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Guideline on setting specifications for related impurities in antibiotics

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Executive summary

Antibiotics active substances currently on the market are produced by fermentation, by fermentation followed by one or more synthetic steps (semi-synthetic substances) or by chemical synthesis. Fermentation processes are, in comparison to synthetic processes, more variable and less controllable, so the impurity profile of an active substance whose manufacturing process involves fermentation may be more complex and less predictable than that of a purely synthetic product. For this reason fermentation products and semi-synthetic substances are not included in the scope of the ICH Q3 and the VICH GL10/GL11 guidelines, which set thresholds for the identification, reporting and qualification of related impurities in active substances manufactured by chemical synthesis.

This guideline has been developed in order to provide guidance on how specifications for related impurities in antibiotics that are fermentation products or semi-synthetic substances derived from fermentation products, and are therefore not included in the scope of the (V)ICH guidelines mentioned above, should be set.

Thresholds are given in the guideline for reporting, identification and qualification of related impurities for antibiotics medicinal products whose active substance is produced by fermentation or semi-synthesis. In cases where the active substance consists of a mixture of closely related compounds, where it may be difficult to apply general thresholds, general guidance is given on how to set specific thresholds and specifications and on how to qualify impurity profiles. The relationships between the requirements in the guideline and the applicable Ph.Eur. chapters and monographs are also addressed.

1. Introduction (background)

Most of the antibiotics currently on the market are produced by fermentation or chemical synthesis. In certain cases the chemical structure of the antibiotics obtained by fermentation is further modified by some synthetic steps, before the substance is used as an active substance in the manufacture of medicinal products (semi-synthetic substances).

Fermentation processes involve biological systems which are less predictable, less controllable and more complex than straightforward chemical reactions. Because of this, the variability in products derived by fermentation is often greater than in products derived by chemical synthesis. Thus, the impurity profile of a fermentation product may be more complex and less predictable than that of a synthetic product.

For this reason, fermentation products and semi-synthetic substances derived from them are not included in the scope of the ICH Q3 and the VICH GL10/GL11 guidelines that set thresholds for the identification, reporting and qualification of related impurities in active substances manufactured by chemical synthesis. These thresholds are defined in the guidelines as limits above which an impurity has to be either identified, reported or qualified, and the same limits are applied in the Ph.Eur. general monograph 'Substances for Pharmaceutical Use'. Fermentation products and their semi synthetic derivatives are also excluded from the scope of this general monograph.

In the absence of other guidance, related impurities in these products have been assessed on a case-by-case basis, which has resulted in the acceptance of different impurity thresholds for the same antibiotic and for different compounds within the same class (e.g. cephalosporins). There is also a need to ensure that the authorisation of new antibiotics is enabled by consistent approaches in setting limits for their impurities.

It is therefore necessary to provide guidance, based on current practice and experience, to formulate general recommendations for impurity thresholds in antibiotics produced by fermentation or semi-synthesis. These are presented in this guideline.

Even so, it is acknowledged that in some cases higher thresholds may be acceptable if necessary and justified taking account of use and exposure of the drug substance/product. This would also include analytical problems (see Annex 1: Explanatory note regarding thresholds).

2. Scope

This document provides guidance for marketing authorisation applications on setting specifications for related impurities in antibiotics (i.e. antibacterial substances) that are fermentation products or semi-synthetic substances derived from fermentation products. It is foreseen to widen the scope to other antibiotics (e.g. antifungal substances) at a later stage.

It provides guidance for the content and qualification of related impurities in both active substances and medicinal products. The guideline is not intended to apply to new active substances used in investigational medicinal products used in clinical trials.

In this guideline thresholds are given for reporting, identification and qualification of related impurities. For antibiotics where the active substance consists of a mixture of closely related compounds where it may be difficult to apply these general thresholds, general guidance is given on how to set thresholds and specifications and how to qualify impurity profiles. The thresholds given in this guideline would represent a general set of requirements, and this could be subject, for specific substances or products, to adaptation to the specific situation. Further requirements might be introduced when considered necessary, e.g. for safety reasons.

This guideline does not cover residues from the fermentation process, i.e. residues from the producer micro-organism, culture media, substrates and precursors; this is covered by the Ph.Eur. general monograph 'Products of fermentation'. (This monograph applies to substances manufactured by fermentation only, and not to substances manufactured by semi-synthesis).

