Impurity Control in the European Pharmacopoeia

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Agenda

- Which impurities are controlled?
- Analytical techniques and general texts/monographs
- Control of organic impurities
- What about validation?
- Control of elemental impurities / Q3D
- Summary

Control of impurities in the Ph. Eur.

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

Impurities in substances for pharmaceutical use: *General texts and monographs*

- **Organic impurities:**

- Represent an essential part of the specific monograph
- Control strategy follows ICH Q3 A
- 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 a total of impurities
- General chapters and texts, like 5.10: « *Control of impurities in substances for pharmaceutical use* »: helps to interpret the test for related substances in monographs on active substances

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?

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.

General monograph 2034

Related substances

- 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

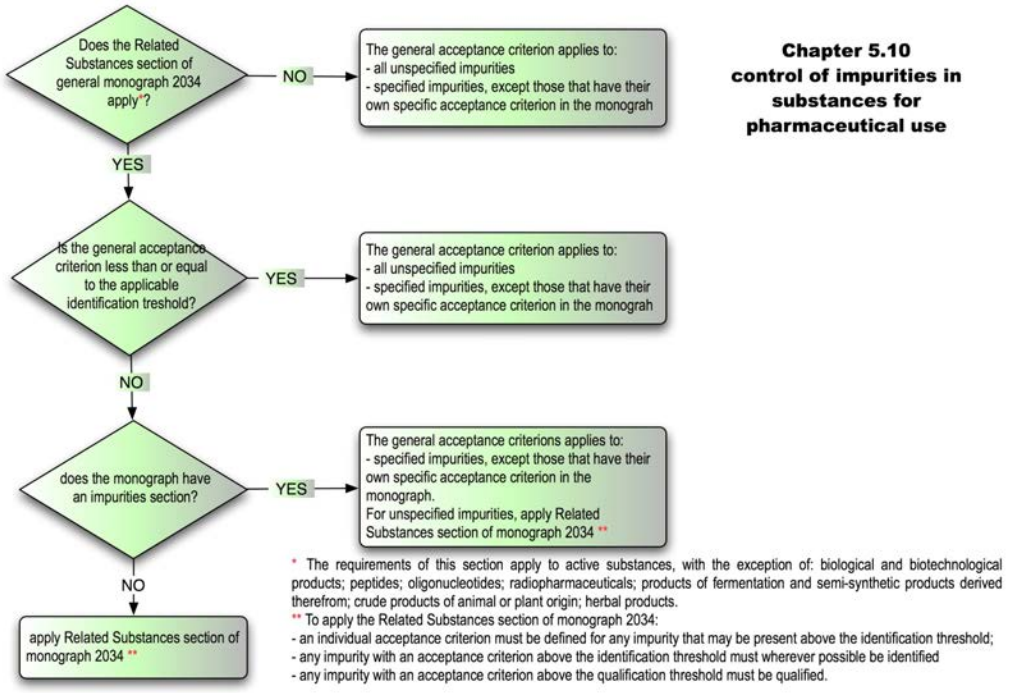
Use	Maximum daily dose	Reporting threshold	Identification threshold	Qualification threshold
Human or human and veterinary	≤ 2 g /day	>0.05 per cent	>0.10 per cent or daily intake >1.0 mg (whichever lower)	>0.15 per cent or daily intake >1.0 mg (whichever lower)
Human or human and veterinary	> 2 g/day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



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

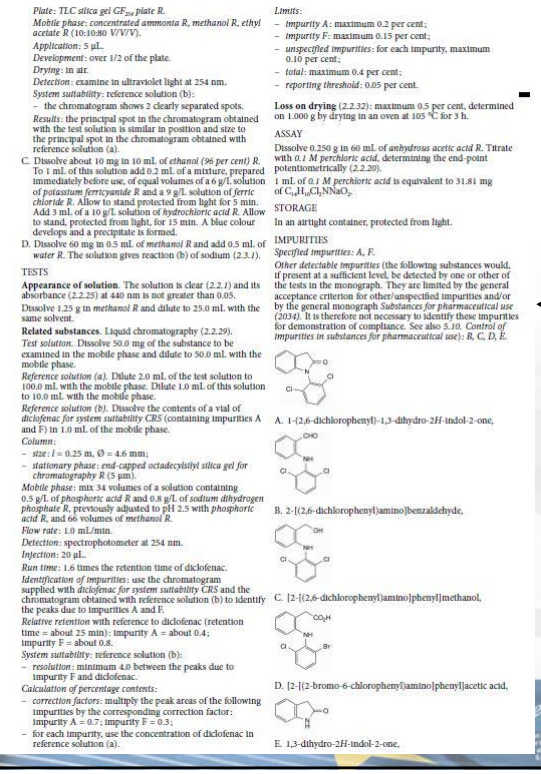


Plate: TLC silica gel GF_{254} plate R.
Mobile phase: concentrated ammonia R, methanol R, ethyl acetate R (10:10:80 V/V/V).
Application: 5 µL.
Development: over 1/2 of the plate.
Drying: in air.
Detection: examine in ultraviolet light at 254 nm.
System suitability: reference solution (b):
– the chromatogram 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)

