# Related substances: how to characterise and control the impurity profile of an API

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# Summary

- Main applicable guideline and the European Pharmacopoeia monographs and chapters
- Related impurities in antibiotics
- Suitability of Ph.Eur monographs to control the impurity profile of the substance, with reference to CEP applications
- Examples

# Impurities in substances for pharmaceutical use

- Organic impurities:
- Control strategy follows ICH Q3A
- Principles are laid down in general monograph 2034
  « Substances for pharmaceutical use »
- « Transparency list » at the end of a monograph: provides list of the impurities which are controlled by the test(s) described in the monograph
- Limits defined for specified, unspecified and total impurities
- General chapters and texts, like 5.10: « *Control of impurities in substances for pharmaceutical use* »

#### Plate: TLC stitca gel GF254 plate R.

Mobile phase: concentrated ammonia R, methanol R, ethyl acetate R (10:10:80 V/V/V).

Application: 5 µL.

Development: over 1/2 of the plate.

Drying: in air.

Detection: examine in ultraviolet light at 254 nm.

System suitability: reference solution (b):

 the chromatogram shows 2 clearly separated spots. *Results*: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

- C. Dissolve about 10 mg in 10 mL of ethanol (96 per cent) R. To 1 mL of this solution add 0.2 mL of a mixture, prepared immediately before use, of equal volumes of a 6 g/L solution of potassium ferricyanide R and a 9 g/L solution of ferric chloride R. Allow to stand protected from light for 5 min. Add 3 mL of a 10 g/L solution of hydrochioric acid R. Allow to stand, protected from light, for 15 min. A blue colour develops and a precipitate is formed.
- D. Dissolve 60 mg in 0.5 mL of methanol R and add 0.5 mL of water R. The solution gives reaction (b) of sodium (2.3.1).

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 1.25 g in methanol R and dilute to 25.0 mL with the same solvent.

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:

stze: l = 0.25 m, Ø = 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.

Run time: 1.6 times the retention time of diclofenac.

Identification of impurities: use the chromatogram supplied with diciofenac 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; impurity F = about 0.8.

System suitability: reference solution (b):

 resolution: minimum 4.0 between the peaks due to impurity F 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).

#### 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 perchioric acid is equivalent to 31.81 mg of  $\rm C_{14}H_{10}Cl_2NNaO_2.$ 

#### STORAGE

In an airtight container, protected from light.

#### IMPURITIES

#### Specified impurities: A, F.

Other detectable impurities (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-2H-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-dthydro-2H-tndol-2-one,





### Limits for specified, unspecified and total impurities

# **General monograph 2034**

Related substances (aligned with ICH Q3A)

- Unless otherwise prescribed, organic impurities in active substances are to be reported, identified wherever possible, and qualified as indicated in Table 2034.-1. (general) or in table 2034.-2 (for peptides obtained by chemical synthesis).
- Specific thresholds may be applied for impurities known to be unusually potent or to produce toxic or unexpected pharmacological effects.

Table 2034.-1. – Reporting, identification and qualification of<br/>organic impurities in active substances

Use	Maximum daily dose	Report- ing threshold	Identification threshold	Qualification threshold
Human use or	$\leq 2 \text{ g/day}$	> 0.05 per cent	> 0.10 per cent or a	> 0.15 per cent or a
human and veterinary use			daily intake of > 1.0 mg (whichever is the lower)	daily intake of > 1.0 mg (whichever is the lower)
Human use or human and veterinary use	> 2 g/day	> 0.03 per cent	> 0.05 per cent	> 0.05 per cent
Veterinary use only	Not applicable	> 0.10 per cent	> 0.20 per cent	> 0.50 per cent

Reporting	Identification	Qualification
threshold	threshold	threshold
> 0.1 per cent	> 0.5 per cent	> 1.0 per cent

Table 2034.-2. – Reporting, identification and qualification oforganic impurities in peptides obtained by chemical synthesis

The requirements above do not apply to biological and biotechnological products, oligonucleotides, products of fermentation and semi-synthetic products derived therefrom, to crude products of animal or plant origin or herbal products.

