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# Guidance for Industry

## Analytical Procedures and Methods Validation

**Chemistry, Manufacturing, and Controls Documentation**

### *DRAFT GUIDANCE*

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For questions on the contents of this draft document contact (CDER) Radhika Rajagopalan, 301-827-5849 or (CBER) Alfred Del Grosso, 301-435-4988.

**U.S. Department of Health and Human Services  
Food and Drug Administration  
Center for Drug Evaluation and Research (CDER)  
Center for Biologics Evaluation and Research (CBER)**

**July 2000  
CMC #**

# Guidance for Industry

## Analytical Procedures and Methods Validation

### Chemistry, Manufacturing, and Controls Documentation

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**U.S. Department of Health and Human Services  
Food and Drug Administration  
Center for Drug Evaluation and Research (CDER)  
Center for Biologics Evaluation and Research (CBER)  
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**Guidance for Industry<sup>1</sup>**

**Analytical Procedures and Methods Validation**

*If you plan to submit comments on this draft guidance, to expedite FDA review of your comments, please:*

- *Clearly explain each issue/concern and, when appropriate, include a proposed revision and the rationale and/or justification for the proposed change.*
- *Identify specific comments by line numbers; use the pdf version of the document whenever possible.*
- *If possible, e-mail an electronic copy (Word or WordPerfect) of the comments you have submitted to the docket to [cunninghamp@cder.fda.gov](mailto:cunninghamp@cder.fda.gov).*

1 **I. INTRODUCTION**

2 This guidance provides recommendations to applicants on submitting analytical procedures,<sup>2</sup>  
3 validation data, and samples to support the documentation of the identity, strength, quality,  
4 purity, and potency of drug substances and drug products.<sup>3</sup> This guidance is intended to assist  
5 applicants in assembling information, submitting samples, and presenting data to support  
6 analytical methodologies. The recommendations apply to drug substances and drug products  
7 covered in new drug applications (NDAs), abbreviated new drug applications (ANDAs),  
8 biologics license applications (BLAs), product license applications (PLAs), and supplements to  
9 these applications.<sup>4</sup> The principles also apply to drug substances and drug products covered in  
10 Type II drug master files (DMFs). If a different approach is chosen, the applicant is encouraged

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<sup>1</sup> This guidance has been prepared by the Analytical Methods Technical Committee of the Chemistry, Manufacturing, and Controls Coordinating Committee (CMC CC) in the Center for Drug Evaluation and Research (CDER) and the Center for Biologics Evaluation and Research (CBER) at the Food and Drug Administration (FDA). This guidance represents the Agency's current thinking on analytical procedures, validation data, and samples. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statutes, regulations, or both.

<sup>2</sup> *Analytical procedure* is interchangeable with *method* or *test procedure*.

<sup>3</sup> The terms *drug substance* and *drug product*, as used in this guidance, refer to human drugs and biologics.

<sup>4</sup> Sponsors preparing investigational new drug applications (INDs) should also consider the recommendations in this guidance. However, the amount and depth of the information that should be submitted to support an IND depends in large part on the phase of the investigation and the specific testing proposed in humans (see section V).

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11 to discuss the matter in advance with the center with product jurisdiction to prevent the  
12 expenditure of resources on preparing a submission that may later be determined to be  
13 unacceptable.

14 The principles of methods validation described in this guidance apply to all types of analytical  
15 procedures. However, the specific recommendations in this guidance may not be applicable to  
16 certain unique analytical procedures for products such as biological, biotechnological, botanical,  
17 or radiopharmaceutical drugs. For example, many bioassays are based on animal challenge  
18 models, immunogenicity assessments, or other immunoassays that have unique features that  
19 should be considered when submitting analytical procedure and methods validation information.  
20 Furthermore, specific recommendations for biological and immunochemical tests that may be  
21 necessary for characterization and quality control of many drug substances and drug products are  
22 beyond the scope of this guidance document. Although this guidance does not specifically  
23 address the submission of analytical procedures and validation data for raw materials,  
24 intermediates, excipients, container closure components, and other materials used in the  
25 production of drug substances and drug products, validated analytical procedures should be used  
26 to analyze these materials. For questions on appropriate validation approaches for analytical  
27 procedures or submission of information not addressed in this guidance, applicants should  
28 consult with the appropriate chemistry review staff at FDA.

29 This guidance, when finalized, will replace the FDA guidance for industry on *Submitting*  
30 *Samples and Analytical Data for Methods Validation* (February 1987).

31 **II. BACKGROUND**

32 Each NDA and ANDA must include the analytical procedures necessary to ensure the identity,  
33 strength, quality, purity, and potency of the drug substance and drug product, including  
34 bioavailability of the drug product (21 CFR 314.50(d)(1) and 314.94(a)(9)(i)). Data must be  
35 available to establish that the analytical procedures used in testing meet proper standards of  
36 accuracy and reliability (21 CFR 211.165(e) and 211.194(a)(2)).

37 *Methods validation* is the process of demonstrating that analytical procedures are suitable for  
38 their intended use. The methods validation process for analytical procedures begins with the  
39 planned and systematic collection by the applicant of the validation data to support the analytical  
40 procedures. The review chemist evaluates the analytical procedures and validation data  
41 submitted in the NDA or ANDA. On request from FDA, an NDA or ANDA applicant must  
42 submit samples of drug product, drug substance, noncompendial reference standards, and blanks  
43 so that the applicant's drug substance and drug product analytical procedures can be evaluated by  
44 FDA laboratories (21 CFR 314.50(e) and 314.94(a)(10)). The FDA laboratory analysis  
45 demonstrates that the analytical procedures are reproducible by laboratory testing. The review

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46 chemists and laboratory analysts determine the suitability of the analytical procedures for  
47 regulatory purposes. FDA investigators inspect the analytical laboratory testing sites to ensure  
48 that the analytical procedures used for release and stability testing comply with current good  
49 manufacturing practices (CGMPs) (21 CFR part 211) or good laboratory practices (GLPs) (21  
50 CFR part 58), as appropriate.

51 Each BLA and PLA must include a full description of the manufacturing methods, including  
52 analytical procedures, that demonstrate that the manufactured product meets prescribed standards  
53 of safety, purity, and potency (21 CFR 601.2(a) and 601.2(c)(1)(iv)). Data must be available to  
54 establish that the analytical procedures used in testing meet proper standards of accuracy and  
55 reliability (21 CFR 211.194(a)(2)). For BLAs, PLAs, and their supplements, the analytical  
56 procedures and their validation are submitted as part of the license application or supplement and  
57 are evaluated by the review committee. Representative samples of the product must be submitted  
58 and summaries of results of tests performed on the lots represented by the submitted sample must  
59 be provided (21 CFR 601.2(a) and 601.2(c)(1)(vi)). The review committee chair may request  
60 analytical testing by CBER laboratory analysts to evaluate the applicant's analytical procedures  
61 and verify the test results.

62 All analytical procedures are of equal importance from a validation perspective. In general,  
63 validated analytical procedures should be used, irrespective of whether they are for in-process,  
64 release, acceptance, or stability testing. Each quantitative analytical procedure should be  
65 designed to minimize assay variation.

66 Analytical procedures and validation data are submitted in the sections of the application on  
67 analytical procedures and controls. Recommendations on information to be submitted are  
68 included in sections III through IX and XI of this guidance. Information on submission of the  
69 *methods validation package* to the NDA or ANDA and samples to the FDA laboratories is  
70 provided in section X.

