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*Evaluation of Medicines for Human Use*

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**COMMITTEE FOR PROPRIETARY MEDICINAL PRODUCTS  
(CPMP)**

**NOTE FOR GUIDANCE ON  
THE INVESTIGATION OF BIOAVAILABILITY AND  
BIOEQUIVALENCE**

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**Note:**

This revised Note for Guidance will replace the previous guideline adopted in December 1991.

Any comments should be sent to the EMEA, EWP Secretariat (Fax No. 44-20-74188613) before the end of March 2001.

# INVESTIGATION OF BIOAVAILABILITY AND BIOEQUIVALENCE

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# 1 INTRODUCTION

2 To exert an optimal therapeutic action an active moiety should be delivered to its site of  
3 action in an effective concentration for the desired period. To allow reliable prediction of the  
4 therapeutic effect the performance of the dosage form containing the active substance should  
5 be well characterised.

6 In the past, several therapeutic misadventures related to differences in bioavailability (e.g.  
7 digoxin, phenytoin, primidone) testify to the necessity of testing the performance of dosage  
8 forms in delivering the active substance to the systemic circulation and thereby to the site of  
9 action. Thus the bioavailability of an active substance from a pharmaceutical product should  
10 be known and reproducible. This is especially the case if one product containing one active  
11 substance is to be used instead of its innovator product. In that case the product should show  
12 the same therapeutic effect in the clinical situation. It is generally cumbersome to assess this  
13 by clinical studies.

14 Comparison of therapeutic performances of two medicinal products containing the same  
15 active substance is a critical means of assessing the possibility of alternative use between the  
16 innovator and any essentially similar medicinal product. Assuming that in the same subject an  
17 essentially similar plasma concentration time course will result in essentially similar  
18 concentrations at the site of action and thus in an essentially similar effect, pharmacokinetic  
19 data instead of therapeutic results may be used to establish equivalence: bioequivalence.

20 It is the objective of this guidance to define, for products with a systemic effect, when  
21 bioavailability or bioequivalence studies are necessary and to formulate requirements for their  
22 design, conduct, and evaluation. The possibility of using *in vitro* instead of *in vivo* studies  
23 with pharmacokinetic end points is also envisaged.

24 This guideline should be read in conjunction with Directive 75-318/EEC, as amended, and  
25 other pertinent elements outlined in current and future EU and ICH guidelines and regulations  
26 especially those on:

- 27 • Pharmacokinetic Studies in Man
- 28 • Modified Release Oral and Transdermal Dosage Forms: Section I (Pharmacokinetic and  
29 Clinical Evaluation)
- 30 • Modified Release Oral and Transdermal Dosage Forms: Section II (Quality)
- 31 • Investigation of Chiral Active Substances.
- 32 • Fixed Combination Medicinal Products
- 33 • Clinical Requirements for Locally Applied, Locally Acting Products Containing  
34 Known Constituents.
- 35 • The Investigation of Drug Interactions
- 36 • Development Pharmaceuticals
- 37 • Process Validation
- 38 • Manufacture of the Finished Dosage Form
- 39 • Validation of analytical procedures: Definitions and Terminology (ICH topic Q2A)
- 40 • Validation of analytical procedures: Methodology (ICH topic Q2B)
- 41 • Structure and Content of Clinical Study Reports (ICH topic E3)
- 42 • Good Clinical Practice: Consolidated Guideline (ICH topic E6)
- 43 • General Considerations for Clinical Trials (ICH topic E8)
- 44 • Statistical Principles for Clinical Trials (ICH topic B9)
- 45 • Choice of Control Group in Clinical Trials (ICH topic E10)
- 46 • Amendments to Commission Regulation on (EC) 542/95
- 47 • Common Technical Document (ICH topic M4)

48 For medicinal products not intended to be delivered into the general circulation the common

49 systemic bioavailability approach cannot be applied. Under these conditions the (local)  
50 availability may be assessed, where necessary, by measurements quantitatively reflecting the  
51 presence of the active substance at the site of action using methods specially chosen for that  
52 combination of active substance and localisation (see section 5.1.8). In this case, as well as in  
53 others, alternative methods may be required such as studies using pharmacodynamic end  
54 points. Furthermore, where specific requirements for different types of products are needed,  
55 the appropriate exceptions are mentioned therein.

56 This Note for Guidance does not explicitly apply to biological products.

## 57 **2 DEFINITIONS**

58 Before defining bioavailability and related terminology some definitions pertaining to dosage and  
59 chemical forms are given:

### 60 **2.1 Pharmaceutical equivalence**

61 Medicinal products are pharmaceutically equivalent if they contain the same amount of the  
62 same active substance(s) in the same dosage forms that meet the same or comparable  
63 standards.

64 Pharmaceutical equivalence does not necessarily imply bioequivalence as differences in the  
65 excipients and/or the manufacturing process can lead to faster or slower dissolution and/or  
66 absorption.

### 67 **2.2 Pharmaceutical alternatives**

68 Medicinal products are pharmaceutical alternatives if they contain the same active moiety but  
69 differ in chemical form (salt, ester, etc.) of that moiety or in the dosage form or strength.

### 70 **2.3 Bioavailability**

71 Bioavailability means the rate and extent to which the active substance or active moiety is  
72 absorbed from a pharmaceutical form and becomes available at the site of action.

73 In the majority of cases substances are intended to exhibit a systemic therapeutic effect, and a  
74 more practical definition can then be given, taking into consideration that the substance in the  
75 general circulation is in exchange with the substance at the site of action:

76 -Bioavailability is understood to be the extent and the rate to which a substance or its  
77 active moiety is delivered from a pharmaceutical form and becomes available in the  
78 general circulation.