# Chapter 5.10: Control of impurities in substances for pharmaceutical use (1)

- Provides:
  - Basis for monographs and impurities control
  - Terminology
  - Interpretation of related substances tests
  - Other aspects of impurities control

# Chapter 5.10: Control of impurities in substances for pharmaceutical use (2)

- how to interpret "general acceptance criteria" in relation with the Impurities section of the monograph
- decision tree to help the users
- general acceptance criterion may be expressed in various ways in the monographs: "any other impurity", "other impurities", "any impurity", "any spot", "any band", etc.

# **Organic impurities in Ph. Eur.**

General Chapter 5.10 defines:

**Specified impurity:** an impurity that is individually listed and limited with a specific acceptance criterion in a monograph. A specified impurity can be either identified or unidentified.

**Unspecified impurity:** an impurity that is limited by a general acceptance criterion and not individually listed with its own acceptance criterion

#### CELIPROLOL HYDROCHLORIDE

#### Celiprololi hydrochloridum



M\_416.0

#### C<sub>20</sub>H<sub>34</sub>ClN<sub>3</sub>O<sub>4</sub> [57470-78-7]

#### DEFINITION

3-[3-Acetyl-4-[(2RS)-3-[(1,1-dimethylethyl)amino]-2hydroxypropoxy]phenyl]-1,1-diethylurea hydrochloride. Content: 99.0 per cent to 101.0 per cent (dried substance).

#### CHARACTERS

Appearance: white or very slightly yellow, crystalline powder. Solubility: freely soluble in water and in methanol, soluble in ethanol (96 per cent), very slightly soluble in methylene chloride.

It shows polymorphism (5.9).

#### IDENTIFICATION

A. Infrared absorption spectrophotometry (2.2.24).

Comparison: celiprolol hydrochloride CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in methanol R, evaporate to dryness and record new spectra using the residues.

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

#### TESTS

Optical rotation (2.2.7): - 0.10° to + 0.10°.

Dissolve 1.0 g in water R and dilute to 10.0 mL with the same solvent.

Related substances. Liquid chromatography (2.2.29). Prepare the solutions immediately before use.

Test solution. Dissolve 0.100 g of the substance to be examined in mobile phase A and dilute to 20.0 mL with mobile phase A.

Reference solution (a). Dissolve 2 mg of the substance to be examined and 2 mg of acebutolol hydrochloride R in mobile phase A and dilute to 50.0 mL with mobile phase A.

Reference solution (b). Dissolve 10 mg of the substance to be examined in 2 mL of mobile phase A and allow to stand for 24 h (for identification of impurity A).

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

Reference solution (d). Dissolve 10 mg of celiprolol for peak identification CRS in mobile phase A and dilute to 2 mL with mobile phase A.

Reference solution (e). This solution is only prepared if required (see below) and is used to determine the identity of impurity I which co-elutes with impurity H (the 2 impurities originate from different routes of synthesis). Dissolve the contents of a vial of celiprolol impurity I CRS in mobile phase A and dilute to 2.0 mL with mobile phase A.

Column

- size: l = 0.15 m,  $\emptyset = 4.6 \text{ mm}$ .
- stationary phase: octylsilyl silica gel for chromatography R (5 µm),
- temperature: 30 °C.
- Mobile phase:
- mobile phase A: mix 91 mL of tetrahydrofuran R, 63 mL of acetonitrile R1, 0.6 mL of pentafluoropropanoic acid R and 0.2 mL of trifluoroacetic acid R; dilute to 1000 mL with water R:

mobile phase B: acetonitrile R1;

Time	Mobile phase A	Mobile phase B (per cent V/V)	
(min)	(per cent V/V)		
0 - 50	$100 \rightarrow 80$	$0 \rightarrow 20$	
50 - 51	$80 \rightarrow 100$	$20 \rightarrow 0$	
51 - 65	100	0	

#### Flow rate: 1.4 mL/min.

Detection: spectrophotometer at 232 nm.

#### Injection: 10 µL.

Identification of impurities: use the chromatogram supplied with celiprolol for peak identification CRS and the chromatogram obtained with reference solution (d) to identify the peaks due to impurities B, E and F.

Relative retention with reference to celiprolol (retention time = about 10 min): impurity A = about 0.3; impurity D = about 0.7; impurity G = about 1.2; impurity B = about 1.4; impurity F = about 1.6; impurity C = about 2.2; impurity H or I = about 2.5; impurity E = about 3.9.