This guideline applies to new active substances and for new sources of existing active substances. It is the Applicant's responsibility to demonstrate that the active substance has already been marketed in the EU when relevant.

The guideline should not be applied retrospectively, but it is intended that this guideline will act as a stimulus to establish best practice and to initiate the revision of relevant Ph.Eur. monographs (i.e. for registered products revised requirements according to the monograph will apply when the monograph is introduced/revised). For new sources of existing active substances this guideline should be read in conjunction with any existing Ph.Eur. monograph for the active substance. It should be noted that comparison with impurity levels/profiles of active substance sources or products approved in the EU is one of the options for qualifying impurities.

It is foreseen to re-evaluate the Scope when more experience has been obtained.

3. Legal basis

This guideline has to be read in conjunction with the introduction and general principles (4) and part 1 of the Annex Is to Directives 2001/82/EC and 2001/83/EC as amended.

4. General requirements

The impurity profile depends very much on the manufacturing process; even for the same strain of a micro-organism, impurity profiles may be different. In general, purification steps including column chromatography and ultra-filtration steps may be crucial to achieve a sufficiently pure active substance.

Semi-synthetic substances are not within the scope of the Ph.Eur. general monograph 'Products of fermentation'. However, the specification of the fermented starting material should be justified with reference to current guidance, including general concepts described in this general monograph, if necessary. (The thresholds in this guideline are not intended to apply for fermented starting materials).

The shorter the synthetic route after the fermentation and the more complex the fermented starting material, the more relevance the general monograph has. Therefore, a detailed description of the fermentation steps as well as other aspects addressed in the general monograph, in particular purification steps, should be presented for semi-synthetic antibiotics, unless justified by the non-complexity of the fermented starting material and the number and/or nature of the synthetic steps following fermentation.

These synthetic steps should contribute to a relevant depletion and inactivation of fermentation by-products in the final active substance, so for example, esterification, etherification and salification of fermentation products (e.g., erythromycin derivatives like erythromycin ethylsuccinate or erythromycin lactobionate) are not considered as significant synthetic steps which would justify an omission of a detailed description of the fermentation process, in particular of the purification.

In cases where the fermented starting material is not complex and taking into consideration the number and nature of the synthetic steps after fermentation, it may be sufficient to have a suitable specification for the fermented starting material including assay, component distribution (if relevant) and related impurities (specified, unspecified, and total). This should be in any case justified. For active substances manufactured by semi-synthesis, the impurity profile of the fermented starting material should be critically evaluated for its contribution to the impurity profile of the final active substance.

Related impurities observed after fermentation include by-products, intermediates and degradation products. For semi-synthesis the impurities also include the fermented starting material and related substances in this starting material, synthesis by-products (including those derived from impurities in the starting material), synthesis intermediates and degradation products.

Specifications should be given for any critical intermediates (this also includes intermediates between different purification steps). These specifications should include limits for specified and single unspecified impurities. Impurities that contribute to the impurity profile of the active substance should be specified. The applicant should provide a discussion on potential impurities, how they are removed and which impurities appear in the active substance.

Even if manufactured by fermentation or semi-synthesis, an antibiotic may be structurally well defined as a mono component substance, and thus it may be efficiently purified. For each class of antibiotic, it is considered preferable to optimise purification steps as far as possible, in order to decrease the level of impurity to below the qualification threshold, than to provide (additional) safety data.

For antibiotics manufactured by fermentation, the active substance may consist of a mixture of closely related compounds that show the relevant biological activity. In such cases it may be difficult to decide whether a compound is part of the active substance or should be regarded as an impurity when setting specifications (e.g. gentamicin). The definition of which substances are components of the active

substance should be based on pre-clinical and clinical studies unless the active substance is described in a Ph. Eur. monograph where the active substance components are defined. Related compounds that are not defined to be components of the active substance are regarded as related impurities.

The thresholds given in the ICH Q3 and VICH GL10/GL11 guidelines and in the guideline 'Chemistry of New Active Substances' (CPMP/QWP/130/96 Rev 1, EMEA/CVMP/541/03) do not apply to fermentation products and semi-synthetic substances derived from fermentation products. For other aspects, where specific guidance is not given in the present guideline, reference is made to the principles described in these guidelines.