79 It may be useful to distinguish between the "absolute bioavailability" of a given dosage form  
80 as compared with that (100%) following intravenous administration (e.g. oral solution *vs.* iv.),  
81 and the "relative bioavailability" as compared with another form administered by the same or  
82 another non intravenous route (e.g. tablets *vs.* oral solution).

### 83 **2.4 Bioequivalence**

84 Two medicinal products are bioequivalent if they are pharmaceutically equivalent or  
85 pharmaceutical alternatives and if their bioavailabilities after administration in the same molar  
86 dose are similar to such degree that their effects, with respect to both efficacy and safety, will  
87 be essentially the same.

88 Alternatively to classical bioavailability studies using pharmacokinetic end points to assess  
89 bioequivalence, other types of studies can be envisaged, e.g. human studies with clinical or  
90 pharmacodynamic end points, studies using animal models or in vitro studies as long as they  
91 are appropriately justified and/or validated.

## 92 **2.5 Essentially similar products**

93 The current EU definition for essentially similar products is as follows (see "The rules  
94 governing medicinal products in the European Union", Notice to Applicants, Vol. 2A in  
95 accordance with the December 1998 European Court of Justice ruling in the "Generics"  
96 case):

97 "A medicinal product is essentially similar to an original product where it satisfies the criteria  
98 of having the same qualitative and quantitative composition in terms of active substances, of  
99 having the same pharmaceutical form, and of being bioequivalent unless it is apparent in the  
100 light of scientific knowledge that it differs from the original product as regards safety and  
101 efficacy".

102 By extension, it is generally considered that for immediate release products the concept of  
103 essential similarity also applies to different oral forms (tablets and capsules) with the same  
104 active substance.

105 The need for a comparative bioavailability study to demonstrate bioequivalence is identified  
106 under 5.1. Concerns about differences in essentially similar medicinal products lie on the use  
107 of different excipients and methods of manufacture that ultimately might have an influence on  
108 safety and efficacy. A bioequivalence study is the widely accepted means of demonstrating  
109 that these differences have no impact on the performance of the formulation in promoting  
110 absorption in the case of immediate release dosage forms. It is desirable that excipients must  
111 be devoid of any effect or their safe use is ensured by appropriate warning in the package  
112 label – see guideline on excipients in the label and package leaflet: "The Rules Governing  
113 Medicinal Products in the European Union", 1998, Vol. 3B, - and not interfere with either the  
114 release or the absorption process.

115 An essentially similar product can be used instead of its innovator product. An 'innovator'  
116 product is a medicinal product authorised and marketed on the basis of a full dossier i.e.  
117 including chemical, biological, pharmaceutical, pharmacological-toxicological and clinical  
118 data. A 'Reference Product' must be an 'innovator' product (see 3.5).

## 119 **2.6 Therapeutic equivalence**

120 A medicinal product is therapeutically equivalent with another product if it contains the same  
121 active substance or therapeutic moiety and, clinically, shows the same efficacy and safety as  
122 that product, whose efficacy and safety has been established.

123 In practice, demonstration of bioequivalence is generally the most appropriate method of  
124 substantiating therapeutic equivalence between medicinal products, which are  
125 pharmaceutically equivalent or pharmaceutical alternatives, provided they contain excipients  
126 generally recognised as not having an influence on safety and efficacy and comply with  
127 labelling requirements with respect to excipients. (see 2.5).

128 However, in some cases where similar extent of absorption but different rates of absorption  
129 are observed the products can still be judged therapeutically equivalent if those differences are  
130 not of therapeutic relevance. A clinical study to prove that differences in absorption rate are  
131 not therapeutically relevant may be necessary.

## 132 **3 DESIGN AND CONDUCT OF STUDIES**

133 In the following sections, requirements for the design and conduct of bioavailability or  
134 bioequivalence studies are formulated. It is assumed that the applicant is familiar with  
135 pharmacokinetic theories underlying bioavailability studies. The design should be based on a  
136 reasonable knowledge of the pharmacodynamics and/or the pharmacokinetics of the active  
137 substance in question. For the pharmacokinetic basis of these studies reference is made to the  
138 recommendation "Pharmacokinetic studies in man". The design and conduct of the study

139 should follow EU-regulations on Good Clinical Practice, including reference to an Ethics  
140 Committee.

141 A bioequivalence study is basically a comparative bioavailability study designed to establish  
142 equivalence between test and reference products. The following sections apply mainly to  
143 bioequivalence studies. Since bioavailability studies are comparative in nature, the contents of  
144 the following sections apply to these studies as well, with the necessary adaptations in  
145 accordance with the aim of each specific study. Where necessary, specific guidance  
146 concerning bioavailability studies will be given.

147 The methodology of bioequivalence studies can be used to assess differences in the  
148 pharmacokinetic parameters in pharmacokinetic studies such as drug-drug or food–drug  
149 interactions or to assess differences in subsets of the population. In this case the relevant  
150 guidelines should be followed and the selection of subjects, the design and the statistical  
151 analysis should be adjusted accordingly.

152 **3.1 Design**

153 The study should be designed in such a way that the formulation effect can be distinguished  
154 from other effects. If the number of formulations to be compared is two, a two-period, two-  
155 sequence crossover design is often considered to be the design of choice.

156 However, under certain circumstances and provided the study design and the statistical  
157 analyses are scientifically sound alternative well-established designs could be considered such  
158 as parallel design for very long half-life substances and replicate designs for substances with  
159 highly variable disposition.

160 In general, single dose studies will suffice, but there are situations in which steady-state  
161 studies

- 162 • are required, e.g. in the case of
  - 163 - dose- or time-dependent pharmacokinetics (mainly for bioavailability studies),
  - 164 - modified release products (in addition to single dose investigations),
- 165 • or can be considered, e.g.
  - 166 - if problems of sensitivity preclude sufficiently precise plasma concentration  
167 measurements after single dose administration.
  - 168 - if the intra-individual variability in the plasma concentration or disposition  
169 precludes the possibility of demonstrating bioequivalence in a reasonably sized  
170 single dose study.

