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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### *DRAFT GUIDANCE*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)**

**August 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)**

**August 2000  
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# Guidance for Industry<sup>1</sup>

## Analytical Procedures and Methods Validation

This draft guidance, when finalized, will represent the Food and Drug Administration's current thinking on this topic. 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.

If you plan to submit comments on this draft guidance, to expedite FDA review of your comments, please: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

### I. INTRODUCTION

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

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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).

<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).

32 chosen, the applicant is encouraged to discuss the matter in advance with the center with product  
33 jurisdiction to prevent the expenditure of resources on preparing a submission that may later be  
34 determined to be unacceptable.

35  
36 The principles of methods validation described in this guidance apply to all types of analytical  
37 procedures. However, the specific recommendations in this guidance may not be applicable to certain  
38 unique analytical procedures for products such as biological, biotechnological, botanical, or  
39 radiopharmaceutical drugs. For example, many bioassays are based on animal challenge models,  
40 immunogenicity assessments, or other immunoassays that have unique features that should be  
41 considered when submitting analytical procedure and methods validation information. Furthermore,  
42 specific recommendations for biological and immunochemical tests that may be necessary for  
43 characterization and quality control of many drug substances and drug products are beyond the scope  
44 of this guidance document. Although this guidance does not specifically address the submission of  
45 analytical procedures and validation data for raw materials, intermediates, excipients, container closure  
46 components, and other materials used in the production of drug substances and drug products,  
47 validated analytical procedures should be used to analyze these materials. For questions on  
48 appropriate validation approaches for analytical procedures or submission of information not  
49 addressed in this guidance, applicants should consult with the appropriate chemistry review staff at  
50 FDA.

51  
52 This guidance, when finalized, will replace the FDA guidance for industry on *Submitting Samples and*  
53 *Analytical Data for Methods Validation* (February 1987).

54  
55

## 56 **II. BACKGROUND**

57

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

63

64 *Methods validation* is the process of demonstrating that analytical procedures are suitable for their  
65 intended use. The methods validation process for analytical procedures begins with the planned and  
66 systematic collection by the applicant of the validation data to support the analytical procedures. The  
67 review chemist evaluates the analytical procedures and validation data submitted in the NDA or  
68 ANDA. On request from FDA, an NDA or ANDA applicant must submit samples of drug product,  
69 drug substance, noncompendial reference standards, and blanks so that the applicant's drug substance  
70 and drug product analytical procedures can be evaluated by FDA laboratories (21 CFR 314.50(e)  
71 and 314.94(a)(10)). The FDA laboratory analysis demonstrates that the analytical procedures are  
72 reproducible by laboratory testing. The review chemists and laboratory analysts determine the  
73 suitability of the analytical procedures for regulatory purposes. FDA investigators inspect the  
74 analytical laboratory testing sites to ensure that the analytical procedures used for release and stability

75 testing comply with current good manufacturing practices (CGMPs) (21 CFR part 211) or good  
76 laboratory practices (GLPs) (21 CFR part 58), as appropriate.

77  
78 Each BLA and PLA must include a full description of the manufacturing methods, including analytical  
79 procedures, that demonstrate that the manufactured product meets prescribed standards of safety,  
80 purity, and potency (21 CFR 601.2(a) and 601.2(c)(1)(iv)). Data must be available to establish that  
81 the analytical procedures used in testing meet proper standards of accuracy and reliability (21 CFR  
82 211.194(a)(2)). For BLAs, PLAs, and their supplements, the analytical procedures and their  
83 validation are submitted as part of the license application or supplement and are evaluated by the  
84 review committee. Representative samples of the product must be submitted and summaries of results  
85 of tests performed on the lots represented by the submitted sample must be provided (21 CFR  
86 601.2(a) and 601.2(c)(1)(vi)). The review committee chair may request analytical testing by CBER  
87 laboratory analysts to evaluate the applicant's analytical procedures and verify the test results.

88  
89 All analytical procedures are of equal importance from a validation perspective. In general, validated  
90 analytical procedures should be used, irrespective of whether they are for in-process, release,  
91 acceptance, or stability testing. Each quantitative analytical procedure should be designed to minimize  
92 assay variation.

93  
94 Analytical procedures and validation data are submitted in the sections of the application on analytical  
95 procedures and controls. Recommendations on information to be submitted are included in sections  
96 III through IX and XI of this guidance. Information on submission of the *methods validation*  
97 *package* to the NDA or ANDA and samples to the FDA laboratories is provided in section X.

98  
99

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

101

#### 102 **A. Regulatory Analytical Procedure**

103

104 *A regulatory analytical procedure* is the analytical procedure used to evaluate a defined  
105 characteristic of the drug substance or drug product. The analytical procedures in the *U.S.*  
106 *Pharmacopeia/National Formulary* (USP/NF) are those legally recognized under section  
107 501(b) of the Food, Drug, and Cosmetic Act (the Act) as the regulatory analytical procedures  
108 for compendial items. For purposes of determining compliance with the Act, the regulatory  
109 analytical procedure is used.