In qualifying an impurity or a given impurity profile at the level specified, several possibilities exist: appropriate battery of non-clinical tests, literature based data; comparison with impurity levels/profiles of active substance sources/products approved in the EU; or proving that the relevant impurity is a significant metabolite of the active substance.

5. Impurity profiling and reporting, identification and qualification thresholds

For antibiotic drug substances, the impurity profile should be characterised according to the guidance described in ICH Q3A (VICH GL10).

In accordance with that guidance, with respect to related substances, limits should be set for:

- Each specified identified impurity;
- Each specified unidentified impurity;
- Any unspecified impurity, with an acceptance criterion of not more than the identification threshold;
- Total impurities.

Exceptionally, if it is shown that it is not practically possible to identify an individual impurity, sufficient evidence of its structure should be provided (e.g. by HPLC/mass spectrometry to show that it may be satisfactorily classified as a related substance of the parent compound. In this case, it should be specified using an appropriate analytical marker e.g. HPLC Relative Retention Time (RRT), as a specified unidentified impurity. As a general principle, for impurities which are not structurally closely related (see section 5.3 below) to the parent compound, thresholds as given by ICH Q3A (VICH GL10) should be applied unless stated differently in the following sections.

For the reasons discussed in section 4 above, and taking into account that the duration of treatment with antibiotics is in most cases limited, for antibiotic related substances the thresholds to be applied may be higher than those stated in ICH Q3A/VICH GL10, and also different for each of the different classes of antibiotic. These thresholds are given below.

5.1. Active substances manufactured by semi-synthesis

Semi-synthetic substances are obtained from a fermented starting material by a process involving at least cleavage and formation of covalent bonds and including extraction/purification steps. Acceptance criteria for related impurities should be set in accordance with the thresholds given below.

The ICH Q3A thresholds for reporting, identification and qualification apply.

Reporting threshold: 0.05%/0.03%

Identification threshold: 0.10%/0.05%

Qualification threshold: 0.15%/0.05%

If the semi-synthetic active substance consists of a family of closely related compounds it may be necessary to apply requirements up to the thresholds described for substances manufactured by fermentation, family of compounds (see 5.3). A justification should be given.

5.2. Active substances manufactured by fermentation, single compound

Acceptance criteria for related impurities should be set in accordance with the thresholds given below.

Reporting threshold: 0.10%

Identification and qualification thresholds: 0.15%

5.3. Active substances manufactured by fermentation, family of compounds

Acceptance criteria for related impurities should be set in accordance with the thresholds given below.

Reporting threshold: 0.10%

Identification threshold: 0.15%

Qualification threshold: 0.50%/0.2%

The qualification threshold of 0.50% for structurally closely related impurities (see definition) is combined with a qualification threshold of 0.2% for other related impurities. Justification for claiming that a related impurity (compound not defined to be included in the active substance) is structurally closely related to the parent compounds should at least be based on evidence such as HPLC/mass spectrometry or the use of reference materials. The proposed 0.50%/0.2% limits are suggested to apply even for daily doses of ≥ 2 g, which may be relevant for some of these antibiotics.

5.4. Peptides manufactured by fermentation/semi-synthesis

For antibiotics that are peptides (e.g. gramicidin, tyrothricin and bacitracin) the same thresholds as for synthetic peptides as given in the Ph. Eur. General Monograph Substances for Pharmaceutical would be considered acceptable without any justification. Other thresholds should be justified. These thresholds do not apply to certain modified peptides containing other moieties than amino acids (e.g. glycopeptides).

5.5. Active substances for veterinary use

For active substances used in veterinary medicine only the VICH GL 10 thresholds for reporting, identification and qualification (of 0.10%, 0.20% and 0.50%, respectively) apply. For active substances used in veterinary medicines only, manufactured by fermentation and consisting of a family of compounds, the thresholds may be justified and assessed on a case-by-case basis.

For active substances used in both veterinary and human medicine, the thresholds given for the respective human classes above apply.

5.6. Special cases for very complex impurity profiles

In case of a very complex impurity profile or where two impurities are very similar, it may not be technically feasible to obtain peak separation. In such cases it may be necessary to set a limit for a combination of unresolved peaks. In this case, where possible, thresholds should be applied for the

combination of peaks. For qualification, the composition of the batches used in the toxicological studies should be taken into account.