171 In such steady-state studies the administration scheme should follow the usual dosage  
172 recommendations.

173 The number of subjects required is determined by

- 174 a) the error variance associated with the primary characteristic to be studied as estimated  
175 from a pilot experiment, from previous studies or from published data,
- 176 b) the significance level desired,
- 177 c) the expected deviation from the reference product compatible with bioequivalence and
- 178 d) the required power.

179 The clinical and analytical standards imposed may also influence the statistically determined  
180 number of subjects. However, generally the minimum number of subjects should be not  
181 smaller than 12 unless justified.

182 Subsequent treatments should be separated by adequate wash out periods. In steady-state  
183 studies wash out of the previous treatment last dose can overlap with the build-up of the  
184 second treatment, provided the build-up period is sufficiently long (at least three times the  
185 terminal half-life).

186 The sampling schedule should be planned to provide an adequate estimation of  $C_{max}$  and to  
187 cover the plasma concentration time curve long enough to provide a reliable estimate of the  
188 extent of absorption. This is generally achieved if the AUC derived from measurements is at  
189 least 80% of the AUC extrapolated to infinity. If a reliable estimate of terminal half-life is  
190 necessary, it should be obtained by collecting at least three to four samples during the  
191 terminal log linear phase.

192 In order to study bioavailability under steady-state conditions when differences between  
193 morning and evening or nightly dosing are known, (e.g. if it is known that the circadian  
194 rhythm is known to have an influence on bioavailability), sampling should be carried out over  
195 a full 24 hours cycle.

196 For drugs with a long half-life, relative bioavailability can be adequately estimated using  
197 truncated AUC as long as the total collection period is justified. In this case the sample  
198 collection time should be adequate to ensure comparison of the absorption process.

## 199 **3.2 Subjects**

### 200 **3.2.1 Selection of subjects**

201 The subject population for bioequivalence studies should be selected with the aim to minimise  
202 variability and permit detection of differences between pharmaceutical products. Therefore,  
203 the studies should normally be performed with healthy volunteers. The inclusion/exclusion  
204 criteria should be clearly stated in the protocol.

205 Subjects could belong to both sexes; however, the risk to women of childbearing potential  
206 should be considered on an individual basis.

207 In general, subjects should be between 18 - 55 years old and of weight within the normal  
208 range according to accepted normal values for the Body Mass Index. They should be screened  
209 for suitability by means of clinical laboratory tests, an extensive review of medical history,  
210 and a comprehensive medical examination. Depending on the drug's therapeutic class and  
211 safety profile special medical investigations may have to be carried out before, during and  
212 after the completion of the study. Subjects should preferably be non-smokers and without a  
213 history of alcohol or drug abuse. If moderate smokers are included (less than 10 cigarettes per  
214 day) they should be identified as such and the consequences for the study results should be  
215 discussed.

### 216 **3.2.2 Standardisation of the study**

217 The test conditions should be standardised in order to minimise the variability of all factors  
218 involved except that of the products being tested. Therefore, standardisation of the diet, fluid  
219 intake, exercise and posture is recommended. Subjects should preferably be fasting at least  
220 during the night prior to administration of the products. If the Summary of Product  
221 Characteristics of the reference product contains specific recommendations in relation with  
222 food intake the study should be designed accordingly.

223 The time of day for ingestion should be specified and as fluid intake may profoundly  
224 influence gastric passage, the volume of fluid (at least 150 ml) should be constant. All meals  
225 and fluids taken after the treatment should also be standardised in regard to composition and  
226 time of administration during the sampling period. The subjects should not take other  
227 medicines during a suitable period before and during the study and should abstain from food  
228 and drinks, which may interact with circulatory, gastrointestinal, liver or renal function (e.g.

229 alcoholic or xanthine-containing beverages or certain fruit juices). As the bioavailability of an  
230 active moiety from a dosage form could be dependent upon gastrointestinal transit times and  
231 regional blood flows, posture and physical activity may need to be standardised.

### 232 3.2.3 Inclusion of patients

233 If the investigated active substance is known to have adverse effects and the pharmacological  
234 effects or risks are considered unacceptable for healthy volunteers it may be necessary to use  
235 patients instead, under suitable precautions and supervision. In this case the applicant should  
236 justify the alternative.

### 237 3.2.4 Genetic phenotyping

238 Phenotyping and/or genotyping of subjects should be considered for exploratory  
239 bioavailability studies and all studies using parallel group design. It may be considered as  
240 well in crossover studies (e.g. bioequivalence, dose proportionality, food interaction studies  
241 etc.) for safety or pharmacokinetic reasons. If a drug is known to be subject to major genetic  
242 polymorphism, studies could be performed in panels of subjects of known phenotype or  
243 genotype for the polymorphism in question.

## 244 3.3 Characteristics to be investigated

245 In most cases evaluation of bioequivalence will be based upon the measured concentrations of  
246 the parent compound. In some situations, however, measurements of an active or inactive  
247 metabolite may be necessary instead of the parent compound. Such situations include cases  
248 where the use of a metabolite may be advantageous to determine the extent of drug input, e.g.  
249 if the concentration of the active substance is too low to be accurately measured in the  
250 biological matrix (e.g. major difficulty in analytical method, product unstable in the biological  
251 matrix or half-life of the parent compound too short) thus giving rise to significant variability.