System suitability: reference solution (a):

- resolution: minimum 4.0 between the peaks due to celiprolol and acebutolol.

Limits:

 correction factors: for the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 4.0; apurity B = 1.5; impurity E = 2.3; impurity F = 0.5; impurity I = 1.7;

- any impurity: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent), and not more than 1 such peak has an area greater than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent);
- tal: not more than 5 times the seea of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);
- if any of the above limits are exceeded and if a peak occurs with a relative retention of about 2.5 (impurity H or I), the identity of this peak has to be clarified by use of a UV spectrum recorded with a diode array detector; if this spectrum is different from the one obtained with reference solution (e), no correction factor is applied;
- disregard limit: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (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 0.350 g under an atmosphere of nitrogen in 50 mL of ethanol (96 per cent) R and add 1.0 mL of 0.1 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 41.60 mg of C<sub>m</sub>H<sub>4</sub>ClN<sub>3</sub>O<sub>4</sub>.

#### STORAGE

Protected from light.

#### IMPURITIES

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



A. 1-[5-amino-2-[(2RS)-3-[(1,1-dimethylethyl)amino]-2hydroxypropoxy]phenyl]ethanone,



B. 1,3-bis[3-acetyl-4-[3-[(1,1-dimethylethyl)amino]-2hydroxypropoxy]phenyl]urea,



C. 1-[3-acetyl-4-[(2RS)-3-[(1,1-dimethylethyl)amino]-2hvdroxypropoxylphenyl]-3-(1,1-dimethylethyl)urea,



D. 3-[3-acetyl-4-[(2RS)-3-(diethylamino)-2hydroxypropoxy]phenyl]-1,1-diethylurea,



E. 1,1'-[[(1,1-dimethylethyl)imino]bis[(2-hydroxypropane-1,3-diyl)oxy(3-acetyl-1,4-phenylene)]]bis(3,3-diethylurea),



F. 3-(3-acetyl-4-hydroxyphenyl)-1,1-diethylurea,



G. 3-[3-acetyl-4-[[(RS)-oxiranyl]methoxy]phenyl]-1,1diethylurea,



H. 3-[3-acetyl-4-[(2RS)-3-bromo-2-hydroxypropoxy]phenyl]-1,1-diethylurea (bromhydrin compound),



I. 1-acetyl-1-(4-ethoxyphenyl)-3,3-diethylurea.



### Antibiotics (products of fermentation and semisynthetic substances)

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

#### 5.1. Active substances manufactured by semi-synthesis

Reporting threshold: 0.05%/0.03% Identification threshold: 0.10%/0.05% Qualification threshold: 0.15%/0.05%

#### 5.2. Active substances manufactured by fermentation, single compound

Reporting threshold: 0.10% Identification and qualification thresholds: 0.15%

#### 5.3. Active substances manufactured by fermentation, family of compounds

Reporting threshold: 0.10% Identification threshold: 0.15% Qualification threshold: 0.50%/0.2%

#### DICLOXACILLIN SODIUM

#### Dicloxacillinum natricum



#### C<sub>19</sub>H<sub>16</sub>Cl<sub>2</sub>N<sub>3</sub>NaO<sub>5</sub>S,H<sub>2</sub>O [13412-64-1]

#### DEFINITION

Sodium (2S,5R,6R)-6-[[[3-(2,6-dichlorophenyl)-5-methyl-1,2oxazol-4-yl]carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1azabicyclo[3.2.0]heptane-2-carboxylate monohydrate.

Semi-synthetic product derived from a fermentation product.

Content: 95.0 per cent to 102.0 per cent (anhydrous substance).

#### CHARACTERS

Appearance: white or almost white, hygroscopic, crystalline powder.

Solubility: freely soluble in water, soluble in ethanol (96 per cent) and in methanol.

#### IDENTIFICATION

First identification : A, D.

Second identification : B, C, D.

A. Infrared absorption spectrophotometry (2.2.24). Preparation: discs.

Comparison: dicloxacillin sodium CRS.

B. Thin-layer chromatography (2.2.27).

Test solution. Dissolve 25 mg of the substance to be examined in 5 mL of water R.

Reference solution (a). Dissolve 25 mg of dicloxacillin sodium CRS in 5 mL of water R.

