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5 **Guideline on setting specifications for related impurities in**  
6 **antibiotics**  
7 **Draft**

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14 **in Antibiotics**

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## 43 **Executive summary**

44 Antibiotics active substances currently on the market are produced by chemical synthesis,  
45 fermentation or fermentation followed by one or more synthetic steps (semi-synthetic substances).  
46 Fermentation processes are, in comparison to synthetic processes, more variable and less controllable,  
47 so the impurity profile of an active substance whose manufacturing process involve fermentation may  
48 be more complex and less predictable than that of a purely synthetic product. For these reasons  
49 fermentation products and semi-synthetic substances are not included in the scope of the ICH Q3 and  
50 the VICH 10/11 guidelines, that set thresholds for identification, reporting and qualification of related  
51 impurities in active substances manufactured by chemical synthesis.

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

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

## 62 **1. Introduction (background)**

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

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

72 For these reasons, fermentation products and semi-synthetic substances derived from them are not  
73 included in the scope of the ICH Q3 and the VICH 10/11 guidelines that set thresholds for  
74 identification, reporting and qualification of related impurities in active substances manufactured by  
75 chemical synthesis. These thresholds are defined in the guidelines as limits above which an impurity  
76 has to be either identified reported or qualified, and the same limits are applied in the Ph.Eur. general  
77 monograph 'Substances for pharmaceutical use'. Fermentation products and their semi synthetic  
78 derivatives are also excluded from the scope of this general monograph.

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

84 It is therefore necessary to provide guidance, based on current practice and experience, to formulate  
85 general recommendations for impurity thresholds in antibiotics produced through fermentation. These  
86 are presented in this guideline.

87 Even so, it is acknowledged that in some cases higher thresholds may be acceptable if necessary and  
88 justified taking account of use and exposure of the drug substance/product.

## 89 **2. Scope**

90 This document provides guidance for marketing authorisation applications on setting specifications for  
91 related impurities in antibiotics (i.e. antibacterial substances) that are fermentation products or semi-  
92 synthetic substances derived from fermentation products. It is foreseen to widen the scope to other  
93 antibiotics (e.g. antifungal substances) at a later stage. It provides guidance for the content and  
94 qualification of related impurities in active substances and medicinal products. The guideline is not  
95 intended to apply to new active substances used in investigational medicinal products used in clinical  
96 trials.

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

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

108 This guideline applies for new applications for marketing authorisation and for new manufacturer  
109 variations. The guideline will not be applied retrospectively, but it is intended that this guideline will act  
110 as a stimulus to establish best practice and to initiate the revision of relevant Ph.Eur. monographs. For  
111 new applications this guideline should be read in conjunction with any existing Ph.Eur. monograph for  
112 the active substance.

## 113 **3. Legal basis**

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

## 116 **4. General requirements**

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

121 Semi-synthetic substances are not within the scope of the Ph.Eur. general monograph 'Products of  
122 fermentation'. However, the specification of the fermented starting material should be justified with  
123 reference to current guidance, including general concepts described in this general monograph, if  
124 necessary.

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

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

136 In cases where the fermented starting material is not complex and taking into consideration the  
137 number and nature of the synthetic steps after fermentation, it may be sufficient to have a suitable  
138 specification for the fermented starting material including assay, component distribution (if relevant)  
139 and related impurities (specified, unspecified, and total). This should be in any case justified.

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

144 Specifications should be given for critical intermediates. These specifications should include limits for  
145 specified and single unknown impurities. The applicant should provide a discussion on potential  
146 impurities, how they are removed and which impurities appear in the active substance.

147 Even if manufactured by fermentation or semi-synthesis, an antibiotic may be structurally well defined,  
148 and thus it may be efficiently purified. For active substances manufactured by semi-synthesis, the  
149 quality of the fermented starting material may be important.

150 For antibiotics manufactured by fermentation, the active substance may consist of a mixture of closely  
151 related compounds that show the relevant biological activity. In such cases it may be difficult to decide  
152 whether a compound is part of the active substance or should be regarded as an impurity when setting  
153 specifications (e.g. gentamicin). The definition of which substances are components of the active  
154 substance should be based on pre-clinical and clinical studies unless the active substance is described  
155 in a Ph.Eur. monograph where the active substance components are defined. Related compounds that  
156 are not defined to be components of the active substance are regarded as related impurities.

157 The thresholds given in the ICH Q3 and VICH GL 10/11 guidelines and in the guideline 'Chemistry of  
158 New Active Substances' (CPMP/QWP/130/96 Rev 1, EMEA/CVMP/541/03) do not apply to fermentation  
159 products and semi-synthetic substances derived from fermentation products. For other aspects, where  
160 specific guidance is not given in the present guideline, reference is made to the principles described in  
161 these guidelines.

## 162 **5. Impurity profiling and reporting, identification and** 163 **qualification thresholds**

164 For antibiotic drug substances, the impurity profile should be characterised according to the guidance  
165 described in ICH 3QA (VICH GL10).