252 Bioequivalence determinations based on metabolites should be justified in each case bearing  
253 in mind that the aim of a bioequivalence study is intended to compare the *in vivo* performance  
254 of test and reference products. In particular if metabolites significantly contribute to the net  
255 activity of an active substance and the pharmacokinetic system is non-linear, it is necessary to  
256 measure both parent drug and active metabolite plasma concentrations.

257 In bioavailability studies, the shape of and the area under the plasma concentration *versus*  
258 time curves or the cumulative renal excretion and excretion rate are mostly used to assess  
259 extent and rate of absorption. The use of urine excretion data may be advantageous in  
260 determining the extent of drug input but has to be justified when used to estimate the rate of  
261 absorption. Sampling points or periods should be chosen, such that the time- concentration  
262 profile is adequately defined so as to allow the estimation of relevant parameters.

263 From the primary results, the bioavailability characteristics desired are estimated, namely  
264  $AUC_t$ ,  $AUC_\infty$ ,  $C_{max}$ ,  $t_{max}$ ,  $Ae_t$ ,  $Ae_\infty$ , or any other justifiable characteristics (cf. Appendix I).  
265 The method of estimating AUC-values should be specified. For additional information  $t_{1/2}$   
266 and MRT can be estimated. For studies in steady state  $AUC_\tau$ ,  $C_{max}$ ,  $C_{min}$  and fluctuation  
267 should be provided.

268 The exclusive use of modelled characteristics is not recommended.

269 If pharmacodynamic effects are used as characteristics the measurements should provide a  
270 sufficiently detailed time course, the initial values in each period should be comparable and  
271 the complete effect curve should remain below the maximum physiological response.  
272 Specificity, accuracy and reproducibility of the measurements should be sufficient. The non-  
273 linear character of the dose/response relationship should be taken into account and base line  
274 corrections should be considered during data analysis.

### 275 **3.4 Chemical analysis**

276 The bioanalytical methods used to determine the active moiety and/or its biotransformation  
277 product(s) in plasma, serum, blood or urine or any other suitable matrix must be well  
278 characterised, fully validated and documented to yield reliable results that can be satisfactorily  
279 interpreted. The main objective of method validation is to demonstrate the reliability of a  
280 particular method for the quantitative determination of an analyte(s) concentration in a  
281 specific biological matrix. The characteristics of a bioanalytical method essential to ensure the  
282 acceptability of the performance and the reliability of analytical results are: (1) stability of the  
283 stock solutions and of the analyte(s) in the biological matrix under processing conditions and  
284 during the entire period of storage; (2) specificity; (3) accuracy; (4) precision (5) limit of  
285 quantification and (6) response function.

286 The validation of a bioanalytical method should comprise two distinct phases: (1) the pre-  
287 study phase in which the compliance of the assay with the six characteristics listed above is  
288 verified and (2) the study phase itself in which the validated bioanalytical method is applied to  
289 the actual analysis of samples from the biostudy mainly in order to confirm the stability,  
290 accuracy and precision.

291 A calibration curve should be generated for each analyte in each analytical run and it should  
292 be used to calculate the concentration of the analyte in the unknown samples in the run. A  
293 number of separately prepared Quality Control samples should be analysed with processed  
294 test samples at intervals based on the total number of samples. In addition, it is necessary to  
295 validate the method of processing and handling the biological samples.

296 All procedures should be performed according to pre-established Standard Operating  
297 Procedures (SOPs). All relevant procedures and formulae used to validate the bioanalytical  
298 method should be submitted and discussed. Any modification of the bioanalytical method  
299 before and during analysis of study specimens requires adequate revalidation; all  
300 modifications should be reported and the scope of revalidation justified.

301 According to the requirements of the note for guidance on the "Investigation of Chiral Active  
302 Substances", bioequivalence studies supporting applications for essentially similar medicinal  
303 products containing chiral active substances should be based upon enantiomeric bio-analytical  
304 methods unless (1) both products contain the same stable single enantiomer; (2) both  
305 products contain the racemate and both enantiomers show linear pharmacokinetics.

### 306 **3.5 Reference and test product**

307 Test products are normally compared with the corresponding dosage form of an innovator  
308 (see 2.5) medicinal product (reference product). The choice of reference product should be  
309 justified by the applicant.

310 For an abridged application claiming essential similarity to a reference product, application to  
311 numerous Member States based on bioequivalence with a reference product from one  
312 Member State can be made.

313 Such an application can be considered acceptable unless there is a significant difference  
314 between the reference products originating from the same manufacturer (or its subsidiaries),  
315 in terms of the qualitative and quantitative composition in excipients. Concerned Member  
316 States may request information from the first Member State on the reference product, namely  
317 on the composition, manufacturing process and finished product specification.

318 Where additional bioequivalence studies are required, they should be carried out using the  
319 product registered in the concerned Member State as the reference product

320 It should be remembered that the development of the test product should always take into  
321 account the Note for Guidance on "Development Pharmaceuticals".

322 The test products used in the biostudy must be prepared in accordance with GMP-rules.  
323 Batch control results of the test product should be reported.

324 In the case of oral solid forms for systemic action the test product should usually originate  
325 from a batch of at least 1/10 of production scale or 100 000 units, whichever is greater, unless  
326 otherwise justified. The production of batches used should provide a high level of assurance  
327 that the product and process will be feasible on an industrial scale; in case of production batch  
328 smaller than 100 000 units, a full production batch will be required. If the product is subjected  
329 to further scale-up this should be properly validated.

330 Samples of the product from full production batches should be compared with those of the  
331 test batch, and should show similar *in vitro* dissolution profiles when employing suitable  
332 dissolution test conditions (see Appendix II).

333 The study sponsor will have to retain a sufficient number of all investigational product  
334 samples in the study for one year in excess of the accepted shelf life or two years after  
335 completion of the trial or until approval whichever is longer to allow re-testing, if it is  
336 requested by the authorities.