Reference solution (b). Dissolve 25 mg of cloxacillin sodium CRS, 25 mg of dicloxacillin sodium CRS and 25 mg of flucloxacillin sodium CRS in 5 mL of water R.

Plate: TLC silanised silica gel plate R.

Mobile phase: mix 30 volumes of acetone R and 70 volumes of a 154 g/L solution of ammonium acetate R adjusted to pH 5.0 with glacial acetic acid R.

Application: 1 uL.

Development: over a path of 15 cm.

Drying: in air.

Detection: expose to iodine vapour until the spots appear and examine in daylight. absorbance (2.2.25) at 430 nm is not greater than 0.04.

pH (2.2.3): 5.0 to 7.0 for solution S.

Specific optical rotation (2.2.7): + 128 to + 143 (anhydrous substance).

Dissolve 0.250 g in *water R* and dilute to 25.0 mL with the same solvent.

Related substances. Liquid chromatography (2.2.29).

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

M<sub>7</sub> 510.3 Test solution (b). Dilute 5.0 mL of test solution (a) to 50.0 mL with the mobile phase.

> Reference solution (a). Dissolve 50.0 mg of dicloxacillin sodium CRS in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 50.0 mL with the mobile phase.

Reference solution (b). Dilute 5.0 mL of test solution (b) to 50.0 mL with the mobile phase.

Reference solution (c). Dissolve 5 mg of flucloxacillin sodium CRS and 5 mg of dicloxacillin sodium CRS in the mobile phase, then dilute to 50.0 mL with the mobile phase. Column:

- size: l = 0.25 m, Ø = 4 mm;
- stationary phase: octadecylsilyl silica gel for chromatography R (5 μm).

Mobile phase: mix 25 volumes of acetonitrile R and 75 volumes of a 2.7 g/L solution of potassium dihydrogen phosphate R adjusted to pH 5.0 with dilute sodium hydroxide solution R. Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 225 nm.

Injection:  $20 \ \mu L$  of test solution (a) and reference solutions (b) and (c).

Run time: 5 times the retention time of dicloxacillin.

Retention time: dicloxacillin = about 10 min.

System suitability: reference solution (c):

- resolution: minimum 2.5 between the peaks due to flucloxacillin (1<sup>st</sup> peak) and dicloxacillin (2<sup>nd</sup> peak).
- Limits:
- any impurity: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);
- total: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (5 per cent);

 disregard limit: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

#### IMPURITIES



A. (4S)-2-[carboxy[[[3-(2,6-dichlorophenyl)-5-methyl-1,2-oxazol-4-yl]carbonyl]amino]methyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid (penicilloic acids of dicloxacillin),



B. (2RS,4S)-2-[[[[3-(2,6-dichlorophenyl)-5-methyl-1,2oxazol-4-yl]carbonyl]amino]methyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid (penilloic acids of dicloxacillin),



C. (2S,5R,6R)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid),



D. 3-(2,6-dichlorophenyl)-5-methyl-1,2-oxazole-4-carboxylic acid.

### Antibiotics (products of fermentation and semisynthetic substances)

→Limit for "any other impurity" of the individual monograph applies to impurities of the monograph and to any other specified impurity. A suitable limit for "unspecified impurities" has to be proposed by applicant.

## Related substances and suitability of the Monograph to control the impurity profile of a substance

- Suitability (or unsuitability) of the method(s) of the monograph to control all the related substances should be demonstrated; in particular where additional impurities (those not listed in the transparency statement of the monograph) are found above relevant reporting threshold or disregard limit of the monograph.
- If the Ph. Eur. method is not suitable to control in-house impurities then it has to be supplemented with an additional (validated) method, unless absence of the concerned impurities is demonstrated.

# Suitability of the Monograph to control the impurity profile of a substance



### Absence of cross validation between Ph.Eur. and in-house method for the control of related substances

Alternative methods may be used but they have to be shown to give equivalent results comparing to the corresponding Ph.Eur methods:

- They have to be fully validated in line with ICH Q2B
- Cross-validation on the same batches against the corresponding Ph.Eur method (using spiked solutions if necessary)
- A discussion on the reasons why an alternative method is proposed is expected
- Comparative and typical chromatograms

Modifications to the Ph.Eur methods allowed within the ranges set by the Ph.Eur 2.2.46.

### Thank you very much for your attention!