### 71 **III. TYPES OF ANALYTICAL PROCEDURES**

#### 72 **A. Regulatory Analytical Procedure**

73 *A regulatory analytical procedure* is the analytical procedure used to evaluate a defined  
74 characteristic of the drug substance or drug product. The analytical procedures in the  
75 *U.S. Pharmacopeia/National Formulary* (USP/NF) are those legally recognized under  
76 section 501(b) of the Food, Drug, and Cosmetic Act (the Act) as the regulatory analytical  
77 procedures for compendial items. For purposes of determining compliance with the Act,  
78 the regulatory analytical procedure is used.

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### 79 **B. Alternative Analytical Procedure**

80 An *alternative analytical procedure* is an analytical procedure proposed by the applicant  
81 for use instead of the regulatory analytical procedure. A validated alternative analytical  
82 procedure should be submitted only if it is shown to perform equal to or better than the  
83 regulatory analytical procedure. If an alternative analytical procedure is submitted, the  
84 applicant should provide a rationale for its inclusion and identify its use (e.g., release,  
85 stability testing), validation data, and comparative data to the regulatory analytical  
86 procedure.

### 87 **C. Stability-Indicating Assay**

88 A *stability-indicating assay* is a validated quantitative analytical procedure that can detect  
89 the changes with time in the pertinent properties of the drug substance and drug product.  
90 A stability-indicating assay accurately measures the active ingredients, without  
91 interference from degradation products, process impurities, excipients, or other potential  
92 impurities. If an applicant submits a non-stability-indicating analytical procedure for  
93 release testing, then an analytical procedure capable of qualitatively and quantitatively  
94 monitoring the impurities, including degradation products, should complement it. Assay  
95 analytical procedures for stability studies should be stability-indicating, unless  
96 scientifically justified.

## 97 **IV. REFERENCE STANDARDS**

### 98 **A. Types of Standards**

99 A *reference standard* (i.e., primary standard) may be obtained from the USP/NF or other  
100 official sources (e.g., CBER, 21 CFR 610.20). If there are questions on whether a source  
101 of a standard would be considered by FDA to be an official source, applicants should  
102 contact the appropriate chemistry review staff. When there is no official source, a  
103 reference standard should be of the highest possible purity and be fully characterized.

104 A *working standard* (i.e., in-house or secondary standard) is a standard that is qualified  
105 against and used instead of the reference standard.

### 106 **B. Certificate of Analysis**

107 A certificate of analysis (COA) for reference standards from non-official sources should  
108 be submitted in the section of the application on analytical procedures and controls. For

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109 standards from official sources, the user should ensure the suitability of the reference  
110 standard. The standard should be stored correctly and used within the established use  
111 interval.

### **C. Characterization of a Reference Standard**

112  
113 Reference standards from USP/NF and other official sources do not require further  
114 characterization. A reference standard that is not obtained from an official source should  
115 be of the highest purity that can be obtained by reasonable effort, and it should be  
116 thoroughly characterized to ensure its identity, strength, quality, purity, and potency. The  
117 qualitative and quantitative analytical procedures used to characterize a reference  
118 standard are expected to be different from, and more extensive than, those used to control  
119 the identity, strength, quality, purity, and potency of the drug substance or the drug  
120 product. Analytical procedures used to characterize a reference standard should not rely  
121 solely on comparison testing to a previously designated reference standard.

122 Generally, this characterization information should include:

- 123 ● A brief description of the manufacture of the reference standard, if the  
124 manufacturing process differs from that of the drug substance. Any additional  
125 purification procedures used in the preparation of the reference standard should be  
126 described.
- 127 ● Legible reproductions of the relevant spectra, chromatograms, thin-layer  
128 chromatogram (TLC) photographs or reproductions, and other appropriate  
129 instrumental recordings.
- 130 ● Data establishing purity. The data should be obtained by using appropriate tests,  
131 such as TLC, gas chromatography (GC), high-pressure liquid chromatography  
132 (HPLC), phase solubility analysis, appropriate thermometric analytical  
133 procedures, and others as necessary.
- 134 ● Appropriate chemical attribute information, such as structural formula, empirical  
135 formula, and molecular weight. Information to substantiate the proof of structure  
136 should include appropriate analytical tests, such as elemental analysis, infrared  
137 spectrophotometry (IR), ultraviolet spectrophotometry (UV), nuclear magnetic  
138 resonance spectroscopy (NMR), and mass spectrometry (MS), as well as  
139 applicable functional group analysis. Detailed interpretation of the test data in  
140 support of the claimed structure should be provided.
- 141 ● A physical description of the material, including its color and physical form.

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- Appropriate physical constants such as melting range, boiling range, refractive index, dissociation constants (pK values), and optical rotation.
  - A detailed description of the analytical procedures used to characterize the reference standard.

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For biotechnological/biological product reference standards, the recommendations on characterization information above may apply and should be considered. However, additional and/or different tests would be important to assess physicochemical characteristics, structural characteristics, biological activity, and/or immunochemical activity. Physicochemical determinations may include isoform, electrophoretic, and liquid chromatographic patterns, as well as spectroscopic profiles. Structural characterization may include a determination of amino acid sequence, amino acid composition, peptide map, and carbohydrate structure. Biological and/or immunochemical activity should be assessed using the same analytical procedures used to determine product potency. These can include animal-based, cell culture-based, biochemical, or ligand/receptor-binding assays. While these tests may be needed for complete characterization of certain reference standards, specific recommendations for validation of biological and immunochemical tests are not contained in this guidance document.

### 161 **V. METHODS VALIDATION FOR INDs**

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For an investigational new drug, sufficient information is required in each phase of an investigation to ensure proper identification, quality, purity, strength, and/or potency. The amount of information on analytical procedures and methods validation necessary will vary with the phase of the investigation (21 CFR 312.23(a)(7)).

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For general guidance on analytical procedures and methods validation information to be submitted for phase 1 studies, sponsors should refer to the FDA guidance for industry on *Content and Format of Investigational New Drug Applications (INDs) for Phase 1 Studies of Drugs, Including Well-Characterized, Therapeutic, Biotechnology-Derived Products* (November 1995). General guidance regarding analytical procedures and methods validation information to be submitted for phase 2 or phase 3 studies will be provided in the FDA guidance for industry *INDs for Phase 2 and 3 Studies of Drugs, Including Specified Therapeutic Biotechnology-Derived Products, Chemistry, Manufacturing, and Controls Content and Format*, when finalized (draft guidance published April 1999).

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175 All analytical procedures should be fully developed and validation completed when the NDA,  
176 ANDA, BLA, or PLA is submitted.

177 **VI. CONTENT AND FORMAT OF ANALYTICAL PROCEDURES FOR NDAs,**  
178 **ANDAs, BLAs, AND PLAs**

179 Any analytical procedure submitted in an NDA, ANDA, BLA, or PLA should be described in  
180 sufficient detail to allow a competent analyst to reproduce the necessary conditions and obtain  
181 results comparable to the applicant's. Aspects of the analytical procedure that require special  
182 attention should be described. If the analytical procedure used is in the current revision of the  
183 USP/NF or other FDA recognized standard references (e.g., AOAC International *Book Of*  
184 *Methods*) and the referenced analytical procedure is not modified, a statement indicating the  
185 analytical procedure and reference may be provided rather than a description of the method (21  
186 CFR 211.194). A description of analytical procedures from any other published sources should  
187 be provided, because the referenced sources may not be readily accessible to the reviewer.

188 The following is a list of information that should typically be included in a description of an  
189 analytical procedure.

190 **A. Principle**

191  
192 A statement of the principle of the analytical procedure should be included. For example,  
193 separation is based on isocratic reversed phase HPLC with detection by UV.

194  
195 **B. Sampling**

196 The number of samples (e.g., vials, tablets) selected, how they are used (i.e., as individual  
197 or composite samples), and the number of replicate analyses per sample should be  
198 described.