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

- 167 • Each specified identified impurity

- 168 • Each specified unidentified impurity
- 169 • Any unspecified impurity, with an acceptance criterion of not more than the identification  
170 threshold
- 171 • Total impurities.

172 Exceptionally, if it is shown that it is not practically possible to identify an individual impurity, then as a  
173 minimum, sufficient evidence of its structure should be provided to show that it may be satisfactorily  
174 classified as a related substance of the parent compound. In this case, it should be specified using an  
175 appropriate analytical marker e.g. HPLC Relative Retention Time, as a specified unidentified impurity.

176 In case of a very complex impurity profile or where two impurities are very similar, it may not be  
177 technically feasible to obtain peak separation. In such cases it may be necessary to set a limit for a  
178 combination of unresolved peaks. In this case, where possible, thresholds should be applied for the  
179 combination of peaks. For qualification, the composition of the batches used in the toxicological studies  
180 should be taken into account.

181 As a general principle, for impurities which are not structurally closely related (see section 5.3 below)  
182 to the parent compound, thresholds as given by ICH Q3A (VICH GL10) should be applied unless stated  
183 differently in the following sections.

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

### 188 ***5.1. Active substances manufactured by semi-synthesis***

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

192 The Q3A thresholds for reporting, identification and qualification apply. For active substances used in  
193 veterinary medicine only the VICH GL 10 thresholds for reporting, identification and qualification (of  
194 0.10%, 0.20% and 0.50%, respectively) apply.

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

### 198 ***5.2. Active substances manufactured by fermentation, single compound***

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

200 Reporting threshold: 0.10%

201 Identification threshold: 0.15%

202 Qualification threshold: 0.15%

203 For active substances used in veterinary medicine only the VICH GL 10 thresholds for reporting,  
204 identification and qualification (of 0.10%, 0.20% and 0.50%, respectively) apply.

205 **5.3. Active substances manufactured by fermentation, family of**  
206 **compounds**

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

208 Reporting threshold: 0.10%

209 Identification threshold: 0.15%

210 Qualification threshold: 0.50%/0.15%

211 For active substances used in veterinary medicine only, the following thresholds apply:

212 Reporting threshold: 0.10%

213 Identification threshold: 0.20%

214 Qualification threshold: 0.50%

215 The qualification threshold of 0.50% for structurally closely related compounds is combined with a  
216 qualification threshold of 0.15% for other related compounds. Justification for claiming that a related  
217 impurity (compound not defined to be included in the active substance) is structurally closely related to  
218 the parent compounds should at least be based on evidence such as HPLC/mass spectrometry or  
219 HPLC/diode-array detection or the use of reference materials. The proposed 0.50%/0.15% limits are  
220 suggested to apply even for daily doses of  $\geq 2$  g, which may be relevant for some of these antibiotics.

221 For each class of antibiotic, it is better and may be easier, to optimise purification steps, in order to  
222 decrease the level of impurity to below the qualification threshold, than providing safety data.

223 It is also acknowledged for known active substances that comparative impurity profiles with innovator  
224 material may be supportive in the characterisation of impurity profiles.

225 **6. New applications and variations**

226 **6.1. New active substances**

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

229 **6.2. Known active substances, not subject to a Ph.Eur. monograph**

230 The impurity profile should be characterised and individual impurities should be identified and, if  
231 necessary, qualified by an appropriate battery of non-clinical tests and other suitable means, including  
232 reference to already approved material.

233 **6.3. Active substances subject to a Ph.Eur. monograph**

234 **6.3.1. Known active substances subject to a Ph.Eur. monograph with**  
235 **transparency statement and the means of their identification<sup>1</sup>**

236 The impurity profile should be characterised and individual impurities identified.

237 Known impurities should be controlled according to the monograph requirements.

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

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<sup>1</sup> Some monographs include a section, "Identification of impurities", where relative retention with reference to the main peak is described for the principal impurity peaks. In some cases the use of Ph.Eur. CRS is described.

239 New impurities should be qualified when necessary to comply with this guideline by the appropriate  
240 battery of non-clinical tests or other suitable means.

### 241 **6.3.2. Known active substances subject to Ph.Eur. monograph, with** 242 **transparency statement, but no means of their identification**

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

245 Known impurities should be controlled according to the monograph requirements.

246 Any new impurities should be identified and qualified, when necessary to comply with this guideline, by  
247 the appropriate battery of non-clinical tests or by other appropriate means, including reference to  
248 innovator material.

### 249 **6.3.3. Known active substances subject to Ph.Eur. monograph, without** 250 **transparency statement**

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

253 Impurities should be qualified, when necessary to comply with this guideline, by the appropriate  
254 battery of non-clinical tests and by other means, including reference to innovator material.

### 255 **6.3.4. Revision of monographs**

256 A revision of the monograph should be initiated when:

- 257 • The means of identification of known impurities have been established
- 258 • New impurities have been identified or qualified.

## 259 **7. Specifications for medicinal products**

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

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

264 Acceptance criteria for related impurities should be set within the thresholds given below. The same  
265 specifications should apply to the product after any opening/reconstitution/dilution (in-use shelf life) as  
266 for the finished product, unless justified by suitable qualification data e.g. by comparison to levels  
267 found in the original product.