337 In accordance with Annex 13 to the EU guide to GMP, reference and test product must be  
338 packed in an individual way for each subject included in the bioequivalence trial. Every effort  
339 should be made to allow a precise tracking of administration of the reference and test products  
340 to the subjects, for instance by the use of labels with a tear-off portion.

### 341 **3.6 Data analysis**

342 The primary concern of bioequivalence assessment is to quantify the difference in  
343 bioavailability between the reference and test products and to demonstrate that any clinically  
344 important difference is unlikely.

#### 345 **3.6.1 Statistical analysis**

346 The statistical method for testing bioequivalence is based upon the 90% confidence interval  
347 for the ratio of the population means (Test/Reference), for the parameters under consideration.

348 This method is equivalent to the corresponding two one-sided test procedure with the null  
349 hypothesis of bioinequivalence at the 5% significance level. The statistical analysis (e.g.  
350 ANOVA) should take into account sources of variation that can be reasonably assumed to  
351 have an effect on the response variable. A statistically significant sequence effect should be  
352 handled appropriately.

353 Pharmacokinetic parameters derived from measures of concentration, e.g. AUC,  $C_{max}$  should  
354 be analysed using ANOVA. The data should be transformed prior to analysis using a  
355 logarithmic transformation. The analysis technique for  $t_{max}$  should be non-parametric and  
356 should be applied to untransformed data. For all pharmacokinetic parameters of interest in  
357 addition to the appropriate 90% confidence intervals for the comparison of the two  
358 formulations, summary statistics such as median, minimum and maximum should be given.

#### 359 **3.6.2 Acceptance range for pharmacokinetic parameters**

360 The pharmacokinetic parameters to be tested, the procedure for testing and the acceptance  
361 ranges should be stated beforehand in the protocol.

362 In studies to determine average bioequivalence the acceptance intervals for the main  
363 characteristics are detailed as follows:

##### 364 AUC-ratio

365 The 90% confidence interval for this measure of relative bioavailability should lie within an  
366 acceptance interval of 0.80-1.25. In case of an especially narrow therapeutic range the  
367 acceptance interval may need to be tightened.

368 In rare cases a wider acceptance range may be acceptable if it is based on sound clinical  
369 justification.

#### 370 C<sub>max</sub>-ratio

371 The 90% confidence interval for this measure of relative bioavailability should lie within an  
372 acceptance interval of 0.80-1.25. In case of an especially narrow therapeutic range the  
373 acceptance interval may need to be tightened.

374 In certain cases a wider interval may be acceptable. The interval must be prospectively  
375 defined e.g. 0.75-1.34 and justified addressing in particular any safety or efficacy concerns for  
376 patients switched between formulations.

#### 377 t<sub>max</sub>-diff

378 Statistical evaluation of t<sub>max</sub> only makes sense if there is a clinically relevant claim for rapid  
379 release or action or signs related to adverse effects. The non-parametric 90% confidence  
380 interval for this measure of relative bioavailability should lie within a clinically determined  
381 range.

#### 382 Others

383 For other (see 3.3) pharmacokinetic parameters (e.g. C<sub>min</sub>, Fluctuation, t<sub>1/2</sub>, etc.)  
384 considerations analogous to those for AUC, C<sub>max</sub> or t<sub>max</sub> apply, taking into consideration the  
385 use of log-transformed or untransformed data, respectively.

### 386 **3.6.3 Handling deviations from the study plan**

387 The method of analysis should be planned in the protocol. The protocol should also specify  
388 methods for handling drop-outs and for identifying biologically implausible outliers. Post hoc  
389 exclusion of outliers is generally not accepted. If modelling assumptions made in the protocol  
390 (e.g. for extrapolating AUC to infinity) turn out to be invalid, a revised analysis in addition to  
391 the planned analysis (if this is feasible) should be presented and discussed.

### 392 **3.6.4 A remark on individual and population bioequivalence**

393 To date, most bioequivalence studies are designed to evaluate average bioequivalence.  
394 Experience with population and individual bioequivalence studies is limited. Therefore, no  
395 specific recommendation is given on this matter.

### 396 **3.7 In vitro dissolution complementary to a bioequivalence study**

397 The results of "in vitro" dissolution tests, obtained with the batches of test and reference  
398 products that were used in the bioequivalence study should be reported. The results should be  
399 reported as profiles of percent of labelled amount dissolved versus time.

400 The specifications for the *in vitro* dissolution of the product should be derived from the  
401 dissolution profile of the batch that was found to be bioequivalent to the reference product and  
402 would be expected to be similar to those of the reference product (see Appendix II).

403 For immediate release products, if the dissolution profile of the test product is dissimilar  
404 compared to that of the reference product and the in vivo data remain acceptable the  
405 dissolution test method should be re-evaluated and optimised. In case that no discriminatory  
406 test method can be developed which reflects in vivo bioequivalence a different dissolution  
407 specification for the test product could be set.

### 408 **3.8 Reporting of results**

409 The report of a bioavailability or a bioequivalence study should give the complete  
410 documentation of its protocol, conduct and evaluation complying with GCP-rules and related  
411 EU and ICH E3 guidelines. This implies that the authenticity of the whole of the report is  
412 attested by the signature of the principal investigator. The responsible investigator(s), if any,

413 should sign for their respective sections of the report.

414 Names and affiliations of the responsible investigator (s), site of the study and period of its  
415 execution should be stated. The names and batch numbers of the products used in the study  
416 as well as the composition(s), finished product specifications and comparative dissolution  
417 profiles should be provided. In addition, the applicant should submit a signed statement  
418 confirming that the test product is the same as the one that is submitted for marketing  
419 authorisation.