Exceptionally, for controlling very complex impurity profiles where identification of individual peaks is impossible, the applicant should propose a combination of different tests that give a reasonable batch consistency with regard to impurities. Where an applicant has qualified a drug substance (from a given manufacturer) and needs to ensure consistency with future batches then as a minimum it could be possible to characterise the impurity profiles by a descriptive specification based on a sufficient number of manufactured batches.

A descriptive specification could consist of the following parameters: (1) 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. (2) 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%). (3) 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 (NMT) C%, but NMT one peak above D% whereby $C > D$, e.g. $C = 2.0\%$, $D = 1.5\%$ or similar dependent on drug substance and drug product profile). This approach should be restricted to active substances consisting of a mixture of components. An example is given in Annex 3 to this guideline.

6. New applications and variations

6.1. New active substances

The impurity profile should be characterised and individual impurities should be identified and, if necessary, qualified by an appropriate battery of non-clinical and clinical tests.

6.2. Existing active substances, not subject to a Ph. Eur. monograph

The impurity profile should be characterised and individual impurities should be identified and, if necessary, qualified as described in the General requirements section.

6.3. Active substances subject to a Ph. Eur. monograph

6.3.1. Existing active substances subject to a Ph. Eur. monograph with transparency statement and availability of a CRS for peak identification or relative retention times for the related substances

The impurity profile should be characterised and individual impurities identified.

Specified impurities should be controlled according to the monograph requirements.

New impurities should be identified, when necessary to comply with this guideline.

New impurities should be qualified when necessary to comply with this guideline as described in the General requirements section.

6.3.2. Existing active substances subject to Ph. Eur. monograph, with transparency statement, but no availability of a CRS for peak identification or relative retention times for the related substances

The impurity profile should be characterised and individual impurities identified, when necessary to comply with this guideline, using as reference the transparency statement of the monograph.

Specified impurities should be controlled according to the monograph requirements.

Any new impurity should be identified and qualified, when necessary, to comply with this guideline as described in the General requirements section.

6.3.3. Existing active substances subject to Ph. Eur. monograph, without transparency statement

The impurity profile should be characterised and individual impurities identified, when necessary to comply with this guideline.

Impurities should be qualified, when necessary to comply with this guideline as described in the General requirements section.

6.3.4. Revision of Ph. Eur. monographs

A revision of the Ph. Eur. monograph should be initiated when:

- The means of identification of known impurities have been established
- New impurities have been identified or qualified

According to Directives 2001/82/EC and 2001/83/EC as amended, the Pharmacopoeia should be informed by the relevant authority when a monograph is insufficient to control the quality of a substance.

7. Specifications for medicinal products

Specifications should be set for related impurities that are degradation products. Impurities originating from the manufacture of the drug substance should not be specified unless they are also degradation products.

Information on the impurity profile may be obtained from the source of the active substance.

Acceptance criteria for related impurities should be set within the thresholds given below. The same specifications should apply to the product after any opening/reconstitution/dilution (in-use shelf life) as for the finished product, unless justified by suitable qualification data e.g. by comparison to levels found in an approved product. New degradants not included in the finished product specification should be listed in a specification for the reconstituted product. Data for in-use shelf life after reconstitution/dilution should be provided.

Active substance manufactured by semi-synthesis:

Reporting threshold: 0.1%

Identification and qualification thresholds: 0.2%

Active substance manufactured by fermentation, single compound:

Reporting threshold: 0.15%

Identification and qualification thresholds: 0.2%

Active substance manufactured by fermentation, family of compounds:

Reporting threshold: 0.15%

Identification threshold: 0.2%

Qualification threshold: 0.5%/0.2% (see explanation in section 5.3)

For all three groups of active substances, higher acceptance criteria for identification and qualification may be set according to the doses/thresholds in ICH Q3B for low doses.

For veterinary medicinal products the VICH GL11 thresholds for reporting, identification and qualification (0.3%, 1.0% and 1.0%, respectively) should be applied. For active substances used in veterinary medicines only, manufactured by fermentation and consisting of a family of compounds, thresholds may be justified and assessed on a case-by-case basis.

8. Analytical procedures

When analysing the final active substance and the medicinal product, whenever possible, an external standard should be used calculating w/w to evaluate and exclude any possible mass imbalance. If using area normalisation the relevant components and related impurities should give similar responses in the detector (see Ph. Eur. 2.2.46). Otherwise correction factors should be used (the use of correction factors is always expected for impurities which do not give similar response to any standard used, except for unknown impurities).