268

269 Active substance manufactured by semi-synthesis:

270 Reporting threshold: 0.1%

271 Identification and qualification thresholds: 0.2%

272

273 Active substance manufactured by fermentation, single compound:



274 Reporting threshold: 0.15%  
275 Identification threshold: 0.2%  
276 Qualification threshold: 0.2%

277

278 Active substance manufactured by fermentation, family of compounds:

279 Reporting threshold: 0.15%  
280 Identification threshold: 0.2%  
281 Qualification threshold: 0.5%/0.2%

282

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

285 For veterinary medicinal products the VICH GL 11 thresholds for reporting, identification and  
286 qualification (0.3%, 1.0% and 1.0%, respectively) should be applied.

## 287 **8. Analytical procedures**

288 When analysing the final active substance and the medicinal product, whenever possible, an external  
289 standard should be used calculating w/w to evaluate and exclude any possible mass imbalance. If  
290 using area normalisation the relevant components and related impurities should give similar responses  
291 in the detector. Otherwise response factors should be used.

292 Area normalisation may be acceptable for certain active substances consisting of a family of  
293 compounds. In area normalisation the area percentage is calculated on the basis of the total area in  
294 the chromatogram, instead of using an external standard. This may be used when analysing relevant  
295 intermediates. When using area normalisation linearity for the intended range should be demonstrated  
296 and an unambiguously defined disregard criterion should be given.

297 When performing qualification of an impurity profile versus the innovator product, a sufficiently specific  
298 analytical procedure should be used. For complicated mixtures the separation technique (e.g. HPLC)  
299 should be combined with mass spectrometry (or diode-array detection, where justified). For routine  
300 testing simpler procedures may be used, if justified.

301 The quantitation limit for the analytical procedure should be not more than ( $\leq$ ) the reporting threshold.  
302 For substances having weak chromophores, this will in some cases lead to high reporting thresholds.

## 303 **Definitions**

304 **Fermented active substances:** Primary or secondary metabolites of micro-organisms such as  
305 bacteria, yeast, fungi and micro-algae, irrespective of whether or not the micro-organism have been  
306 modified by traditional procedures or by recombinant DNA technology.

## 307 **References**

- 308 1. 'Impurities in new drug substances (revised)' (CPMP/ICH/2737/99) (ICH Q3A(R))
- 309 2. 'Impurities in new drug products' (CPMP/ICH2738/99) (ICH Q3B(R))
- 310 3. 'Control of impurities of pharmacopoeial substances' (CPMP/QWP/1529/04 and  
311 EMEA/CVMP/059/04-FINAL)

- 312 4. 'Specifications: test Procedures and acceptance criteria for new drug substances and new drug  
313 products: chemical substances' (CPMP/ICH/367/96) (ICH Q6A)
- 314 5. 'Assessment of the quality of medicinal products containing existing/known active substances'  
315 (EMA/CHMP/CVMP/QWP/296289/2008)
- 316 6. 'Impurities in new veterinary drug substances' (EMA/CVMP/VICH/837/99-Rev.1) (VICH 10(R))
- 317 7. 'Impurities in new veterinary medicinal products' (EMA/CVMP/VICH/838/99-Rev.1) (VICH  
318 11(R))
- 319 8. 'Test procedures and acceptance criteria for new veterinary drug substances and new medicinal  
320 products: chemical substances' (EMA/CVMP/VICH/810/04-corrigendum) (VICH 39)
- 321 9. 'Chemistry of New Active Substances' (CPMP/QWP/130/96 Rev 1)
- 322 10. 'New impurities control: setting specifications for antibiotics and synthetic peptides:  
323 proceedings from EDQM symposium, Strasbourg 21-22 September 2006'
- 324 11. European Pharmacopoeia general monograph 'Substances for Pharmaceutical Use'
- 325 12. European Pharmacopoeia general chapter 5.10 'Control of impurities in substances for  
326 pharmaceutical use'
- 327 13. European Pharmacopoeia general monograph 'Products of fermentation'

## 328 **Annex: explanatory note regarding thresholds**

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

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

336 *Active substances manufactured by semi-synthesis:*

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

341 *Active substances manufactured by fermentation, single compound:*

342 Consisting of only one active compound these substances are relatively easy to purify, and as a  
343 consequence of this it is possible to set relatively low thresholds.

344 *Active substances manufactured by fermentation, family of compounds:*

345 When the active substance is a mixture of closely related compounds it is difficult to purify this mixture  
346 from other closely related compounds present (and excessive purification could also lead to a different  
347 component distribution). Some of these other components have the same antibiotic activity as the  
348 components defined to be included in the active substance, while other components do not have the  
349 same activity. These components are handled as related impurities, but due to the complex situation it  
350 is difficult to set very low thresholds. It is proposed to have a relatively low qualification threshold, and

351 to include the possibility of having a wider qualification threshold for structurally closely related  
352 substances (based on evidence such as HPLC/mass spectrometry).  
353