110

#### 111 **B. Alternative Analytical Procedure**

112

113 *An alternative analytical procedure* is an analytical procedure proposed by the applicant for  
114 use instead of the regulatory analytical procedure. A validated alternative analytical procedure  
115 should be submitted only if it is shown to perform equal to or better than the regulatory  
116 analytical procedure. If an alternative analytical procedure is submitted, the applicant should  
117 provide a rationale for its inclusion and identify its use (e.g., release, stability testing), validation

118 data, and comparative data to the regulatory analytical procedure.

119  
120 **C. Stability-Indicating Assay**

121  
122 *A stability-indicating assay* is a validated quantitative analytical procedure that can detect  
123 the changes with time in the pertinent properties of the drug substance and drug product. A  
124 stability-indicating assay accurately measures the active ingredients, without interference from  
125 degradation products, process impurities, excipients, or other potential impurities. If an  
126 applicant submits a non-stability-indicating analytical procedure for release testing, then an  
127 analytical procedure capable of qualitatively and quantitatively monitoring the impurities,  
128 including degradation products, should complement it. Assay analytical procedures for  
129 stability studies should be stability-indicating, unless scientifically justified.

130  
131  
132 **IV. REFERENCE STANDARDS**

133  
134 **A. Types of Standards**

135  
136 *A reference standard* (i.e., primary standard) may be obtained from the USP/NF or other  
137 official sources (e.g., CBER, 21 CFR 610.20). If there are questions on whether a source of  
138 a standard would be considered by FDA to be an official source, applicants should contact  
139 the appropriate chemistry review staff. When there is no official source, a reference standard  
140 should be of the highest possible purity and be fully characterized.

141  
142 *A working standard* (i.e., in-house or secondary standard) is a standard that is qualified  
143 against and used instead of the reference standard.

144  
145 **B. Certificate of Analysis**

146  
147 A certificate of analysis (COA) for reference standards from non-official sources should be  
148 submitted in the section of the application on analytical procedures and controls. For  
149 standards from official sources, the user should ensure the suitability of the reference standard.  
150 The standard should be stored correctly and used within the established use interval.

151  
152 **C. Characterization of a Reference Standard**

153  
154 Reference standards from USP/NF and other official sources do not require further  
155 characterization. A reference standard that is not obtained from an official source should be of  
156 the highest purity that can be obtained by reasonable effort, and it should be thoroughly  
157 characterized to ensure its identity, strength, quality, purity, and potency. The qualitative and  
158 quantitative analytical procedures used to characterize a reference standard are expected to  
159 be different from, and more extensive than, those used to control the identity, strength, quality,  
160 purity, and potency of the drug substance or the drug product. Analytical procedures used to

161 characterize a reference standard should not rely solely on comparison testing to a previously  
162 designated reference standard.

163

164 Generally, this characterization information should include:

165

166 ! A brief description of the manufacture of the reference standard, if the manufacturing  
167 process differs from that of the drug substance. Any additional purification  
168 procedures used in the preparation of the reference standard should be described.

169

170 ! Legible reproductions of the relevant spectra, chromatograms, thin-layer  
171 chromatogram (TLC) photographs or reproductions, and other appropriate  
172 instrumental recordings.

173

174 ! Data establishing purity. The data should be obtained by using appropriate tests, such  
175 as TLC, gas chromatography (GC), high-pressure liquid chromatography (HPLC),  
176 phase solubility analysis, appropriate thermometric analytical procedures, and others  
177 as necessary.

178

179 ! Appropriate chemical attribute information, such as structural formula, empirical  
180 formula, and molecular weight. Information to substantiate the proof of structure  
181 should include appropriate analytical tests, such as elemental analysis, infrared  
182 spectrophotometry (IR), ultraviolet spectrophotometry (UV), nuclear magnetic  
183 resonance spectroscopy (NMR), and mass spectrometry (MS), as well as applicable  
184 functional group analysis. Detailed interpretation of the test data in support of the  
185 claimed structure should be provided.

186

187 ! A physical description of the material, including its color and physical form.

188

189 ! Appropriate physical constants such as melting range, boiling range, refractive index,  
190 dissociation constants (pK values), and optical rotation.

191

192 ! A detailed description of the analytical procedures used to characterize the reference  
193 standard.