Area normalisation (2.2.46) may (instead of using an external standard) be acceptable for certain active substances consisting of a family of compounds. This may be used when analysing relevant intermediates. When using area normalisation, linearity for the intended range should be demonstrated and an unambiguously defined disregard criterion should be given.

When performing qualification of an impurity profile versus an approved product, a sufficiently specific analytical procedure should be used. For complicated mixtures the separation technique (e.g. HPLC) should be combined with mass spectrometry (or other techniques). For routine testing, simpler procedures may be used, if justified.

The quantitation limit for the analytical procedure should be not more than (\leq) the reporting threshold. For substances having weak chromophores, other detection methods than UV absorption should be used. If in some cases it is not possible to report or identify impurities at the thresholds given in this guideline, this will be taken into account in the assessment.

Definitions

Product of fermentation: Indirect gene products (primary or secondary metabolites) of micro-organisms such as bacteria, yeast, fungi and micro-algae, irrespective of whether or not the micro-organism have been modified by traditional procedures or by recombinant DNA technology.

Semi-synthesis: One or more synthesis steps following fermentation. A synthesis step involves cleavage and formation of covalent bonds.

Single compound: The active substance consists of only one compound, as usual.

Family of compounds: The active substance consists of a mixture of compounds. The composition regarding names and amounts of relevant components is defined in the active substance specification in accordance with composition used in pre-clinical and clinical studies. The composition will appear in any Ph. Eur. monograph available.

Complex/not complex fermented starting material for semi-synthesis: The starting material is not complex if the molecular entity is simple, comprehensive characterisation is possible and the

impurity profile can be provided. The required level of documentation for a complex starting material depends on whether there is a significant number of chemical process steps compared to few biological ones and whether the process is capable of reducing related impurities arising from the biological process to a level that they do not contribute to the impurity profile of the active substance.

Structurally closely related impurity: A higher threshold for qualification of 0.50% applies if an impurity is structurally closely related to an active substance containing more than one active compound. All related compounds that are not included in the definition of the active substance are regarded as impurities. Factors that should be discussed when claiming structural similarity include: (1) The impurity should be a specified identified impurity. If it is not identified, the qualification threshold of 0.2% applies. (2) It should have the same physico-chemical (including spectral) characteristics as the compounds constituting the active substance. (3) It should have major characteristic parts in common with the compounds of the active substance. (4) Only variations within the moieties that vary between the active substance components are allowed. Typical variations could be minor differences in alkyl chains (different branching, one less or additional methylene unit) or exchange of a hydrogen atom with a methyl group not only in an alkyl chain. (5) No new genotoxic structural alerts of very high toxicological concern, e.g. N-nitroso, epoxy or azoxy.

References

1. 'Impurities in new drug substances (revised)' (CPMP/ICH/2737/99) (ICH Q3A(R))
2. 'Impurities in new drug products' (CPMP/ICH/2738/99) (ICH Q3B(R))
3. 'Control of impurities of pharmacopoeial substances' (CPMP/QWP/1529/04 and EMEA/CVMP/059/04-FINAL)
4. 'Specifications: test Procedures and acceptance criteria for new drug substances and new drug products: chemical substances' (CPMP/ICH/367/96) (ICH Q6A)
5. 'Assessment of the quality of medicinal products containing existing/known active substances' (EMEA/CHMP/CVMP/QWP/296289/2008)
6. 'Impurities in new veterinary drug substances' (EMEA/CVMP/VICH/837/99-Rev.1) (VICH GL10(R))
7. 'Impurities in new veterinary medicinal products' (EMEA/CVMP/VICH/838/99-Rev.1) (VICH GL11(R))
8. 'Test procedures and acceptance criteria for new veterinary drug substances and new medicinal products: chemical substances' (EMEA/CVMP/VICH/810/04-corrigendum) (VICH 39)
9. 'Chemistry of New Active Substances' (CPMP/QWP/130/96 Rev 1)
10. 'New impurities control: setting specifications for antibiotics and synthetic peptides: proceedings from EDQM symposium, Strasbourg 21-22 September 2006'
11. European Pharmacopoeia general monograph 'Substances for Pharmaceutical Use'
12. European Pharmacopoeia general chapter 5.10 'Control of impurities in substances for pharmaceutical use'
13. European Pharmacopoeia general monograph 'Products of fermentation'
14. 'Development and Manufacture of Drug Substances (chemical entities and biotechnological/biological entities)' (CHMP/ICH/425213/2011) (ICH Q11)

Annex 1: Explanatory note regarding thresholds

In setting up the thresholds, the antibiotics have been classified by the method of their preparation (whether they are prepared by fermentation only or the fermentation is followed by synthetic steps) and their composition (whether the antibiotic is a single substance or a mixture of closely related compounds). Thus, the differences in thresholds for reporting, identification and qualification between these different classes of antibiotics are mainly for technical/practical reasons.

