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

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Impurity Control in the European Pharmacopoeia

2019 Training Session "The European Pharmacopoeia"

10 – 11 September 2019, Iselin, New Jersey, USA

Agenda

- Which impurities are controlled?
- Analytical techniques and general texts/monographs
- Control of organic impurities
- Validation
- Elemental impurities
- DNA reactive impurities
- Summary

Control of impurities in Ph. Eur.

Organic impurities	Inorganic impurities
Volatile impurities, Water and residual solvents	Special groups, e. g. genotoxic (DNA reactive) imps, inorganics subjected to Q3D

Organic impurities: *General texts and monographs*

- General monograph 2034 « Substances for pharmaceutical use »
  describes general requirements for control of organic and inorganic impurities, volatiles, DNA reactive impurities
- General text 5.10: Control of impurities in substances for pharmaceutical use
  helps to interprete the test for related substances, provides definitions, explanations, recommendations
- General text 5.4: Residual solvents: refers to ICH Q3C
- General text 5.20: Elemental impurities: refers to ICH Q3D
- Several general chapters

QUIZ

- **Organic impurities:**

1. What is the difference between « disregard limit » and « reporting threshold »?
2. What would you consider a suitable resolution criterion?
3. What is the meaning of a « correction factor » in the quantitative determination of an organic impurity?
4. How would you identify an impurity in a chromatographic system?

Related substances

- 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) – implementation of ICH Q3 A which becomes legally binding
- Specific thresholds may be applied for impurities known to be unusually potent or to produce toxic or unexpected pharmacological effects.
- If the individual monograph does not provide suitable control for a new impurity, a suitable test for control must be developed and included in the specification for the substance

Requirements for active substances except synthetic peptides, Table 2034.1

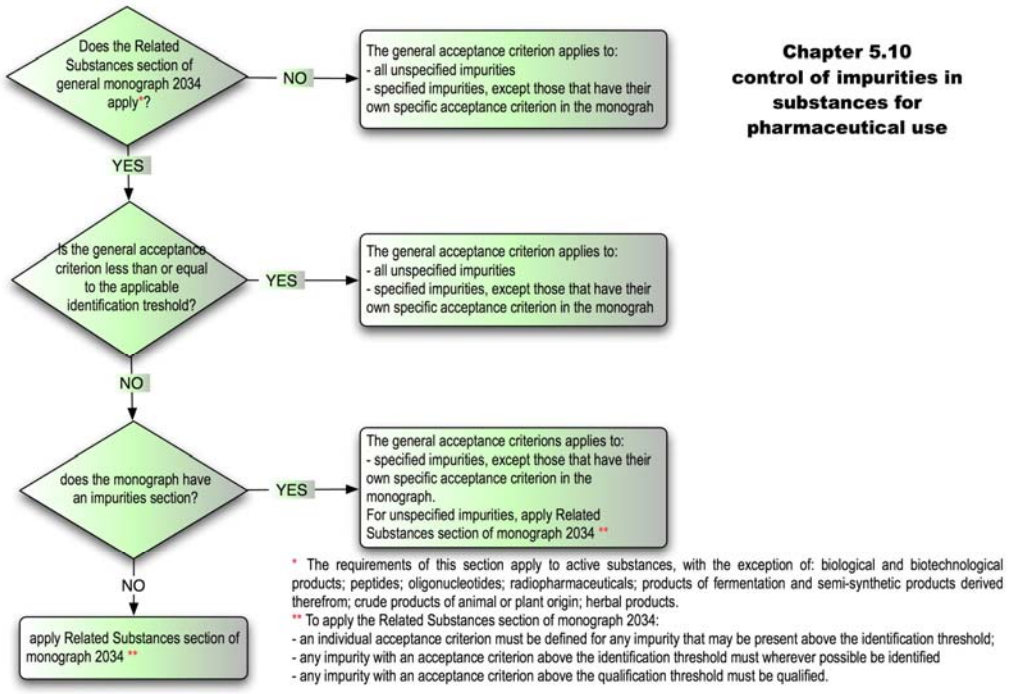
Use	Maximum daily dose	Reporting threshold	Identification threshold	Qualification threshold
Human or human and veterinary	≤ 2 g /day	>0.05 per cent	>0.10 per cent or daily intake >1.0 mg (whichever lower)	>0.15 per cent or daily intake >1.0 mg (whichever lower)
Human or human and veterinary	> 2 g/day	>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

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)