199 **C. Equipment and Equipment Parameters**

200  
201 A listing of all equipment (e.g., instrument type, detector, column type, dimensions)  
202 should be included, as well as a list of equipment parameters (e.g., flow rate,  
203 temperatures, run time, wavelength settings). A drawing representing the experimental  
204 configuration (e.g., illustrating positions for a spray pattern analytical procedure) should  
205 be provided, when appropriate.

206  
207 **D. Reagents**

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208 A list of reagents and their grades (e.g., USP/NF, American Chemical Society (ACS)  
209 Analytical Reagent) should be included. If in-house or modified commercial reagents are  
210 used, directions for their preparation should be included. Unstable or potentially  
211 hazardous reagents should be identified, and storage conditions, directions for safe use,  
212 and usable shelf life for these reagents should be specified.

### **E. System Suitability Testing**

214 System suitability test parameters and acceptance criteria are based on the concept that  
215 the equipment, electronics, analytical operations, and samples to be analyzed constitute an  
216 integrated system. System suitability testing ensures that the system is working properly  
217 at the time of analysis. Appropriate system suitability criteria should be defined and  
218 included in the analytical procedure.

219 All chromatographic analytical procedures should include system suitability testing and  
220 criteria. Parameters typically used in system suitability evaluations are defined and  
221 discussed in the CDER reviewer guidance on *Validation of Chromatographic Methods*  
222 (November 1994).

223 System suitability testing is recommended as a component of any analytical procedure,  
224 not just those that involve chromatographic techniques. Regardless of the type of  
225 analytical procedure, testing should be used to confirm that the system will function  
226 correctly independent of the environmental conditions. For example, titration analytical  
227 procedures should always include the evaluation of a blank (commonly referred to as a  
228 *blank titration*).

### **F. Preparation of Standards**

230 Procedures for the preparation of all standard solutions (e.g., stock, working standard  
231 solutions, internal standards) should be included.

### **G. Preparation of Samples**

233 Sample preparation for individual tests should be clearly described. Specific details  
234 should be provided for unusual sample preparations (e.g., solid-phase extraction,  
235 derivatization).

### **H. Procedure**

237 A step-by-step description of the procedure should be provided. The description should  
238 include, where appropriate, equilibration times, injection sampling sequence, and system

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239 suitability or start-up parameters. Unusual hazards should be identified.

240 **I. Calculations**

241 Representative calculations, with a tabulation defining all symbols and numerical factors,  
242 and specific instructions for the calculation of degradation products and impurities should  
243 be included. Any mathematical transformations or formulas used in data analysis should  
244 be described in detail. These may include logarithmic transformations used to obtain a  
245 linear relationship from exponential data, or the use of multiple order regression analyses.

246 **J. Reporting of Results**

247 *1. General*

248 The format used to report results (e.g., percent label claim, weight/weight,  
249 weight/volume, parts per million (ppm)) including the specific number of  
250 significant figures to be reported should be provided.

251 *2. Impurities Analytical Procedures*

252 The name and location/identifier (e.g., retention time (RT), relative retention time  
253 (RRT)) of impurities and the type of impurity (e.g., process, degradant, excipient  
254 degradant) should be included in the analytical procedures for impurities in the  
255 drug substance and drug product. The detection limit (DL) or quantitation limit  
256 (QL) should be stated, as appropriate. The DL or QL can be set using the drug  
257 substance's detection response.

258 Reporting of organic impurities should cover (1) specified identified impurities by  
259 name, (2) specified unidentified impurities by location/identifier, (3) any  
260 unspecified impurities, and (4) total impurities. The total organic impurities for  
261 the drug product or drug substance is the sum of all impurities equal to or greater  
262 than their individual QL. See recommendations regarding appropriate QLs in  
263 FDA impurities guidances (see references). Inorganic impurities and residual  
264 solvents should also be addressed.

265 For the drug product, drug substance process impurities may be excluded from  
266 reporting if an acceptable rationale is provided in the sections on analytical  
267 procedures and controls. Drug product impurities from the drug product  
268 manufacturing process, packaging, and labeling should be addressed.

269 The above reporting information may not be strictly applicable to all products

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270 (e.g., biological, biotechnological, botanical, radiopharmaceutical drugs), but any  
271 significant process and product-related impurities should be determined and  
272 reported.

273 **VII. METHODS VALIDATION FOR NDAs, ANDAs, BLAs, AND PLAs**

274 **A. Noncompendial Analytical Procedures**

275 In an NDA, ANDA, BLA, or PLA, data must be submitted to establish that the analytical  
276 procedures used in testing meet proper standards of accuracy and reliability (21 CFR  
277 211.194(a)(2)). *Methods validation* is the process of demonstrating that analytical  
278 procedures are suitable for their intended use. At the time of submission, the NDA,  
279 ANDA, BLA, or PLA should contain methods validation information to support the  
280 adequacy of the analytical procedures.

281  
282 The International Conference on Harmonisation (ICH) guidance *Q2A Text on Validation*  
283 *of Analytical Procedures* (March 1995) and *Q2B Validation of Analytical Procedures:*  
284 *Methodology* (November 1996) provide recommendations on validation of analytical  
285 procedures. Analytical procedures outside the scope of the ICH guidances should still be  
286 validated.

287 *1. Validation Characteristics*

288  
289 Applicants should submit information on the validation characteristics of their  
290 proposed analytical procedures (see ICH *Q2A* and ICH *Q2B*). Although not all of  
291 the validation characteristics are needed for all types of tests (see section VII.A.3),  
292 typical validation characteristics are:

- 293 ● Accuracy
- 294 ● Precision (repeatability and intermediate precision)
- 295 ● Specificity
- 296 ● Detection limit
- 297 ● Quantitation limit
- 298 ● Linearity
- 299 ● Range
- 300 ● Robustness

301 *2. Other Methods Validation Information*

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- 302 Methods validation information should also include:
- 303 ● Data to demonstrate the stability of all analytical sample preparations  
304 through the time required to complete the analysis.
  - 305 ● Legible reproductions of representative instrument output or recordings  
306 (e.g., chromatograms) and raw data output (e.g., integrated areas), as  
307 appropriate. Instrument output for placebo, standard, and sample should  
308 also be provided (see section VII.A.2.c).
  - 309 ● Representative calculations using submitted raw data, to show how the  
310 impurities in drug substance are calculated.
  - 311 ● Information from stress studies (see section VII.A.2.b).
  - 312 ● Impurities labeled with their names and location identifiers (e.g., RRT for  
313 chromatographic data) for the impurity analytical procedure.
  - 314 ● For drug substances:
    - 315 ● A discussion of the possible formation and control of polymorphic  
316 and enantiomeric substances.
    - 317 ● Identification and characterization of each organic impurity, as  
318 appropriate. This information may not be needed for all products  
319 (e.g., botanicals). Other impurities (e.g., inorganics, residual  
320 solvents) should be addressed and quantitated.
- 321 Recommendations on submitting information on impurities is  
322 provided in various FDA guidances such as the ICH guidance *Q3A*  
323 *Impurities in New Drug Substances* (January 1996).
- 324 ● A list of known impurities, with structure if available, including  
325 process impurities, degradants, and possible isomers.
  - 326 ● For drug products:
    - 327 ● A degradation pathway for the drug substance in the dosage form,  
328 where possible.
    - 329 ● Data demonstrating recovery from the sample matrix as illustrated

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330 by the accuracy studies.

- 331 • Data demonstrating that neither the freshly prepared nor the  
332 degraded placebo interferes with the quantitation of the active  
333 ingredient.

334 ICH *Q2A* and *Q2B* address almost all of the validation parameters. Areas that  
335 should be provided in more detail are described below.