194

195 For biotechnological/biological product reference standards, the recommendations on  
196 characterization information above may apply and should be considered. However, additional  
197 and/or different tests would be important to assess physicochemical characteristics, structural  
198 characteristics, biological activity, and/or immunochemical activity. Physicochemical  
199 determinations may include isoform, electrophoretic, and liquid chromatographic patterns, as  
200 well as spectroscopic profiles. Structural characterization may include a determination of  
201 amino acid sequence, amino acid composition, peptide map, and carbohydrate structure.  
202 Biological and/or immunochemical activity should be assessed using the same analytical  
203 procedures used to determine product potency. These can include animal-based, cell culture-

204 based, biochemical, or ligand/receptor-binding assays. While these tests may be needed for  
205 complete characterization of certain reference standards, specific recommendations for  
206 validation of biological and immunochemical tests are not contained in this guidance document.  
207

## 208

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

210  
211 For an investigational new drug, sufficient information is required in each phase of an investigation to  
212 ensure proper identification, quality, purity, strength, and/or potency. The amount of information on  
213 analytical procedures and methods validation necessary will vary with the phase of the investigation  
214 (21 CFR 312.23(a)(7)).  
215

216 For general guidance on analytical procedures and methods validation information to be submitted for  
217 phase 1 studies, sponsors should refer to the FDA guidance for industry on *Content and Format of*  
218 *Investigational New Drug Applications (INDs) for Phase 1 Studies of Drugs, Including Well-*  
219 *Characterized, Therapeutic, Biotechnology-Derived Products* (November 1995). General  
220 guidance regarding analytical procedures and methods validation information to be submitted for phase  
221 2 or phase 3 studies will be provided in the FDA guidance for industry *INDs for Phase 2 and 3*  
222 *Studies of Drugs, Including Specified Therapeutic Biotechnology-Derived Products, Chemistry,*  
223 *Manufacturing, and Controls Content and Format*, when finalized (draft guidance published April  
224 1999).  
225

226 All analytical procedures should be fully developed and validation completed when the NDA, ANDA,  
227 BLA, or PLA is submitted.  
228

### 229

### 230 **VI. CONTENT AND FORMAT OF ANALYTICAL PROCEDURES FOR NDAs,**

### 231 **ANDAs, BLAs, AND PLAs**

232  
233 Any analytical procedure submitted in an NDA, ANDA, BLA, or PLA should be described in  
234 sufficient detail to allow a competent analyst to reproduce the necessary conditions and obtain results  
235 comparable to the applicant's. Aspects of the analytical procedure that require special attention  
236 should be described. If the analytical procedure used is in the current revision of the USP/NF or other  
237 FDA recognized standard references (e.g., AOAC International *Book Of Methods*) and the  
238 referenced analytical procedure is not modified, a statement indicating the analytical procedure and  
239 reference may be provided rather than a description of the method (21 CFR 211.194). A description  
240 of analytical procedures from any other published sources should be provided, because the referenced  
241 sources may not be readily accessible to the reviewer.  
242

243 The following is a list of information that should typically be included in a description of an analytical  
244 procedure.  
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#### 246 **A. Principle**

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

**B. Sampling**

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

**C. Equipment and Equipment Parameters**

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

**D. Reagents**

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

**E. System Suitability Testing**

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

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

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

**F. Preparation of Standards**

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

## **G. Preparation of Samples**

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

## **H. Procedure**

A step-by-step description of the procedure should be provided. The description should include, where appropriate, equilibration times, injection sampling sequence, and system suitability or start-up parameters. Unusual hazards should be identified.

## **I. Calculations**

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

## **J. Reporting of Results**

### *1. General*

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

### *2. Impurities Analytical Procedures*

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

Reporting of organic impurities should cover (1) specified identified impurities by name, (2) specified unidentified impurities by location/identifier, (3) any unspecified impurities, and (4) total impurities. The total organic impurities for the drug product or

333 drug substance is the sum of all impurities equal to or greater than their individual QL.  
334 See recommendations regarding appropriate QLs in FDA impurities guidances (see  
335 references). Inorganic impurities and residual solvents should also be addressed.

336  
337 For the drug product, drug substance process impurities may be excluded from  
338 reporting if an acceptable rationale is provided in the sections on analytical procedures  
339 and controls. Drug product impurities from the drug product manufacturing process,  
340 packaging, and labeling should be addressed.

341  
342 The above reporting information may not be strictly applicable to all products (e.g.,  
343 biological, biotechnological, botanical, radiopharmaceutical drugs), but any significant  
344 process and product-related impurities should be determined and reported.

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

### 348 **A. Noncompendial Analytical Procedures**

350  
351 In an NDA, ANDA, BLA, or PLA, data must be submitted to establish that the analytical  
352 procedures used in testing meet proper standards of accuracy and reliability (21 CFR  
353 211.194(a)(2)). *Methods validation* is the process of demonstrating that analytical  
354 procedures are suitable for their intended use. At the time of submission, the NDA, ANDA,  
355 BLA, or PLA should contain methods validation information to support the adequacy of the  
356 analytical procedures.