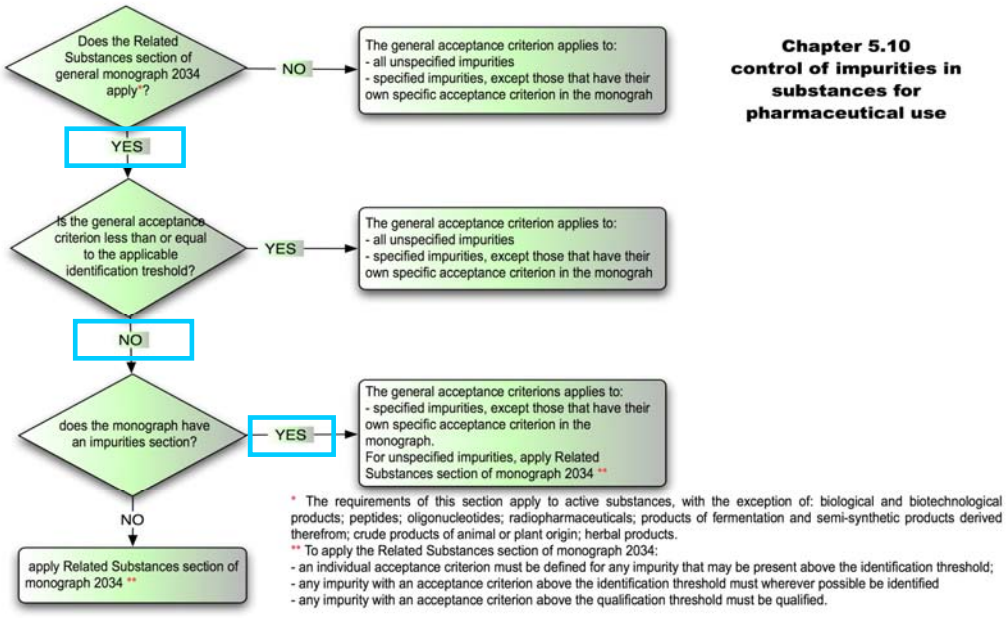
- How to interpret general acceptance criteria in relation with the Impurities section of the monograph
- In older monographs general acceptance criterion may be expressed in various ways in the monographs: "any other impurity", "other impurities", "any impurity", "any spot", "any band", etc.
- Decision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



Example

Substance is an active substance for human use with maximum daily dose ≤ 2 g: identification threshold > 0.10%; qualification threshold >0.15%

- Monograph has:
  - Impurity A ≤ … (2 per cent)
  - Impurity D ≤ … (1 per cent)
  - Any other impurity ≤ … (0.5 per cent)
  - Impurities section:
    - Specified impurities A, B, C, D, E;
    - Other detectable impurities F, G



Example (*continued*)

- Impurities A and D are specified impurities with their own acceptance criteria (2%, 1%)
- "Any other impurity" refers to B, C and E as specified impurities (limit is above identification threshold) (0.5%)
- Apply *Substances for Pharmaceutical Use* for all other impurities (including F, G) (0.10%)*

*Substance is an active ingredient for human use with maximum daily dose ≤ 2 g: identification threshold > 0.10%; qualification threshold >0.15%

Organic impurities in Ph. Eur (1)

- **Analytical Techniques:**

➢ Most often chromatographic methods

HPLC with different detection techniques –
e. g. UV/VIS, RI, MS, Fluorescence, ELSD, MALS, CAD
GC
TLC, HPTLC, mainly in the field of herbals

➢ Rarely UV (e. g. absorbance ratios in riboflavin) or chemical reactions (e. g. test for free acids in testosterone esters)

Organic impurities in Ph. Eur. (2)

- 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*

Reference to general Chapters: 2.2.29

Plate: TLC silica gel GF_{254} 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 *hydrochloric 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:
– *size*: *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 *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; 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 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 (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,