336 a. Robustness

337 Robustness, a measure of the analytical procedure's capability to remain  
338 unaffected by small but deliberate variations, is described in ICH *Q2A* and *Q2B*.  
339 Such testing should be performed during development of the analytical procedure  
340 and the data discussed and/or submitted. In cases where an effect is observed,  
341 representative instrument output (e.g., chromatograms) should be submitted.

342 b. Stress Studies

343 Degradation information obtained from *stress studies* (e.g., products of acid and  
344 base hydrolysis, thermal degradation, photolysis, oxidation) for the drug substance  
345 and for the active ingredient in the drug product should be provided to  
346 demonstrate the specificity of the assay and analytical procedures for impurities.  
347 The stress studies should demonstrate that impurities and degradants from the  
348 active ingredient and drug product excipients do not interfere with the quantitation  
349 of the active ingredient. Stress studies are described in various FDA guidances  
350 relating to the stability of drug products (see references).

351 The design of the stress studies and the results should be submitted to the stability  
352 section of the application. Representative instrument output (e.g.,  
353 chromatograms) and/or other appropriate data (e.g., degradation information  
354 obtained from stress studies) should be submitted in the sections on analytical  
355 procedures and controls.

356 c. Instrument Output/Raw Data

357 i. Organic Impurities

358 Representative data should be submitted to support an assessment of the  
359 organic impurities. Representative data for residual solvents are generally  
360 not needed. Instrument output and the raw numerical values (e.g., peak

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361 area) with appropriate identification and labeling (e.g., RT for  
362 chromatographic peaks, chemical shift ( $\delta$ ) and coupling constant (J) for  
363 NMR) should be provided. The impurity profile should be assessed at the  
364 quantitation limit and the instrument output provided. Additional  
365 information should be provided to confirm that the impurity profile is  
366 adequately characterized. For example, a representative chromatogram  
367 using detection at a low wavelength, such as 205 nm, and double the  
368 proposed total run time could be submitted to support the specificity of the  
369 analytical procedure.

370 For quantitation purposes, the response factor of the drug substance may  
371 be used for impurities without a reference standard. In cases where the  
372 response factors are not close, this practice may still be acceptable,  
373 provided a correction factor is applied or the impurities are, in fact, being  
374 overestimated. Acceptance criteria and analytical procedures used to  
375 estimate identified or unidentified impurities often are based on analytical  
376 assumptions (e.g., equivalent detector response). Assumptions should be  
377 discussed and justified.

378 ii. Drug Substance

379 Data should be submitted showing the separation and detection of  
380 impurities using spiked or stress samples. Complete impurity profiles as  
381 graphic output (e.g., chromatograms) and raw data (e.g., integrated peak  
382 areas) of representative batches should be submitted in the sections on  
383 analytical procedures and controls for the drug substance. For ANDAs  
384 and related submissions, appropriate information for the batches used in  
385 the biobatch or submission batch should be provided. All responses (e.g.,  
386 peaks) should be labeled.

387 The analytical procedure used should be capable of differentiating  
388 changes, if any, between past and present batches. The quantitation limit  
389 and the type of organic impurity (e.g., degradant, process impurity) should  
390 be stated. The analytical procedure number, batch number, manufacturing  
391 date and site, and date of analysis should be provided.

392 iii. Drug Product

393 Information such as instrument output (e.g., chromatograms) and raw data  
394 (e.g., integrated peak areas) from representative batches under long-term  
395 and accelerated stability conditions, and stressed samples should be

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396 submitted in the sections on analytical procedures and controls of the drug  
397 product. For ANDAs and related submissions, appropriate information for  
398 the biobatch or submission batch should be provided. References to the  
399 raw data (e.g., chromatograms) should be included in the stability section  
400 of the application.

401 At a minimum, the submission should include instrument output and raw  
402 data for release testing and at the latest available time point for the same  
403 batch. All responses (e.g., peaks) should be labeled and identified. In  
404 addition, the analytical procedure number, batch number of the drug  
405 product, manufacturing date, date of analysis, source and batch number of  
406 drug substance, manufacturing site, and container/closure information  
407 should be provided. The analytical procedures used should be capable of  
408 differentiating changes, if any, between past and present batches. The  
409 quantitation limit and the type (e.g., degradant, leachables from packaging)  
410 should be reported. Multiple methodologies can be used.

411 If process impurities from the drug substance and excipients with their  
412 related impurities are not reported in the impurities analytical procedure,  
413 the potential locations/identifier (e.g., RT, RRT) of these compounds  
414 should be described and listed in the analytical procedure.

415 3. *Recommended Validation Characteristics for Types of Tests*

416 Table 1 is a summary of the validation characteristics that should be addressed  
417 during validation of different types of analytical procedures. The same  
418 methodology can be used for several purposes. The validation information should  
419 support the intended purpose of the test. For example, if Raman spectroscopy is  
420 the methodology selected to quantitate polymorphic forms as impurities, or chiral  
421 HPLC for enantiomeric impurities, the recommended validation characteristics in  
422 Table 1 under *quantitative testing for impurities* would apply. However, if Raman  
423 spectroscopy or chiral HPLC are used for the purpose of identification or as  
424 specific tests, the recommended validation characteristics listed for those types of  
425 tests would apply.

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426 **Table 1. Recommended Validation Characteristics of the Various Types of Tests.**

427 428 Type of Tests / Characteristics	Identification	Testing for Impurities		Assay Dissolution (Measurement Only), Content/Potency	Specific Tests
		Quantitative	Limit		
429 Accuracy	-	+	-	+	+ <sup>4</sup>
430 431 Precision- Repeatability	-	+	-	+	+ <sup>4</sup>
432 433 Precision-Intermediate Precision	-	+ <sup>1</sup>	-	+ <sup>1</sup>	+ <sup>4</sup>
434 Specificity	+ <sup>2</sup>	+	+	+ <sup>5</sup>	+ <sup>4</sup>
435 Detection Limit	-	- <sup>3</sup>	+	-	-
436 Quantitation Limit	-	+	-	-	-
437 Linearity	-	+	-	+	-
438 Range	-	+	-	+	-
439 Robustness	-	+	- <sup>3</sup>	+	+ <sup>4</sup>

440 **NOTE:**

- 441 - Signifies that this characteristic is not normally evaluated.  
 442 + Signifies that this characteristic is normally evaluated.  
 443 1 In cases where reproducibility has been performed, intermediate precision is not needed.  
 444 2 Lack of specificity for an analytical procedure may be compensated for by the addition of a second  
 445 analytical procedure.  
 446 3 May be needed in some cases.  
 447 4 May not be needed in some cases.  
 448 5 Lack of specificity for an assay for release may be compensated for by impurities testing.

449 a. Identification

450 Identification analytical procedures may include tests such as IR, differential  
 451 scanning calorimetry (DSC), X-ray diffraction (XRD), UV, and HPLC retention  
 452 time. A specific identification test should be included for the active ingredient  
 453 whenever possible. In cases where a nonspecific identification analytical  
 454 procedure is proposed for the active ingredient, two independent analytical  
 455 procedures are generally sufficient, if justified. For other identification tests (e.g.,

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456 a chiral HPLC retention time as confirmation for the presence of an enantiomer,  
457 chloride test for a counterion) a single test is acceptable. This concept of the  
458 number of identification tests is applicable to both the drug substance and drug  
459 product.

460  
461 b. Impurities

462 The validation characteristics under *quantitative testing for impurities*, as  
463 described in Table 1, apply, regardless of which methodology is used to quantitate  
464 impurities. If the same analytical procedure is proposed as a limit test, validation  
465 characteristics under *limit testing for impurities* will apply.

466 c. Assay

467  
468 Assay includes the content of the active ingredient, preservative (if used), and  
469 measurement of content in dissolution and content uniformity samples.