As a background for the proposed thresholds current practice for Ph.Eur. monographs and assessment practice in connection with the issuing of Certificates of Suitability has been considered.

Active substances manufactured by semi-synthesis:

Purification steps and the subsequent synthetic steps make it possible to obtain active substances with low levels of impurities. In many cases, the designated "starting material" for the synthetic steps is a well characterised compound of good purity (e.g. 6-APA and 7-ACA), similar to designated starting materials manufactured by synthesis. Therefore ICH thresholds (Q3A and VICH GL 10) are proposed.

Active substances manufactured by fermentation, single compound:

Consisting of only one active compound these substances are relatively easy to purify, and as a consequence relatively low thresholds are possible.

Active substances manufactured by fermentation, family of compounds:

When the active substance is a mixture of closely related compounds it is difficult to purify this mixture from other closely related compounds present (and excessive purification could also lead to a different component distribution). Some of these other components have similar antibiotic activity as the components defined to be included in the active substance, while other components do not have the same activity. These components are handled as related impurities, but due to the complex situation it is difficult to set very low thresholds. It is proposed to have a relatively low qualification threshold, and to include the possibility of having a wider qualification threshold for structurally closely related substances (based on evidence such as HPLC/mass spectrometry).

In some cases for the fermentation products it may not be possible to adhere to the thresholds in this guideline. Problems regarding reporting threshold are addressed in the chapter Analytical procedures. Problems regarding identification are addressed in the chapter Impurity profiling and reporting, identification and qualification thresholds. Regarding qualification there are several options given, including comparison with products on the market. Some products on the market contain unknown impurities above the identification thresholds stated in this guideline. If the same substance is to be used for a new application, this impurity should preferably be identified. In any case, a revision of the Ph. Eur. monograph should be requested. For especially problematic existing active substances such as teicoplanin and gentamicin it is known that there will be problems in meeting the reporting and identification thresholds. This has been taken into account in the Scope of the guideline and in section 8 Analytical procedures.

Annex 2: Thresholds

Human

Active substances	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%

Products	Semi-synthetic *	Fermentation, single	Fermentation, family	Peptides
Reporting	0.1%	0.15%	0.15%	0.1%
Identification	0.2%	0.2%	0.2%	0.5%
Qualification	0.2%	0.2%	0.5%**/0.2%	1.0%

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

***) Structurally closely related impurity according to definition

Veterinary only

Active substances	Semi-synthetic	Fermentation, single	Fermentation, family
Reporting	0.10%	0.10%	0.10% or case-by-case
Identification	0.20%	0.20%	0.20% or case-by-case
Qualification	0.50%	0.50%	0.50% or case-by-case

Products	Semi-synthetic	Fermentation, single	Fermentation, family
Reporting	0.3%	0.3%	0.3% or case-by-case
Identification	1.0%	1.0%	1.0% or case-by-case
Qualification	1.0%	1.0%	1.0% or case-by-case

Annex 3: Example of “fingerprint chromatogram” approach to control very complex impurity profiles

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 $C > D$, e.g. $C = 2.0 \%$, $D = 1.5 \%$ or similar dependent on drug substance and drug product profile)

Example:

Purity specification of Vancomycin

Vancomycin B: NLT 93.0 %

Ph. Eur. impurity A	NMT 2.0 %
Ph. Eur. impurity B	NMT 2.0 %
Ph. Eur. impurity C	NMT 2.0 %
Ph. Eur. impurity D	NMT 2.0 %
Individual unknown impurities with RRT 0.4 – 0.8	NMT 1.0 %
Individual unknown impurities with RRT 1.4 – 1.7	NMT 1.0 %
Other unknown impurities	NMT 0.3 %
Sum of all impurities	NMT 7.0 %

Corresponding Chromatograms of two API batches