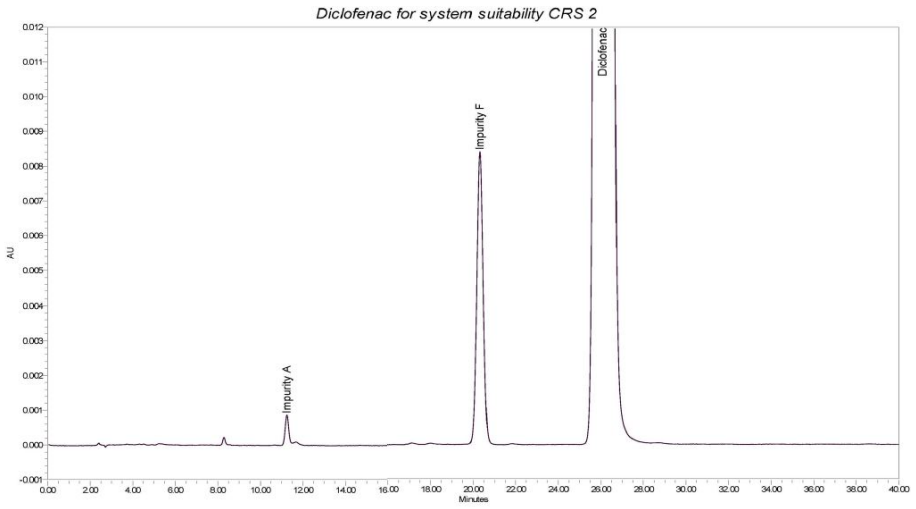
E. 1,3-dihydro-2*H*-indol-2-one,

Transparency list

Organic impurities in Ph. Eur. (3)

- **Monograph Diclofenac sodium**
- *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; 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 sodium 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 in Ph. Eur. (4)



Identification and system suitability test

➢ *Identification:* Specified impurities must be identified in the chromatographic system

- Use of CRS – System suitability CRS or Peak identification CRS
- Retention times and relative retention values:
  only given for information

Identification and system suitability test

- Alternative approach

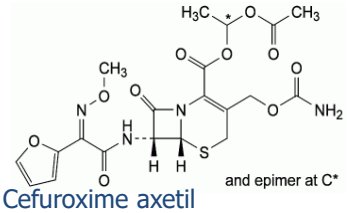
  In situ degradation to form specified impurities

- ✓ Hydrolysis
- ✓ Oxidation
- ✓ Ring-closure
- ✓ Z-E Isomerisation
- ✓ Epimerisation

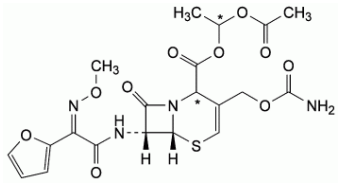
Identification and system suitability test

- Alternative approach

  In situ degradation

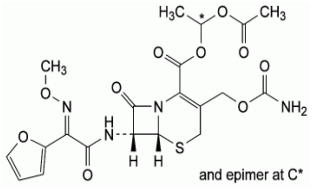


Cefuroxime axetil



Impurity A heat the test solution at 60 °C for 1h

Impurity B



expose the test solution to uv-light at 254 nm for 24 h

Identification and system suitability test

System suitability test

Individual monograph

Resolution test
Peak-to-valley ratio

General chapter 2.2.46
Chromatographic separation techniques

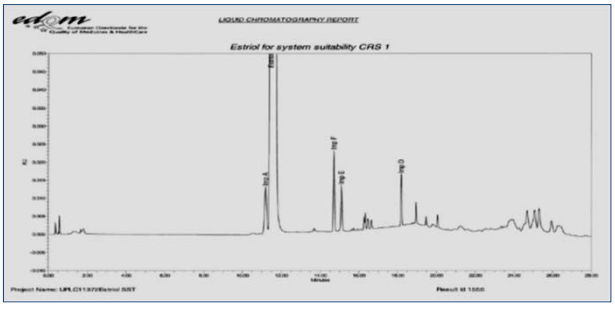
Symmetry factor 0.8 to 1.5
Minimum S/N 10 at reporting threshold
Repeatability requirement for assays

Identification and system suitability test

System suitability test: e. g. peak-to-valley ratio

- Often used for closely eluting peaks when Rs is not possible
- Imitates « real life » situation: example Estriol

Requirement: p/v imp. A - estriol minimum 5.0



Calculation of percentage contents (1)

- Option 1: using an external standard – dilution of the test solution or impurity itself: preferred method in Ph. Eur.

  Attention: dilution of test solution

  ⇒ consider response factor!

- Option 2: peak area normalisation

Calculation of percentage contents (2)

•Response and correction factors:

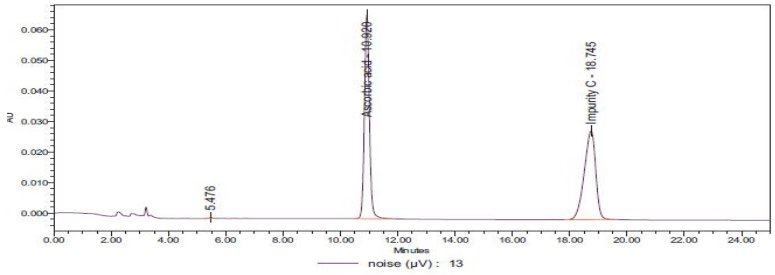
Response factor: Sensitivity of a detector for a given substance relative to a standard substance

- $RRF = Ai/As \times Cs/Ci$
- RRF = response factor
- Ai = area of the peak due to the impurity
- As = area of the peak due to the test substance
- Cs = concentration of the test substance in milligrams per millilitre
- Ci = concentration of the impurity in milligrams per millilitre.