470  
471 d. Specific Tests

472 Specific tests to control the drug substance, excipient, or drug product can include  
473 tests such as particle size analysis, droplet distribution, spray pattern, dissolution  
474 (excludes measurement), optical rotation, and methodologies such as DSC, XRD,  
475 and Raman spectroscopy. The validation characteristics may differ for the various  
476 analytical procedures. For example, accuracy, repeatability, intermediate  
477 precision and robustness should be evaluated for molecular size distribution gel  
478 permeation chromatography (GPC).

479  
480 **B. Compendial Analytical Procedures**

481 The suitability of a compendial analytical procedure must be verified under actual  
482 conditions of use (21 CFR 211.194(a)(2)). Information to demonstrate that USP/NF  
483 analytical procedures are suitable for the drug product or drug substance should be  
484 included in the submission. Information on the specificity, intermediate precision, and  
485 stability of the sample solution should be included. Compendial assay analytical  
486 procedures may not be stability-indicating, and this should be considered when  
487 developing the specification (see section III.C). For compendial items, additional  
488 analytical procedures, such as impurities or osmolality, may be requested to support the  
489 quality of the drug product or drug substance. These additional analytical procedures  
490 should be validated (see section VII.A).

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491 **VIII. STATISTICAL ANALYSIS**

492 **A. General**

493  
494 Methods validation includes an assessment of the adequacy of the analytical procedure.  
495 Statistical analysis (e.g., linear regression analysis, relative standard deviation) of  
496 methods validation data is often used to demonstrate the validity of the method. The  
497 statistical procedures for the analysis of the validation data should be determined prior to  
498 the start of any validation study. The procedure followed, including the amount of data to  
499 collect and the criteria used in determining the acceptability of the analytical procedure,  
500 should be specified.

501 The raw methods validation data and statistical procedures used to analyze the raw data  
502 should be provided and discussed in the sections on analytical procedures and controls.  
503 All statistical procedures used in the analysis of the data should be based on sound  
504 principles and be suitable for evaluating the dataset.

505 **B. Comparative Studies**

506 Comparative studies are performed to evaluate intermediate precision (e.g., different  
507 equipment, analysts, days). Comparative studies are also used to evaluate *between*  
508 *laboratory* variability (i.e., reproducibility) when an analytical procedure is used in more  
509 than one laboratory or to compare and evaluate the precision and accuracy of two  
510 analytical procedures (e.g., regulatory analytical procedure and an alternative analytical  
511 procedure). When comparative studies are performed, homogeneous samples from the  
512 same batch should be used, if feasible. Comparative results should be statistically  
513 analyzed and discussed and any bias explained.

514 **C. Statistics**

515 For information on statistical techniques used in making comparisons, as well as other  
516 general information on the interpretation and treatment of analytical data, appropriate  
517 literature or texts should be consulted (see references) .

518 **IX. REVALIDATION**

519 When sponsors make changes in the analytical procedure, drug substance (e.g., route of  
520 synthesis), or drug product (e.g., composition), the changes may necessitate revalidation of the  
521 analytical procedures. Revalidation should be performed to ensure that the analytical procedure  
522 maintains its characteristics (e.g., specificity) and to demonstrate that the analytical procedure

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523 continues to ensure the identity, strength, quality, purity, and potency of the drug substance and  
524 drug product, and the bioavailability of the drug product. The degree of revalidation depends on  
525 the nature of the change. When a different regulatory analytical procedure is substituted (e.g.,  
526 HPLC for titration), the new procedure should be validated (see section VII).

527 If during each use an analytical procedure can meet the established system suitability  
528 requirements only with repeated adjustments to the operating conditions stated in the analytical  
529 procedure, the analytical procedure should be reevaluated, amended, and revalidated, as  
530 appropriate.

531 FDA intends to provide guidance in the future on postapproval changes in analytical procedures.

### **X. METHODS VALIDATION PACKAGE: CONTENTS AND PROCESSING**

533 Part of the methods validation process may include FDA laboratory analysis to demonstrate that  
534 an analytical procedure is reproducible by laboratory testing. A methods validation package (see  
535 X.A) and samples (see X.B) will be needed for this process.

#### **A. Methods Validation Package**

537 The methods validation package will usually include information copied from pertinent  
538 sections of the application. To aid the review chemist, these copies should retain the  
539 original pagination of the application sections.

540 For ANDA and NDA products, the archival copy and extra copies of the methods  
541 validation packages should be submitted with the application. For ANDAs and related  
542 supplemental applications, one archival copy and two extra copies of the methods  
543 validation package should be submitted. For NDAs and related supplemental  
544 applications, one archival copy and three extra copies should be submitted. For BLAs  
545 and PLAs, a separate methods validation package need not be submitted. Information  
546 similar to that specified here should be included in the BLA or PLA submission.

547 The methods validation package should include:

##### **1. *Tabular List of All Samples to Be Submitted***

549 The list should include the lot number, identity (with chemical name and structure  
550 where required for clarity), package type and size, date of manufacture, and  
551 quantity of the samples.  
552

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### 553 2. *Analytical Procedures*

554 A detailed description of each of the analytical procedures listed in the  
555 specifications should be submitted. The description should be sufficient to allow  
556 the FDA laboratory analysts to perform the analytical procedure (see section VI).

### 557 3. *Validation Data*

558 Appropriate validation data to support the analytical procedures should be  
559 submitted. Individual values as well as summary tables should be provided.  
560 Representative instrument output and raw data and information regarding stress  
561 studies should be included (see section VII).

### 562 4. *Results*

563 The results obtained by the applicant for the submitted samples should be  
564 provided. Alternatively, COAs could be submitted. The dates of analysis should  
565 be stated.

### 566 5. *Composition*

567 The components and composition of the drug product should be provided.

### 568 6. *Specifications*

569 The specifications for the drug substance and the drug product should be included.

### 570 7. *Material Safety Data Sheets*

571 The applicant should include material safety data sheets (MSDSs) for all samples,  
572 standards, and reagents (29 CFR 1910.1200(g)). As appropriate, MSDSs should  
573 be provided for other materials used in the analytical procedures listed in the  
574 methods validation package. In the case of toxic or hazardous materials, MSDSs  
575 should be posted on the outside of the package to facilitate safe handling.

## 576 **B. Selection and Shipment of Samples**

577 On request from CDER, an NDA or ANDA applicant must submit samples of drug  
578 product, drug substance, noncompendial reference standards, and blanks, so that the  
579 suitability of the applicant's drug substance and drug product analytical procedures can be  
580 evaluated by FDA laboratories (21 CFR 314.50(e) and 314.94(a)(10)). For BLAs and

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581 PLAs, representative samples of the product must be submitted, and summaries of the  
582 results of tests performed on the lots represented by the submitted sample must be  
583 provided (21 CFR 601.2(a) and 601.2(c)(1)(vi)).

584 For CDER products, the number of sets of samples that should be submitted for methods  
585 validation will be identified in the instructions forwarded to the applicant by the FDA  
586 laboratory. In general, the quantity of samples in each set should be double the amount  
587 needed to carry out the testing as performed by the applicant. Along with the drug  
588 substance and the drug product samples, the applicant should submit internal standards,  
589 non-USP reference standards, samples of impurities, degradation products, and unusual  
590 reagents. A set of samples will be shipped to each assigned laboratory.

591 For biological products, CBER should be consulted on the submission of samples and  
592 supporting materials.

593 Unless specified differently by the reviewer, samples from any batch, preferably samples  
594 from an aged batch, may be selected for NDAs and NDA supplemental applications. The  
595 submitted drug product samples should be from a batch made with the proposed market  
596 formulation. For original ANDAs and appropriate supplements, a sample of the finished  
597 product from a batch being used to support approval of the submission should be used. If  
598 a sample is selected from a batch not described in the application, an amendment  
599 containing a copy of the batch record and certificate of analysis should be provided to the  
600 ANDA. For supplements that do not require submission and review of an exhibit batch  
601 record and associated data, any commercial batch may be submitted. For biological  
602 products, samples from several consecutively manufactured batches should be submitted.

