described, if available

Challenges

- Balance between number of reference standards and feasibility/sustainability
  - Impurities from different production processes = different reference standards
  - Individual impurities versus (compounded) mixtures
- Candidate material(s)
  - Amount (10 g to 50 g of bulk material)
  - Containing impurities of interest (= specified impurities) at detectable levels
  - Normal production batches expected to be suitable
  - For compounding, 100 mg to 500 mg of individual impurities

PEAK IDENTIFICATION

Challenges

- Manufacture
  - Compounding: solubility, stability, homogeneity → feasibility study
- Confirmation of identity of impurity peaks in mixtures (traceability)
  - CRS 1: availability of authentic samples of impurities for spiking (10 to 50 mg)
  - CRS n+1: spike with CRS n (but not always appropriate for complex profiles)
  - Alternative detection e.g. LC/MS but pre-requisites (mobile phase, ionisation, difference in m/z, …)
- Sustainability
  - Stability of impurities
  - Batch-to-batch: identity, not necessarily content of impurities
  - Evolution in impurity profile/API synthesis → adapt monograph/reference standard
  - Changes in method: need to confirm once more identity of impurity peaks

QUANTIFICATION

Reference standard strategy

- Individual impurity
- Semi-quantitative (e.g. TLC): use of a commercial reagent may be considered, if available sufficiently pure and well defined in corresponding Ph.Eur. Chapter

Challenges

- Candidate material(s)
  - Amount (25 g to 100 g): more material needed due to extensive characterisation and increased amount per vial (sufficient for preparation of two solutions), compared to "peak identification"
  - Content preferably above 95.0 %
- Manufacture
  - Homogeneity → water sorption/desorption study

QUANTIFICATION

Challenges

- Content determination
  - Mass balance/related substances: method corresponding to the intended use (solubility, differences in response, late eluting compounds)
  - Inorganics/residual solvents: high amount of sample required
  - Orthogonal methods (e.g. qNMR): selectivity
  - Hydrates/differences in salt form (stoichiometric conversion factor)
- Sustainability
  - Stability
  - Batch-to-batch: identity and content
  - Changes in method: verify impact on content / change batch if impact

SYSTEM SUITABILITY

Reference standard strategy

- Individual impurity or mixture
- Commercial reagent or *in situ* degradation can be described if available, but *cave* impact
- *Cave* test solution to which an individual impurity is added
- Compliance with monograph is not required

Challenges

- Candidate material(s)
  - Amount (10 g to 50 g of bulk material)
  - Containing impurities of interest at appropriate levels, especially for peak-to-valley ratio criterion (method validation – composition of reference standard is integral part of system suitability test)
  - If impurity is not specified or an impurity level far from specification is needed

SYSTEM SUITABILITY

Challenges

- Confirmation of identity of impurity peaks in mixtures (traceability)
  - Cfr. "Peak identification"
- Sustainability
  - Batch-to-batch: identity and content of impurities
  - In-house compounding provides more control on impurity levels, but feasibility (solubility, stability, homogeneity) needs to be tested
  - If content varies between batches, impact on intended use needs to be assessed

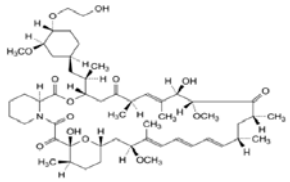
CONTROL OF IMPURITIES

Challenges linked to the establishment of reference standards: example

EVEROLIMUS FOR SYSTEM SUITABILITY CRS 1

EVEROLIMUS

Everolimusum



$C_{53}H_{83}NO_{14}$ M_r 958
[159351-69-6]

(1*R*,9*S*,12*S*,15*R*,16*E*,18*R*,19*R*,21*R*,23*S*,24*E*,26*E*,28*E*,30*S*,32*S*,-35*R*)-1,18-Dihydroxy-12-[(2*R*)-1-[(1*S*,3*R*,4*R*)-4-(2-hydroxyethoxy)-3-methoxycyclohexyl]propan-2-yl]-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-11,36-dioxa-4-azatricyclo[30.3.1.0^{4,9}]hexatriaconta-16,24,26,28-tetraene-2,3,10,14,20-pentone.

Semi-synthetic product derived from a fermentation product. A suitable antioxidant may be added.

Related substances. Liquid chromatography (*2.2.29*). *Carry out the test protected from light. Prepare the solutions immediately before use.*

Reference solution (a). Dissolve 5 mg of *everolimus for system suitability CRS* (containing impurities D, E, F, H, I and J) in 1 mL of *acetonitrile R*.

Identification of impurities: use the chromatogram supplied with *everolimus for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities D, E, F, H, I and J; use

Relative retention with reference to everolimus (retention time = about 15 min): impurity H = about 0.78; impurity I = about 0.81; impurity C = about 0.87; impurity D = about 0.92; everolimus tautomer = about 1.1; impurity E = about 1.34; impurity J = about 1.38; impurity F = about 1.5.

System suitability: reference solution (a):
- *resolution*: minimum 1.5 between the peaks due to impurity D and everolimus; minimum 1.5 between the peaks due to everolimus and everolimus tautomer.

Limits:
- *impurities C, D, I*: for each impurity, maximum 0.8 per cent;
- *impurity H*: maximum 0.4 per cent;
- *sum of impurities E and J*: maximum 0.3 per cent;
- *impurity F*: maximum 0.2 per cent;
- *unspecified impurities*: for each impurity, maximum 0.15 per cent;
- *total*: maximum 2.5 per cent;
- *reporting threshold*: 0.05 per cent; disregard any peak due to everolimus tautomer.