420 All results should be clearly presented and should include data from subjects who eventually  
421 dropped-out. Drop-out and withdrawal of subjects should be fully documented and accounted  
422 for. The method used to derive the pharmacokinetic parameters from the raw data should be  
423 specified. The data used to estimate AUC should be reported. If pharmacokinetic models are  
424 used to evaluate the parameters the model and computing procedure used should be justified.  
425 Deletion of data should be justified.

426 All individual subject data should be given and individual plasma concentration/time curves  
427 presented in linear/linear and log/linear scale. The analytical report should include the results  
428 for all standard and quality control samples as well. A representative number of  
429 chromatograms or other raw data should be included covering the whole concentration range  
430 for all, standard and quality control samples as well as the specimens analysed. The analytical  
431 validation report should be submitted as well.

432 The statistical report should be sufficiently detailed to enable the statistical analysis to be  
433 repeated, e.g. randomisation scheme, demographic data, values of pharmacokinetic  
434 parameters for each subject, descriptive statistics for each formulation and period. A detailed  
435 ANOVA and/or non-parametric analysis, the point estimates and corresponding confidence  
436 intervals including the method of their estimation *should* also be included.

## 437 **4 APPLICATIONS FOR PRODUCTS CONTAINING NEW ACTIVE** 438 **SUBSTANCES**

### 439 **4.1 Bioavailability**

440 In the case of new active substances (new chemical entities) intended for systemic action, the  
441 pharmacokinetic characterisation will have to include the determination of the systemic  
442 availability of the substance in its intended pharmaceutical form in comparison with  
443 intravenous administration. If this is not possible the bioavailability relative to a suitable oral  
444 solution or suspension should be determined. In the case of a prodrug the intravenous  
445 reference solution should preferably be made of the active moiety.

### 446 **4.2 Bioequivalence**

447 During development bioequivalence studies are necessary as bridging studies between (i)  
448 pivotal and early clinical trial formulations; (ii) pivotal clinical trial formulations, especially  
449 those used in the dose finding studies, and the to-be-marketed medicinal product; (iii) other  
450 comparisons depending on the situation. Such studies may be exempted if the absence of  
451 differences in the in vivo performance can be justified by satisfactory in vitro data.

## 452 **5 APPLICATIONS FOR PRODUCTS CONTAINING APPROVED ACTIVE** 453 **SUBSTANCES**

### 454 **Bioequivalence studies**

455 In vivo bioequivalence studies are needed when there is a risk that possible differences in  
456 bioavailability may result in therapeutic inequivalence.

457 The kind of studies to be performed may vary with the type of product, as follows.

458 **5.1.1 Oral Immediate Release Forms with Systemic Action**

459 This section pertains to dosage forms such as tablets, capsules and oral suspensions and takes  
460 into consideration criteria derived from the concepts underlying the Biopharmaceutics  
461 Classification System, i.e. high solubility, high permeability for the active substance and high  
462 dissolution rate for the medicinal product. These criteria, along with a non-critical therapeutic  
463 range should be primarily considered; therefore the following characteristics have to be taken  
464 into account in order to justify the request for exemption from in vivo bioequivalence studies.

465 a) Characteristics related to the active substance:

466 i - risk of therapeutic failure or adverse drug reactions:

467 this risk depends on the requirements of special precautions with respect to precision  
468 and accuracy of dosing of the active substance, e.g. the need for critical plasma  
469 concentrations;

470 ii - risk of bioinequivalence,

471 evidence of bioavailability problems or bioinequivalence exists for some specific  
472 active substances;

473 iii – solubility:

474 This parameter is a major criterion to justify exemption from in vivo studies. When  
475 the active substance is highly water soluble, the product could be in general  
476 exempted from bioequivalence studies unless, considering the other characteristics,  
477 the exemption could entail a potential risk. Polymorphism and particle size are major  
478 determinants of dissolution rate and special attention should be paid to these  
479 characteristics. An active substance is considered highly water soluble if the amount  
480 contained in the highest dose strength of an immediate release product is dissolved in  
481 250 ml of each of three pharmacopoeial buffers within the range of pH 1-8 at 37°C  
482 (preferably at or about pH 1.0, 4.6, 6.8);

483 iv - pharmacokinetic properties

484 linear and complete absorption reduces the possibility of an immediate release  
485 dosage form to influence the bioavailability.

486 b) Characteristics related to the medicinal product:

487 i - rapid dissolution

488 in case of exemption from bioequivalence studies, in vitro data should demonstrate the  
489 similarity of dissolution profile between the test product and the reference product.  
490 However, in cases where more than 85% of the active substance are dissolved within  
491 15 minutes, the similarity of dissolution profiles may be accepted as demonstrated (see  
492 appendix II);

493 ii – excipients

494 the excipients included in the composition of the medicinal product are well  
495 established and no interaction with the pharmacokinetics of the active substance is  
496 expected. In case of atypically large amounts of known excipients or new excipients  
497 being used, additional documentation has to be submitted;

498       iii – manufacture  
499           the method of manufacture of the finished product in relation with critical  
500           physicochemical properties of the active substance (e.g. particle size, polymorphism)  
501           should be adequately addressed and documented in the development pharmaceuticals  
502           section of the dossier.

### 503   **5.1.2 Oral solutions**

504   If the product is an aqueous oral solution at time of administration and contains an active  
505   substance in the same concentration as an oral solution currently approved as a medicinal  
506   product, no bioequivalence study is required, provided the excipients contained in it do not  
507   affect gastrointestinal transit, absorption or in vivo stability of the active substance.

508   In those cases where an oral solution has to be tested against an oral immediate release  
509   formulation a comparative bioavailability study will be required unless an exemption can be  
510   justified (see 5. 1. 1).