603 The drug product should be supplied in its original packaging. Bulk substances (e.g.,  
604 drug substances, impurities, excipients) should be stored in opaque nonreactive  
605 containers. To prevent breakage during shipping, the samples should be adequately  
606 packaged in a sturdy container. Samples shipped from outside the United States should  
607 contain the appropriate customs forms to reduce delay in delivery.

608 If special storage precautions (e.g., freezing, use of an inert gas blanket) are required to  
609 protect sample integrity, arrangements should be made in advance with the validating  
610 laboratory for scheduled direct delivery. If a sample is toxic or potentially hazardous, the  
611 container should be prominently labeled with an appropriate warning and precautionary  
612 handling instructions.

### 614 **C. Responsibilities of the Various Parties**

#### 615 *1. Applicant*

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616 In the sections of the application on analytical procedures and controls, the  
617 applicant should provide a name, address, telephone number, and facsimile  
618 number so that samples can be requested. If this information is not provided, the  
619 contact person and address listed in the NDA, ANDA, BLA, or PLA submission  
620 will be used.

621 The methods validation packages should be compiled and submitted with the  
622 NDA or ANDA submission. For BLAs and PLAs, a separate methods validation  
623 package need not be submitted.

624 When an FDA laboratory contacts the applicant for samples, the applicant should  
625 provide FDA laboratories with the samples within 10 working days. With the  
626 exception of sample delivery arrangements, all communications concerning  
627 validation at the FDA laboratories should be made through or with the knowledge  
628 of the review chemist for CDER applications, or the BLA/PLA committee chair  
629 for CBER applications.

### 630 2. *Review Chemist*

631 The review chemist will review the application to determine that the analytical  
632 procedures are adequate to ensure the identity, strength, quality, purity, and  
633 potency of the drug substance and/or drug product. Any changes in the methods  
634 resulting from the review of the application may require resubmission of the  
635 methods validation package. The review chemist, in coordination with the  
636 appropriate FDA laboratories, will decide which analytical procedures are to be  
637 validated. Comments from the FDA laboratories, if any, will be forwarded by the  
638 review chemist to the applicant on completion of the studies by the laboratories.

### 639 3. *FDA Laboratory*

640 An FDA laboratory will contact applicants with instructions on the submission of  
641 samples and the addresses to which samples should be mailed. The laboratory  
642 will test the samples according to the submitted analytical procedures to determine  
643 whether the analytical procedures are acceptable for quality control and suitable  
644 for regulatory purposes. Results and comments will be forwarded to the review  
645 chemist on completion of the studies.

### 646 4. *Investigator*

647 The investigator inspects the analytical laboratory testing sites where the release  
648 and stability testing are performed to ensure that the analytical procedures are

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649 performed in compliance with CGMP/GLP.

650 **XI. METHODOLOGY**

651 Sections II through IX provide general information on the submission of analytical procedures  
652 and methods validation information, including validation characteristics. Additional information  
653 on certain methodologies is provided below.

654 **A. High-Pressure Liquid Chromatography (HPLC)**

655 The widespread use of HPLC analytical procedures and the multitude of commercial  
656 sources of columns and packings frequently have created problems in assessing  
657 comparability. Many of the following points may also apply to other chromatographic  
658 analytical procedures.

660 *1. Column*

661 The following characteristics are useful for defining a particular column and, if  
662 known, should be included in the analytical procedure description. If method  
663 development has indicated that columns from only one commercial source are  
664 suitable, this information should be included as part of the analytical procedure. If  
665 more than one column is suitable, a listing of columns found to be equivalent  
666 should be included.

667 *a. Column Parameters*

- 668 ● Material: glass, stainless steel, plastic
- 669 ● Dimensions: length, inner diameter
- 670 ● Frit size
- 671 ● Filter type
- 672 ● Precolumn and/or guard column type, if used

673 *b. Packing Material*

- 674 ● Particle type: size, shape, pore diameter
- 675 ● Surface modification (e.g., bonded surface type, surface coverage, percent  
676 carbon, additional silylation)
- 677 ● Recommended pH range for column use

678 *2. System Suitability Testing*

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679 Each analytical procedure submitted should include an appropriate number of  
680 system suitability tests defining the critical characteristics of that system. Criteria  
681 for all system suitability testing should be provided. The system suitability tests  
682 listed below are defined in CDER's reviewer guidance on *Validation of*  
683 *Chromatographic Methods* (November 1994).

- 684 ● Tailing factor
- 685 ● Relative retention
- 686 ● Resolution
- 687 ● Relative standard deviation (RSD)
- 688 ● Capacity factor
- 689 ● Number of theoretical plates

690 The RSD is normally performed at the beginning of the run. However, for assays  
691 with lengthy run times or as otherwise justified by the applicant, the reported  
692 average may be taken from injections at the beginning and end of the run, or at the  
693 beginning, middle, and end of the run.

694 If an internal standard is used, the minimum acceptable resolution between the  
695 internal standard and one or more active ingredients should be specified. If the  
696 analytical procedure is used to control the level of impurities, the minimum  
697 resolution between the active ingredient and the closest eluting impurity, or the  
698 two peaks eluting closest to each other, should be given.

### 700 3. *Operating Parameters*

701 The sequence of injection of blanks, system suitability standards, other  
702 standards, and samples should be defined. Flow rates, temperatures, and  
703 gradients should be described.

704 Complete details should be provided for the preparation of the mobile phase,  
705 including the order of addition of the reagents and the methods of degassing and  
706 filtration. The effect of adjustments in mobile phase composition on retention  
707 times should be included in the analytical procedure. The rationale for the use of  
708 precolumns and/or guard columns should be provided and justified. Any special  
709 requirements, such as the use of inert tubing or injection valves, should be  
710 specified.

### 711 **B. Gas Chromatography (GC)**

712 At a minimum, the following parameters should be included in the description of a GC

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713 procedure. Additional parameters should be specified if required by the analytical  
714 procedure. If method development has indicated that columns from only one  
715 commercial source are suitable, this information should be included as part of the  
716 analytical procedure. If more than one column is suitable, a listing of columns found to  
717 be equivalent should be included.

### 718 1. *Column*

- 719 ● Column dimensions: length, internal diameter, external diameter
- 720 ● Stationary phase
- 721 ● Column material (e.g., silica, glass, stainless steel)
- 722 ● Column conditioning procedure

### 723 2. *Operating Parameters*

- 724 ● Gases: purity, flow rate, pressure
- 725 ● Temperatures: column, injector, detector (including temperature  
726 program, if used)
- 727 ● Injection (e.g., split, splitless, on-column)
- 728 ● Detector
- 729 ● Typical retention time and total run time

### 730 3. *System Suitability Testing*

731 Appropriate system suitability criteria should be defined and included in all  
732 analytical procedures.

733 If an internal standard is used, the minimum acceptable resolution between the  
734 internal standard and one or more active ingredient should be specified. If the  
735 analytical procedure is used to control the level of impurities, the minimum  
736 resolution between the active ingredient and the closest eluting impurity, or the  
737 two peaks eluting closest to each other, should be given.

738 The RSD is normally performed at the beginning of the run. However, for  
739 assays with lengthy run times or as otherwise justified by the applicant, the  
740 reported average may be taken from injections at the beginning and end of the  
741 run, or beginning, middle, and end of the run.

## 742 **C. Spectrophotometry, Spectroscopy, Spectrometry and Related Physical** 743 **Methodologies** 744

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745 These analytical procedures include, but are not limited to, IR spectrophotometry, near  
746 IR spectrophotometry (NIR), UV/visible spectrophotometry (UV/Vis), atomic emission  
747 and atomic absorption, NMR, Raman spectroscopy, MS, and XRD.