- According to Ph. Eur. is negligible when between 0.8 and 1.2
- Correction factor: reciprocal value of response factor

Calculation of percentage contents (3)

- Ascorbic acid and impurity C: example for different response factors (Imp. C is 8 x more concentrated that ascorbic acid)



Peak Results

	Name	RT	RT Ratio	Area	% Area	Height	Resolution	S/N	Int Type
1	Ascorbic acid	10.920		805026	49.89	66675	21.42	10258	BB
2	Impurity C	18.745	1.72	805706	49.93	29006	15.09	4463	BB

Calculation of percentage contents (4)

- Response/correction factors:
- Note: when correction factors are > 5, the quantification should be performed using impurities as external standards (Ph. Eur. Technical Guide)
- **Calculation of response factors:**
- using the mean of the area ratios over the whole range of linearity or the ratio of the slopes of the respective linearity regression equations

Calculation of percentage contents (5)

- Response factor - important points to consider:
  - Take into account the purity of the impurity and of the test substance
  - Purity calculation: Content (%) = [100 – (water + solvents)] x chromatographic purity (%)/100
  - Take into account the form (base/acid or salt) of the impurity and the test substance, an additional correction factor for the molecular mass ratio may be introduced
  - Perform the chromatography at defined wavelength and flow rate

Calculation of percentage contents (6)

Sensitivity:

- It must be assured that the system is sufficiently sensitive to control impurities not only at their acceptance criterion, but down to the reporting threshold: it may be necessary to add a sensitivity criterion in the case of low responding impurities (RRf < 0.8)

Calculation of percentage contents (7)
Reporting threshold (previously disregard limit)

- *Is the limit above which an impurity should be reported (ICH Q3A R2)*
- 2-fold purpose

- - decision criterion for the user whether a peak area or a corrected peak area of an impurity is to be included in the total of impurities
- - general criterion for the **user** to determine compliance of his actual chromatographic system with the requirement of general chapter 2.2.46 → S/N ratio minimum 10 at the disregard limit/reporting threshold (LOQ should be equal or less than reporting threshold)

Validation of impurity tests (1)

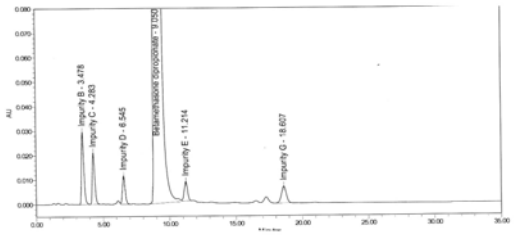
- Follows ICH Q2 (R1)

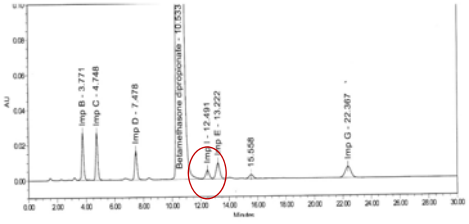
- Different requirements for limit tests (area comparison) and quantitative tests

- Typical parameters:
  - Accuracy
  - Specificity (selectivity)
  - Precision
  - Linearity and range
  - Limit of detection (limit test)
  - Limit of quantitation (quantitative test)
  - Linearity and range (quantitative test)
  - Robustness

Validation of impurity tests (2)

Revision of *Betamethasone sodium phosphate* monograph:
Revision of a related substances test
Addition of specified impurity I, acceptance criterion 0.15 %





Old SST CRS

New SST crs

What to do

Validation of impurity tests (3)

Revision of *Betamethasone sodium phosphate* monograph:

- Addition of a specified impurity I, acceptance criterion 0.15 %
- **Complete re-validation?**

- No, but partial re-validation:

- Selectivity -> new SST
- Sensitivity -> S/N ratio
- Response factor of the new impurity
- Linearity (new impurity)
- Precision, robustness

Differences EP – USP

European Pharmacopoeia	US Pharmacopeia
Monograph Meloxicam	Monograph Meloxicam
- Identification of specified impurities by CRS (imps A, B, C, D)	- Identification of specified impurities by RS (A and B) or by relative retention (C and D)
- Limit for unspecified impurities 0.10 %	- Limit for unspecified impurities 0.1 %
- Comparative style	- Quantitative style
- Impurities A, B each 0.1 %	- Impurities A, B each 0.1 %
- Impurities C, D each 0.05 %	- Impurities C, D each 0.05 %

Difference?