IMPURITIES

Specified impurities: A, C, D, E, F, H, I, J.

H. unknown structure,

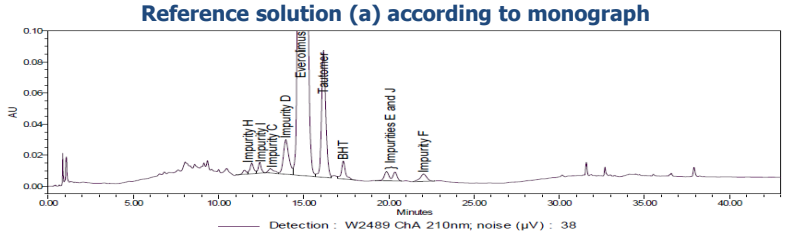
I. unknown structure,

J. unknown structure.

No authentic samples available

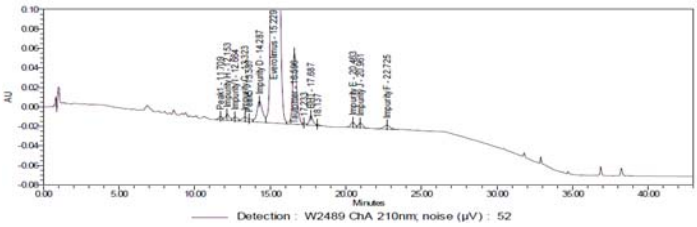
EVEROLIMUS FOR SYSTEM SUITABILITY CRS 1

Reference solution (a) according to monograph



Mobile phase not compatible with LC/MS (KH_2PO_4)

Reference solution (a) with modified mobile phase



EVEROLIMUS FOR SYSTEM SUITABILITY CRS 1

Table 1 – LC/MS results for reference solution (a) (ESI in positive and negative mode)

Retention time	Identity according to monograph	Adduct	Theoretical sum formula (adduct)	Theoretical monoisotopic m/z	Concordance m/z found with theory?	Comment
12.2 min	Impurity H	$[M+NH_4]^+$	N/A*	N/A*	N/A*	Sum formula could be determined
		$[M+HCOO]^-$				
12.7 min	Impurity I	$[M+NH_4]^+$	N/A*	N/A*	N/A*	Sum formula could be determined
		$[M+HCOO]^-$				
14.3 min	Impurity D	$[M+NH_4]^+$	$C_{53}H_{87}N_2O_{14}$	975.6152	Yes	Everolimus and impurities B and D have the same monoisotopic mass and cannot be distinguished by MS
		$[M+HCOO]^-$	$C_{54}H_{84}NO_{16}$	1002.5796	Yes	
20.5 min	Impurity E	$[M+NH_4]^+$	$C_{52}H_{83}N_2O_{14}$	959.5839	**No**	Sum formula could be determined
		$[M+HCOO]^-$	$C_{53}H_{80}NO_{16}$	986.5483	**No**	
21.0 min	Impurity J	$[M+NH_4]^+$	N/A*	N/A*	N/A*	Major signal: sum formula could be determined
						Minor signal: concordant with impurity E
		$[M+HCOO]^-$				Major signal: sum formula could be determined
						Minor signal: concordant with impurity E
22.7 min	Impurity F	$[M+NH_4]^+$	$C_{55}H_{93}N_2O_{16}$	1037.6520	Yes	-
		$[M+HCOO]^-$	$C_{56}H_{90}NO_{18}$	1064.6163	Yes	

* according to the Ph. Eur. monograph for everolimus, the structure of these impurities is unknown.

EVEROLIMUS FOR SYSTEM SUITABILITY CRS 1

- ✓ **Doubts on elution of impurity E**
  - → Due to change in mobile phase?
  - → Feedback to Group of Experts and manufacturer: investigation ongoing
  - → No impact on user since the sum of impurities E and J (peak pair) is specified
- ✓ **Replacement batch (future)**
  - → Keep content of impurity D similar (system suitability)
  - → Spiking probably not sufficient to confirm identity of impurity peaks
- ✓ **Additional impurities (generics) will need to be specified**
  - → Separate reference standard(s)
  - → Perhaps method will change; if so, confirmation of peak identity needed

FINAL REMARKS

- ✓ An important challenge is to cope with the **availability** of suitable candidate material and authentic samples of impurities. Cooperation with manufacturers is key to overcome this challenge.
- ✓ Devising a "**smart**" reference standard **strategy** is of paramount importance:
  - Only describe a reference standard when there is a real need
  - Make best use of what is available (and what can be expected to be available in the future)
  - Keep it simple; don't try to create an ideal, all-purpose reference standard.
- ✓ Do not compromise on confirmation of **identity** of impurity peaks
- ✓ Encourage use of **volatile** mobile phases so that LC/MS can be applied directly.

FINAL REMARKS

- ✓ There is a close **link** between impurity **limits** and reference standards
  - Specify impurities when justified by batch data
  - Increase in number of specified impurities → increase in challenges for reference standards.
- ✓ Some **ideas** / food for thought:
  - Use system suitability reference standards only for that purpose, i.e. keep them separate from peak identification reference standards, where possible.
  - Develop system that allows monitoring of evolution of market quality of batches to anticipate potential reference standard problems for replacement batches.

Thank you for your attention

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