748 Spectrometric analytical procedures may not be stability-indicating. The bias of the  
749 analytical procedure should be evaluated by comparing it with a chromatographic  
750 procedure, where appropriate. When manually operated equipment is used, the  
751 description of the analytical procedure should include an acceptance criterion for the  
752 amount of time that may elapse between sampling and reading. Appropriate system  
753 suitability and/or calibration testing is recommended. Validation criteria should include  
754 specificity (demonstrating no interference of placebo), linearity, repeatability,  
755 intermediate precision, and robustness.

### 756 **D. Capillary Electrophoresis (CE)**

757 At a minimum, the parameters listed below should be specified for a capillary  
758 electrophoretic analytical procedure. Additional parameters may be included as  
759 required by the procedure. If method development has indicated that capillaries from  
760 only one commercial source are suitable, this information should be included as part of  
761 the analytical procedure. If more than one capillary is suitable, a listing of capillaries  
762 found to be equivalent should be included.

#### 763 *1. Capillary*

- 764 ● Capillary dimensions: length, length to detector, internal diameter,  
765 external diameter
- 766 ● Capillary material
- 767 ● Capillary internal coating (if any)

#### 768 *2. Operating Parameters*

- 770 ● Capillary preparation procedure: procedure to be followed before the  
771 first use, before the first run of the day, before each run (e.g., flush with  
772 100 millimolar sodium hydroxide, flush with running buffer)
- 773 ● Running buffer: composition, including a detailed preparation procedure  
774 with the order of addition of the components
- 775 ● Injection: mode (e.g., electrokinetic, hydrodynamic), parameters (e.g.,  
776 voltage, pressure, time)
- 777 ● Detector
- 778 ● Typical migration time and total run time
- 779 ● Model of CE equipment used



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811 Appropriate system suitability and/or calibration testing is recommended. Validation  
812 criteria should include specificity, and intermediate precision.

813 **F. Methodologies Relating to Particle Size Analysis**

814 Particle size analysis is an important element for quality control and regulatory  
815 evaluation of certain drug substances and drug products. The normal concepts of  
816 validation may differ for particle size methodologies as compared to other analytical  
817 methodologies such as HPLC. However, a standard mixture may be used for  
818 calibration.

819 Particle size evaluation can include characteristics of size, morphology, surface, and  
820 population of particles. The following parameters are useful for describing particle size  
821 analysis for characterization of drug substances and drug products.

822 *1. Particle Size Methods*

823 Types of particle size methods include, but are not limited to:

824 a. Nonfractionation methods that evaluate an entire population of particles

- 825 ● Microscopy (optical, electron)  
826 ● Light scattering (dynamic, photon correlation, laser diffraction)  
827 ● Electrozone sensing  
828 ● Photozone sensing  
829

830 b. Fractionation methods that use physical techniques to separate particles  
831 on the basis of size

- 832 ● Sieving  
833 ● Cascade impactor  
834 ● Sedimentation  
835 ● Size exclusion chromatography

836 *2. Calibration and Validation Characteristics*

837 To ensure proper instrument operation, the system should be calibrated  
838 according to the manufacturer's and/or the laboratory's specification, as  
839 appropriate.

840 The methods validation usually involves evaluation of intermediate precision

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841 and robustness. Assurance should be provided that the data generated are  
842 reproducible and control the product's quality. See additional information in  
843 sections V and VII.

### 844 **G. Dissolution**

845 The equipment used for dissolution is covered by USP <711> or USP <724>. The  
846 dissolution procedure description and validation should include the following.

#### 847 *1. Dissolution Medium*

848 A brief discussion of the reasons for selecting the medium.

#### 849 *2. Procedure*

850 A dissolution test consists of a dissolution procedure and method of analysis  
851 (automated on-line analysis or manual sampling followed by HPLC analysis).  
852 The written procedure should cover the following items:

- 853 ● Apparatus
- 854 ● Preparation of standard
- 855 ● Preparation of sample
- 856 ● Method of analysis (e.g., UV, HPLC)
- 857 ● Sampling procedure (e.g., intervals, filtration, handling of samples,  
858 dilutions)
- 859 ● Calculations
- 860 ● Acceptance criteria

861 Regardless of the method of analysis, system suitability criteria should be  
862 described. Blank and standard solution spectra or chromatograms should be  
863 included.

#### 864 *3. Validation Characteristics*

865 Both the dissolution procedure and the method of analysis should be validated.

866 The time needed for the completion of the sample analysis should be stated in  
867 the procedure. Data should be submitted to support the stability of the  
868 dissolution sample during the procedure. If filters are used on-line or during  
869 sample preparation, appropriate recovery studies should be performed and  
870 documented and any bias should be addressed.

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871 **H. Other Instrumentation**

872 *1. Noncommercial Instrumentation*

873 FDA encourages the development and use of the most appropriate  
874 instrumentation. However, the use of rare or exotic systems not only places an  
875 undue burden on the regulatory laboratory, but also may delay the validation  
876 process.

877 When noncommercial instrumentation is used, the instrumentation should be  
878 capable of being constructed from commercially available components at a  
879 reasonable cost, if possible. For unique methodologies or instrumentation  
880 requiring contract fabrication, the applicant's cooperation with the FDA  
881 laboratories in helping facilitate duplication of the analytical procedure is  
882 important. In addition to design and equipment specifications, complete  
883 performance assessment procedures should be provided. Such systems may be  
884 found suitable for regulatory use.

885 *2. Automated Analytical Procedures*

886 The use of automated analytical procedures, although desirable for control  
887 testing, may lead to delay in regulatory methods validation because FDA  
888 laboratories have to assemble and validate the system before running samples.  
889 To avoid this delay, applicants should demonstrate the equivalence of a manual  
890 procedure to the automated procedure based on the same principle whenever  
891 possible.

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**ATTACHMENT A**

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893

**NDA, ANDA, BLA, AND PLA SUBMISSION CONTENTS**

894 The information relating to analytical procedures and methods validation that should be  
895 submitted in NDAs, ANDAs, BLAs, and PLAs is identified below with a cross-reference to the  
896 section of this guidance that provides recommendations and/or discussion on the topics.

897 Information that should be included in the analytical procedures and controls sections

- 898 ● Reference standard information Section IV
- 899 ● Analytical procedures Section III, VI
- 900 ● Validation data Section VII
- 901 ● Stress studies Section VII.A.2.c
- 902 ● Instrument output/raw data for impurities Section VII.A.2.b
- 903 ● Statistical analysis Section VIII
- 904 ● Revalidation, as needed Section IX

905

906 Information that should be included in the methods validation package<sup>5</sup>

- 907 ● Contents of the MV Package Section XI
- 908 ● Representative instrument output/data for stress studies Section VII.A.2.c
- 909 ● Representative instrument output and raw data for initial  
910 and oldest sample of a batch Section VII.A.2.b

911 Information that should be included in the stability section

- 912 ● Stress study designs and results Section VII.A.2.b
- 913 ● Reference (volume and page number of submission)  
914 to instrument output and raw data submitted to the section  
915 dedicated to analytical procedures and controls Section VII.A.2.c

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<sup>5</sup> For BLAs and PLAs, a separate methods validation package need not be submitted. Information similar to what is listed here should be included in the BLA or PLA submission.

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**ATTACHMENT B**

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**METHODS VALIDATION PROBLEMS AND DELAY**

918

Listed below are examples of common problems that can delay successful validation.