Impurities in finished products (1)

Ph. Eur. Policy:

- Follows ICH Q3 B:

➢ Thresholds for identification, reporting and qualification are higher than for APIs

➢ Only degradation impurities above the reporting threshold are reported and taken into account for the total of impurities

➢ Synthetic impurities identified in the chromatographic system (e. g. by CRS) are excluded

Impurities in finished products (2)

Example: *Rosuvastatin tablets, extract of the test for related substances:*

Identification of impurities: use the chromatogram supplied with *rosuvastatin for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B and C; use the chromatogram supplied with *rosuvastatin impurity mixture CRS* and the chromatogram obtained with reference solution (c) to identify the peak due to impurity D; use the chromatogram obtained with reference solution (d) to identify impurity FP-A.

Limits:

– *impurity C*: maximum1.5 per cent;

– *impurity D*: maximum1.5 per cent;

– *impurity FP-A*: maximum 0.5 per cent;

– *unspecified impurities*: for each impurity, maximum 0.2 per cent;

– *total*: maximum 2.5 per cent;

– *reporting threshold* : 0.1 per cent; disregard the peaks due to impurities A and B.

Synthetic impurities A and B not taken into account

Other impurities controlled

- **Inorganics:** are controlled by general tests like sulfated ash, heavy metals (2.4.8, now only for substances for veterinary use), specific tests like AAS, ICP or general chapter 2.4.20
- **Volatiles:** residual solvents are controlled according to general text 5.4 and general chapter 2.4.24. Class 3 solvents may be controlled by LOD (up to 0.5 %). Water is most often controlled by semi-micro determination, coulometry or loss on drying.
- **DNA-reactive (mutagenic) impurities:** as from 1st January 2016 subjected to ICH M7. Control tests in monographs are in the test or production section.

Elemental Impurities (1)

- ICH Q3D fully implemented in Ph. Eur. (Gen. Text 5.20)
- General monographs 2034 (Substances for pharmaceutical use) and 2619 (Pharmaceutical preparations) revised
- Classical heavy metal tests have been deleted from individual monographs (except for monographs on substances only for veterinary use)
- Chapter 2.4.20 « Determination of elemental impurities » under revision in PDG

Elemental Impurities (2)

- Chapter 2.4.20 (corresponds to USP chapter 233)
- This chapter is currently being harmonised within PDG
- New concept: Example procedures are provided (ICP-MS/OES), validation criteria provided (e.g. accuracy, range, precision), user can choose own method, provided validation criteria are fulfilled

Soon public enquiry

Elemental Impurities (3)

- Specific elemental impurity tests

- No systematic deletion from individual monographs
- Particular case: substances of natural origin, e. g. mined excipients:

- Some case-by-case decisions: e. g. Methylthioninium chloride

DNA reactive (mutagenic) impurities (1)

Ph. Eur. follows ICH M7:

Tests are described when there is proof for genotoxicity, not based on structural alerts

- General monograph 2034 Substances for pharmaceutical use:

« For DNA reactive impurities, the requirements of ICH Guideline M7 *Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk* must be complied with for active substances to be used in medicinal products for human use, in cases defined in the scope of the guideline. "

DNA reactive (mutagenic) impurities (2)

Ph. Eur. follows ICH M7:

-Specific monographs:

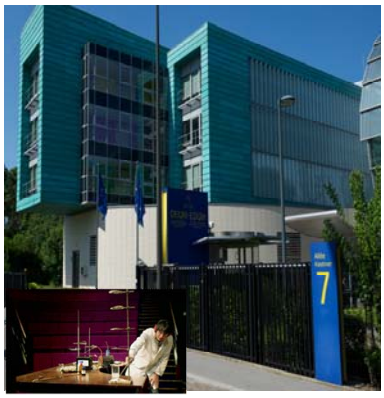
Two options

1. Production section: Either no suitable, selective or sensitive test is known or the test requires too sophisticated equipment. MAH has to ensure the compliance of production with defined requirements.
2. Test section: Test to be included when suitable method is available and limits are known.

Conclusions

- Ph. Eur. impurity control strategy is in line with ICH guidelines
- Impurity tests are validated
- Monographs provide information on all known organic impurities controlled, specified or unspecified
- Limits based on specifications as approved by competent authorities and taking into account batch data. Limits provided for specified, unspecified and sum of impurities
- Nowadays quantitative tests preferred over comparative tests
- Peak identification and system suitability tests in chromatography performed using Ph. Eur. reference standards

Thank you for your attention



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