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;

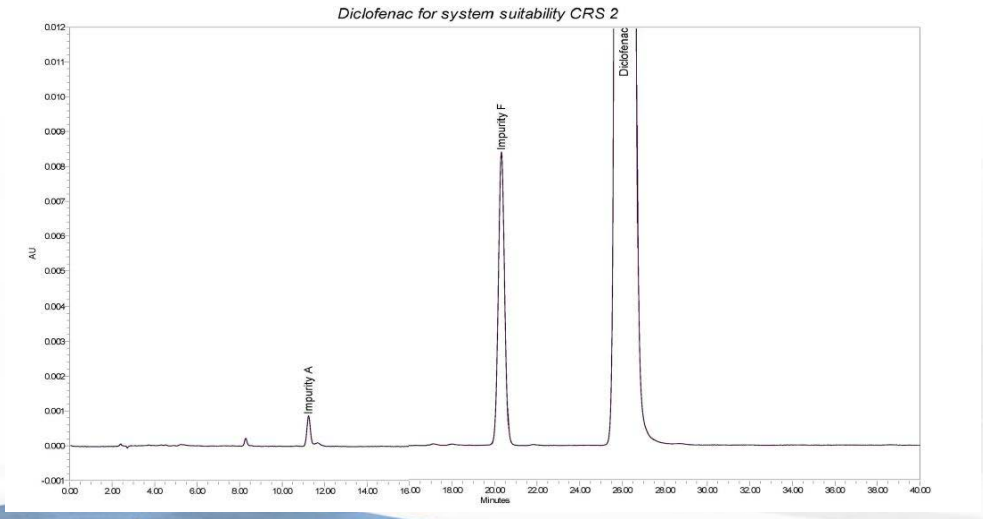
– *impurity F*: maximum 0.15 per cent;

– *unspecified impurities*: for each impurity, maximum 0.10 per cent;

– *total*: maximum 0.4 per cent;

– *reporting threshold*: 0.05 per cent.

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

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

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

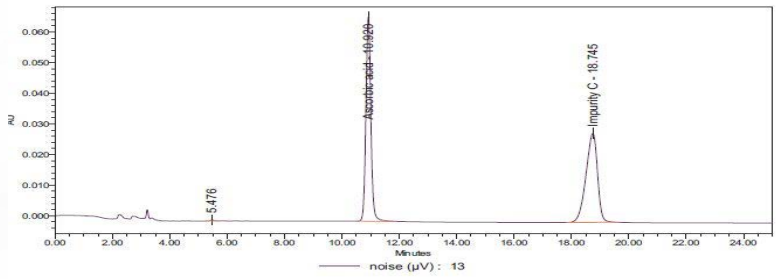
Ci = concentration of the impurity in milligrams per millilitre

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

Tests in Ph. Eur. are validated.

Amount of validation depends on the intended type of test: *quantitative* or *limit* test

Limit test:	**LOD and specificity**
Quantitative test:	**Accuracy, precision, specificity, LOQ, linearity and range**

Validation: Specificity

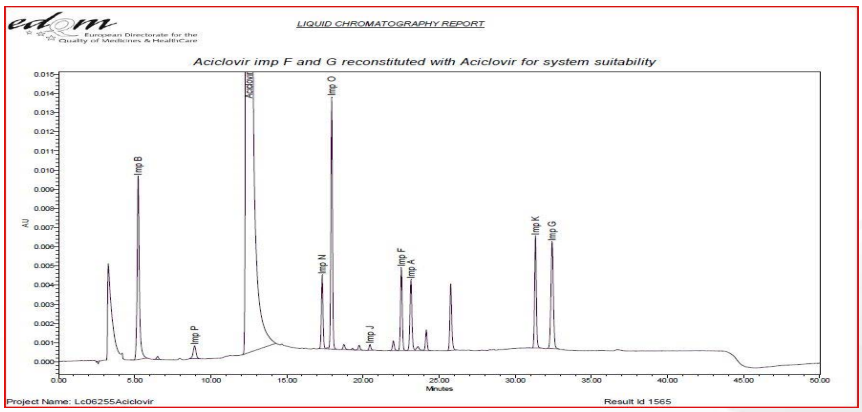
The first thing to look at:

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, degradants, matrix , etc.*

A prerequisite for specificity is *selectivity,* which, in chromatography means the ability of a method to separate the analyte from impurities or related compounds.