### 511   **5.1.3 Non-Oral Immediate Release forms with systemic action**

512   In general bioequivalence studies are required.

### 513   **5.1.4 Modified Release and transdermal dosage forms**

514   Requirements for bioequivalence studies in accordance with the specific guideline

### 515   **5.1.5 Fixed combinations products**

516   Combination products should in general be assessed with respect to bioavailability and  
517   bioequivalence of individual active substances either separately (in the case of a new  
518   combination) or as an existing combination. Criteria under 5.1.1 will apply to individual  
519   components. The study should be designed in such a way that the possibility of a  
520   pharmacokinetic drug-drug interaction could be detected.

### 521   **5.1.6 Parenteral solutions**

522   The applicant is not required to submit a bioequivalence study if the product is to be  
523   administered as an aqueous intravenous solution containing the same active substance in the  
524   same concentration as the currently authorised product.

525   In the case of other parenteral routes, e.g. intramuscular or subcutaneous, if the product is of  
526   the same type of solution (aqueous or oily), contains the same concentration of the same  
527   active substance and the same or comparable excipients as the medicinal product currently  
528   approved, then bioequivalence testing is not required.

### 529   **5.1.7 Gases**

530   If the product is a gas for inhalation a bioequivalence study is not required.

### 531   **5.1.8 Locally applied products**

532   a) Locally acting

533   For products for local use (after oral, nasal, inhalation, ocular, dermal, rectal, vaginal etc.  
534   administration) intended to act without systemic absorption the approach to determine  
535   bioequivalence based on systemic measurements is not applicable and pharmacodynamic or  
536   comparative clinical studies are in principle required. The lack of them should be justified  
537   (see specific Note for Guidance).

538   Whenever systemic exposure resulting from locally applied, locally acting medicinal products  
539   entails a risk of systemic adverse reactions, systemic exposure should be measured.

540 b) Systemically acting

541 For locally applied products with systemic action a bioequivalence study is always required.

### 542 **5.1. In Vitro Dissolution**

543 Dissolution studies are always necessary and consequently required. In some cases those  
544 studies are alone sufficient to assess the bioequivalence but in other cases they are insufficient  
545 and should be completed by in vivo studies. Dissolution studies must follow the guidance as  
546 laid out in Appendix II.

### 547 **5.2. Variations**

548 If a product has been reformulated from the formulation initially approved or the  
549 manufacturing method has been modified by the manufacturer in ways that could be  
550 considered to impact on the bioavailability, a bioequivalence study is required, unless  
551 otherwise justified. Any justification presented should be based upon general considerations,  
552 e.g. as per 5.1.1, or on whether an acceptable in vivo / in vitro correlation has been  
553 established.

554 In cases where the bioavailability of the product undergoing change has been investigated and  
555 an acceptable correlation between in vivo performance and in vitro dissolution has been  
556 established, the requirements for in vivo demonstration of bioequivalence can be waived if the  
557 dissolution rate in vitro of the new product is similar with that of the already approved  
558 medicinal product under the same test conditions as used to establish the correlation (see  
559 Appendix II)

560 In all other cases bioequivalence studies have to be performed.

561 For variations of the innovator product the reference product for use in bioequivalence and  
562 dissolution studies is usually that authorised under the current formula, manufacturing  
563 method, packaging etc. and the product manufactured in line with the proposed changes is  
564 tested against this.

565 When variations to an essentially similar product are made the reference product for the  
566 bioequivalence study should be the innovator product.

### 567 **5.3. Dose proportionality in immediate release oral dosage forms**

568 If a new application concerns several strengths of the active substance a bioequivalence study  
569 investigating only one strength may be acceptable. However the choice of the strength used  
570 should be justified on analytical, pharmacokinetic and safety grounds. Furthermore all of the  
571 following conditions should be fulfilled:

- 572 • the pharmaceutical products are manufactured by the same manufacturer and process;
- 573 • the drug input has shown to be linear over the therapeutic dose range (if this is not the  
574 case the strengths where the sensitivity is largest to identify differences in the two  
575 products should be used);
- 576 • the qualitative composition of the different strengths is the same;
- 577 • the ratio between amounts of active substance and excipients is the same, or, in the case  
578 of preparations containing a low concentration of the active substance, the ratio between  
579 the amounts of excipients is the same;
- 580 • the dissolution profile should be similar under identical conditions for the additional  
581 strengths and the strength of the batch used in the bioequivalence study.

582 If a new strength (within the approved dose range) is applied for on the basis of an already  
583 approved medicinal product and all of the stated conditions hold then a bioequivalence study  
584 is not necessary.

585 **5.4. Suprabioavailability**

586 If suprabioavailability is found, i.e. if the new product displays an extent of absorption  
587 appreciably larger than the approved product, reformulation to a lower dosage strength should  
588 be considered. In this case, the biopharmaceutical development should be reported and a final  
589 comparative bioavailability study of the reformulated new product with the old approved  
590 product should be submitted.

591 In case reformulation is not carried out the dosage recommendations for the suprabioavailable  
592 product will have to be supported by clinical studies if different from the reference product.  
593 Such a pharmaceutical product should not be accepted as therapeutically equivalent to the  
594 existing reference product. If marketing authorisation is obtained, the new product may be  
595 considered as a new medicinal product.

596 To avoid confusion for both prescribers and patients, it is recommended that the name of  
597 suprabioavailable product precludes confusion with the older approved product

598 Suprabioavailable products cannot claim "essential similarity" (see section 2.5) with the  
599 innovator product.

600 **APPENDIX I**

601

602 **Explanation of the symbols in paragraph 3.3**

603

604 **C<sub>max</sub>**: maximal plasma concentration;

605 **C<sub>min</sub>**: minimal plasma concentration;

606 **C<sub>av</sub>**: average plasma concentration;

607 **t<sub>max</sub>**: time passed since administration at which the plasma concentration  
608 maximum occurs;

609 **AUC<sub>t</sub>**: area under the plasma concentration curve from administration to last  
610 observed concentration at time t.