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- Failure to provide a sample of a critical impurity, degradation product, internal standard, or novel reagent

921

- Failure to submit well-characterized reference standards for noncompendial drugs

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- Failure to provide sufficient detail or use of unacceptable analytical procedures. For example:

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- Use of arbitrary arithmetic corrections
- Failure to provide system suitability tests
- Differing content uniformity and assay analytical procedures without showing equivalence factors for defining corrections as required by the current USP chapter <905> - Uniformity of Dosage Units

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- Failure to submit complete or legible data. For example:
  - Failure to label instrument output to indicate sample identity
  - Failure to label the axes

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- Inappropriate shipping procedures. For example:
  - Failure to properly label samples
  - Failure to package samples in accordance with product storage conditions
  - Inadequate shipping forms (e.g., missing customs form for samples from outside the United States)

939

- Failure to describe proper storage conditions on shipping containers

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### **REFERENCES**

940

#### **FDA Documents<sup>6</sup>**

942 Guidance for Industry: *ANDAs: Impurities in Drug Products* (Draft, December 1998).

943 Guidance for Industry: *ANDAs: Impurities in Drug Substances* (February 2000).

944 Guidance for Industry: *CMC Content and Format of INDs for Phase 2 and 3 Studies of Drugs, Including Specified Therapeutic Biotechnology-Derived Products* (Draft, December 1997).

946 Guidance for Industry: *Content and Format of Investigational New Drug Applications (INDs) for Phase 1 Studies of Drugs, Including Well-Characterized, Therapeutic, Biotechnology-derived Products* (February 1995).

949 Guidance for Industry: *Investigating Out of Specification (OOS) Test Results for Pharmaceutical Production* (Draft, September 1998).

951 Guidance for Industry: *Stability Testing of Drug Substances and Drug Products* (Draft, June 1998).

953 Guidance for Industry: *Submission of Chemistry, Manufacturing, and Controls Information for Synthetic Peptide Substances* (November 1994).

955 Guidance for Industry: *Submitting Documentation for the Stability of Human Drugs and Biologics* (February 1987).

957 Reviewer Guidance: *Validation of Chromatographic Methods* (November 1994).

958 FDA CDER MAPP 5221.1 *Requesting Methods Validation for ANDAs* (November 1998).

#### **International Conference on Harmonization Guidances**

960 ICH *Q1A: Stability Testing of New Drug Substances and Products* (November 1994)

961 ICH *Q1B: Photostability Testing of New Drug Substances and Products* (November 1996)

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<sup>6</sup> Draft guidances have been included for completeness only. As draft documents, they are not intended to be implemented until published in final form.

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- 962 ICH *Q1C: Stability Testing for New Dosage Forms* (May 1997)
- 963 ICH *Q2A: Text on Validation of Analytical Procedures* (March 1995)
- 964 ICH *Q2B: Validation of Analytical Procedures: Methodology* (May 1997)
- 965 ICH *Q3A: Impurities in New Drug Substances* (January 1996)
- 966 ICH *Q3B: Impurities in New Drug Products* (May 1997)
- 967 ICH *Q3C: Impurities: Residual Solvents* (December 1997)
- 968 ICH *Q5C: Quality of Biotechnological Products: Stability Testing of*  
969 *Biotechnological/Biological Products* (July 1996)
- 970 ICH *Q6A: Specifications: Test Procedures and Acceptance Criteria for New Drug Substances*  
971 *and New Drug Products: Chemical Substances* (Draft (Step 2) November 1997)
- 972 ICH *Q6B: Specifications: Test Procedures and Acceptance Criteria for*  
973 *Biotechnological/Biological Products* (March 1999)
- 974 **U.S. Pharmacopeia/National Formulary**
- 975 Chapter <621> Chromatography; US Pharmacopeia 23, United States Pharmacopeial  
976 Convention, Inc., Rockville MD: 1994
- 977 Chapter <781> Optical Rotation, US Pharmacopeia 23, United States Pharmacopeial  
978 Convention, Inc., Rockville, MD: 1994
- 979 Chapter <1225> Validation of Compendial Methods; US Pharmacopeia 23, United States  
980 Pharmacopeial Convention, Inc., Rockville MD: 1994
- 981 Interpretation and Treatment of Analytical Data; USP Pharmacopeial Forum, United States  
982 Pharmacopeial Convention, Inc., Rockville MD: 1994, Volume 24, Number 5, pp. 7051 - 7056

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983     **Other**

984     Miller, J.C., J.N. Miller, and E. Horwood, *Statistics for Analytical Chemistry*, 3rd edition,  
985     Prentice Hall, 1993.

986     Saunders, B.D., and R.G. Trapp, *Basic and Clinical Biostatistics*, 2nd edition, Appleton and  
987     Lange, 1994.

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**GLOSSARY**

989 **Acceptance Criteria:** Numerical limits, ranges, or other suitable measures for acceptance of  
990 the results of analytical procedures.

991 **Active moiety:** The molecule or ion, excluding those appended portions of the molecule that  
992 cause the drug to be an ester, salt (including a salt with hydrogen or coordination bonds), or  
993 other noncovalent derivative (such as a complex, chelate, or clathrate) of the molecule,  
994 responsible for the physiological or pharmacological action of the drug substance (21 CFR  
995 314.108(a)). The active moiety is the entire molecule or ion, not the *active site*.

996 **Detection Limit:** The detection limit of an individual analytical procedure is the lowest  
997 amount of analyte in a sample that can be detected, but not necessarily quantitated as an exact  
998 value.

999 **Drug Product:** A finished dosage form, for example, a tablet, capsule, or solution that  
1000 contains a drug substance, generally, but not necessarily, in association with one or more other  
1001 ingredients (21 CFR 314.3(b)).

1002 **Drug Substance/Active Ingredient:** An active ingredient that is intended to furnish  
1003 pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or  
1004 prevention of disease or to affect the structure or any function of the human body. The active  
1005 ingredient does not include intermediates used in the synthesis of such ingredient. The term  
1006 includes those components that may undergo chemical change in the manufacture of the drug  
1007 product and be present in the drug product in a modified form intended to furnish the specified  
1008 activity or effect (21 CFR 210.3(b)(7) and 314.3(b)).

1009 **Placebo (or Blank):** A dosage form that is identical to the drug product except that the drug  
1010 substance is absent or replaced by an inert ingredient or a mixture of the drug product  
1011 excipients quantitatively equivalent to those found in the drug product dosage form.

1012 **Quantitation Limit:** The quantitation limit of an individual analytical procedure is the lowest  
1013 amount of analyte in a sample that can be quantitatively determined with suitable precision and  
1014 accuracy. The quantitation limit is a parameter of quantitative assays for low levels of  
1015 compounds in sample matrices, and is used particularly for the determination of impurities  
1016 and/or degradation products.

1017 **Reagent:** For analytical procedures, any substance used in a reaction for the purpose of  
1018 detecting, measuring, examining, or analyzing other substances.  
1019

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1020 **Specification:** The quality standards (i.e., tests, analytical procedures, and acceptance criteria)  
1021 provided in an approved application to confirm the quality of the drug substances, drug  
1022 products, intermediates, raw materials, reagents, and other components including container  
1023 closure systems, and in-process materials.

1024 **Spiking:** The addition of a small known amount of a known compound to a standard, sample,  
1025 or placebo, typically for the purpose of confirming the performance of an analytical procedure  
1026 or the calibration of an instrument.

1027 **Stability-Indicating Assay:** A validated quantitative analytical procedure that can detect the  
1028 changes with time in the pertinent properties (e.g., active ingredient, preservative level) of the  
1029 drug substance and drug product. A stability-indicating assay accurately measures the active  
1030 ingredients without interference from degradation products, process impurities, excipients, or  
1031 other potential impurities.

1032 **Working Standard:** A standard that is qualified against and used instead of the reference  
1033 standard (also known as *in-house* or *secondary standard*).