(In scientific literature the term « selectivity » is often used instead of the ICH term « specificity »)

Validation: Specificity/Selectivity



Critical separations: Impurities N – O

Impurities F – A

Impurities K - G

Validation: Specificity/Selectivity

How can selectivity be ensured?

Ph. Eur. describes system suitability tests in specific monographs **and** general texts

Resolution factor: …using two closely eluting peaks…there should be minimum base-line separation, i. e. Rs > 1.5

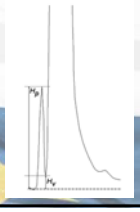
t_{R1}, t_{R2} = retention times of the peaks;

w_{h1}, w_{h2} = peak widths at half-height.

$$R_s = \frac{1.18\left(t_{R2} - t_{R1}\right)}{w_{h1} + w_{h2}}$$

- In cases where several closely eluting impurities are present, it may be useful to describe more than one resolution requirement…(particularly important in gradient systems)
- Peak-to-valley ratio: The minimum requirement for 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.

$$p/v = \frac{H_p}{H_v}$$



Validation: Accuracy

Accuracy: *..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.*

Typically accuracy can be assessed with recovery tests, i. e. a test sample is spiked with known amounts of an impurity which is then quantitatively determined by the chosen analytical procedure.

But how to quantify

Validation: LOD and LOQ

Limit of detection (LOD) and of quantification (LOQ)

Different ways of evaluation:

- Based on visual evaluation
- Based on signal-to-noise (S/N ratio):

  Ph. Eur. approach
- Based on standard deviation of the response and the slope of the calibration curve

Validation: Linearity and range

Linearity and range

The 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.

A linear relationship should be evaluated across the range of the analytical procedure. A minimum of 5 concentrations is recommended. Test results should be evaluated by appropriate statistical methods.

e. g. Impurity testing:

Linearity to be demonstrated in the range from reporting level or 50 % of the specification to 120 % of the specification

Validation: Precision

Precision

Is understood as:

- Repeatability – e. g. minimum 9 determinations covering the range (3 concentrations, 3 replicates)
  - Technical Guide: rsd of peak response of 3 injections of reference
  - solutions 0.1 % not more than 5 %
- Intermediate precision – repetition in one lab, but on different days, using different equipment, with different analysts, etc.
- Reproducibility – assessed by a interlaboratory trial: used for elaboration of standardised methods, e. g. Pharmacopoeias

Validation: Robustness

Robustness

... describes the reliability of an analysis with respect to deliberate variations in method parameters

e. g. variations in pH, mobile phase composition, temperature, choice of columns, flow rate etc.

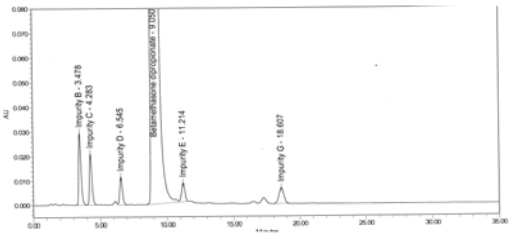
→ system suitability parameters should be maintained under these modified conditions

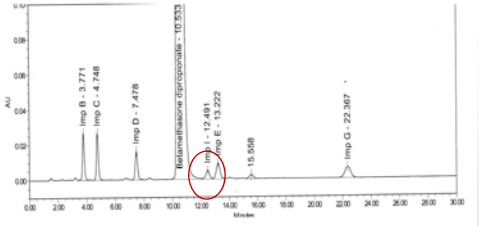
→ robustness should be considered during the development phase

Revision of a related substances test
Example: Betamethasone dipropionate

Revision of the related substances test of a monograph:

Addition of a specified impurity I, acceptance criterion 0.15 %





Old SST CRS

New SST CRS

What to do now?

Revision of a related substances test
Example : Betamethasone dipropionate

Revision of the related substances test of a 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

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.
- **Genotoxic (DNA-reactive) 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 substances only for veterinary use)
- Chapter 2.4.20 « Determination of elemental impurities » under revision in PDG

Elemental Impurities (2)

- Chapter 2.4.20

- This chapter is currently being harmonised in 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.

Elemental Impurities (3)

- Specific metal tests

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

Thank you for your attention !