611 **AUC<sub>∞</sub>**: area under the plasma concentration curve extrapolated to infinite time;

612 **AUC<sub>τ</sub>**: AUC during a dosage interval in steady state;

613 **MRT**: mean residence time;

614 **Ae<sub>t</sub>**: cumulative urinary excretion from administration until time t;

615 **Ae<sub>∞</sub>**: cumulative urinary excretion extrapolated to infinite time;

616 **t<sub>1/2</sub>**: plasma concentration half-life;

617 **Fluctuation**:  $(C_{\max} - C_{\min})/C_{\text{av}}$  or  $(C_{\max} - C_{\min})/C_{\min}$

618

619 **APPENDIX II**

620

621 **Dissolution testing**

622 A medicinal product is composed of drug substance and excipients and the proportion  
623 between them, the type of excipients and the manufacturing method of the final product are  
624 chosen based on both the content, the physicochemical and the bulk properties of the drug and  
625 on its absorption properties. Taken as a whole this gives each product certain dissolution  
626 characteristics.

627 During the development of a medicinal product a dissolution test is used as a tool to identify  
628 formulation factors that are influencing and may have a crucial effect on the bioavailability of  
629 the drug. As soon as the composition and the manufacturing process are defined a dissolution  
630 test is used in the quality control of scale-up and of production batches to ensure both batch-  
631 to-batch consistency and that the dissolution profiles remain similar to those of pivotal  
632 clinical trial batches. Furthermore, a dissolution test can be used to support the bioavailability  
633 of a new drug product, the bioequivalence of an essentially similar product or variations.

634 Therefore, dissolution studies can serve several purposes:

635 i - Quality assurance

- 636 • To get information on the test batches used in bioavailability/bioequivalence studies  
637 and pivotal clinical studies to support specifications for quality control.
- 638 • To be used as a tool in quality control to demonstrate consistency in manufacture
- 639 • To get information on the reference product used in bioavailability/bioequivalence  
640 studies and pivotal clinical studies

641 ii -Bioequivalence surrogate inference

- 642 • To demonstrate similarity between reference products from different Member States
- 643 • To demonstrate similarity between different formulations of an active substance  
644 (variations and new, essentially similar products included) and the reference medicinal  
645 product
- 646 • To collect information on batch to batch consistency of the products (test and  
647 reference) to be used as basis for the selection of appropriate batches for the in vivo  
648 study.

649 The test methodology should be in accordance with pharmacopoeial requirements unless  
650 those requirements are shown to be unsatisfactory. Alternative methods can be considered  
651 when justified that these are discriminatory and able to differentiate between batches with  
652 acceptable and non-acceptable performance of the product in vivo.

653 If an active substance is considered highly soluble, it is reasonable to expect that it will not  
654 cause any bioavailability problems if, in addition, the dosage system is rapidly dissolved in  
655 the physiological pH-interval expected after product administration. A bioequivalence study  
656 may in those situations be waived based on case history and similarity of dissolution profiles  
657 which are based on discriminatory testing, provided that the other exemption criteria in 5.1.1  
658 are met. The similarity should be justified by dissolution profiles, covering at least three time  
659 points, attained at three different buffers (normally pH range 1-6.8; in cases where it is  
660 considered necessary pH range 1-8).

661 In the case of a drug or excipients that are insensitive to pH, profiles from only two buffer  
662 systems are required.

663 If an active substance is considered to have a low solubility and a high permeability, the rate  
CPMP/EWP/QWP/1401/98

664 limiting step for absorption may be dosage form dissolution. This is also the case when one or  
665 more of the excipients are controlling the release and subsequent dissolution step of the active  
666 substance. In those cases a variety of test conditions is recommended and adequate sampling  
667 should be performed until either 90% of the drug is dissolved or an asymptote is reached.  
668 Knowledge of dissolution properties under different conditions e.g. pH, agitation, ionic  
669 strength, surfactants, viscosity, osmotic pressure is important since the behaviour of the solid  
670 system in vivo may be critical for the drug dissolution independent of the physico-chemical  
671 properties of the active substance. An appropriate experimental statistical design may be used  
672 to investigate the critical parameters and for the optimisation of such conditions.

673 Any methods to prove similarity of dissolution profiles are accepted as long as they are  
674 justified.

675 The similarity may be compared by model-independent or model-dependent methods e.g. by  
676 linear regression of the percentage dissolved at specified time points, by statistical comparison  
677 of the parameters of the Weibull function or by calculating a similarity factor e.g. the one  
678 defined below:

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684

$$f_2 = 50 \cdot \log \left( \frac{100}{\sqrt{1 + \frac{\sum_{t=1}^{t=n} [\bar{R}(t) - \bar{T}(t)]^2}{t-1}}} \right)$$

685 In this equation  $f_2$  is the similarity factor,  $n$  is the number of time points,  $R(t)$  is the mean  
686 percent drug dissolved of e.g. a reference product, and  $T(t)$  is the mean percent drug  
687 dissolved of e.g. a test product.

688 The evaluation of similarity is based on the conditions of

- 689 • A minimum of three time points (zero excluded)
- 690 • 12 individual values for every time point for each formulation
- 691 • not more than one mean value of > 85% dissolved for each formulation
- 692 • that the standard deviation of the mean of any product should be less than 10% from  
693 second to last time point.

694 An  $f_2$  value between 50 and 100 suggests that the two dissolution profiles are similar. In cases  
695 where more than 85% of the drug are dissolved within 15 minutes, dissolution profiles may be  
696 accepted as similar without further mathematical evaluation.