357  
358 The International Conference on Harmonisation (ICH) guidance *Q2A Text on Validation of*  
359 *Analytical Procedures* (March 1995) and *Q2B Validation of Analytical Procedures:*  
360 *Methodology* (November 1996) provide recommendations on validation of analytical  
361 procedures. Analytical procedures outside the scope of the ICH guidances should still be  
362 validated.

#### 363 *1. Validation Characteristics*

364  
365 Applicants should submit information on the validation characteristics of their  
366 proposed analytical procedures (see ICH *Q2A* and ICH *Q2B*). Although not all of  
367 the validation characteristics are needed for all types of tests (see section VII.A.3),  
368 typical validation characteristics are:  
369

- 370 ! Accuracy
- 371 ! Precision (repeatability and intermediate precision)
- 372 ! Specificity
- 373 ! Detection limit
- 374 ! Quantitation limit
- 375

- 376 ! Linearity
- 377 ! Range
- 378 ! Robustness

379

380 2. *Other Methods Validation Information*

381

382 Methods validation information should also include:

383

- 384 ! Data to demonstrate the stability of all analytical sample preparations through
- 385 the time required to complete the analysis.

386

- 387 ! Legible reproductions of representative instrument output or recordings (e.g.,
- 388 chromatograms) and raw data output (e.g., integrated areas), as appropriate.
- 389 Instrument output for placebo, standard, and sample should also be provided
- 390 (see section VII.A.2.c).

391

- 392 ! Representative calculations using submitted raw data, to show how the
- 393 impurities in drug substance are calculated.

394

- 395 ! Information from stress studies (see section VII.A.2.b).

396

- 397 ! Impurities labeled with their names and location identifiers (e.g., RRT for
- 398 chromatographic data) for the impurity analytical procedure.

399

- 400 ! For drug substances:

401

- 402 C A discussion of the possible formation and control of polymorphic and
- 403 enantiomeric substances.

404

- 405 C Identification and characterization of each organic impurity, as
- 406 appropriate. This information may not be needed for all products
- 407 (e.g., botanicals). Other impurities (e.g., inorganics, residual solvents)
- 408 should be addressed and quantitated.

409

410 Recommendations on submitting information on impurities is provided

411 in various FDA guidances such as the ICH guidance *Q3A Impurities*

412 *in New Drug Substances* (January 1996).

413

- 414 C A list of known impurities, with structure if available, including process
- 415 impurities, degradants, and possible isomers.

416

- 417 ! For drug products:

418

- 419 C A degradation pathway for the drug substance in the dosage form,  
420 where possible.  
421  
422 C Data demonstrating recovery from the sample matrix as illustrated by  
423 the accuracy studies.  
424  
425 C Data demonstrating that neither the freshly prepared nor the degraded  
426 placebo interferes with the quantitation of the active ingredient.  
427

428 ICH *Q2A* and *Q2B* address almost all of the validation parameters. Areas that should  
429 be provided in more detail are described below.  
430

431 a. Robustness  
432

433 Robustness, a measure of the analytical procedure's capability to remain unaffected by  
434 small but deliberate variations, is described in ICH *Q2A* and *Q2B*. Such testing  
435 should be performed during development of the analytical procedure and the data  
436 discussed and/or submitted. In cases where an effect is observed, representative  
437 instrument output (e.g., chromatograms) should be submitted.  
438

439 b. Stress Studies  
440

441 Degradation information obtained from *stress studies* (e.g., products of acid and base  
442 hydrolysis, thermal degradation, photolysis, oxidation) for the drug substance and for  
443 the active ingredient in the drug product should be provided to demonstrate the  
444 specificity of the assay and analytical procedures for impurities. The stress studies  
445 should demonstrate that impurities and degradants from the active ingredient and drug  
446 product excipients do not interfere with the quantitation of the active ingredient. Stress  
447 studies are described in various FDA guidances relating to the stability of drug  
448 products (see references).  
449

450 The design of the stress studies and the results should be submitted to the stability  
451 section of the application. Representative instrument output (e.g., chromatograms)  
452 and/or other appropriate data (e.g., degradation information obtained from stress  
453 studies) should be submitted in the sections on analytical procedures and controls.  
454

455 c. Instrument Output/Raw Data  
456

457 i. Organic Impurities  
458

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

462 with appropriate identification and labeling (e.g., RT for chromatographic  
463 peaks, chemical shift ( $\delta$ ) and coupling constant (J) for NMR) should be  
464 provided. The impurity profile should be assessed at the quantitation limit and  
465 the instrument output provided. Additional information should be provided to  
466 confirm that the impurity profile is adequately characterized. For example, a  
467 representative chromatogram using detection at a low wavelength, such as  
468 205 nm, and double the proposed total run time could be submitted to  
469 support the specificity of the analytical procedure.

470  
471 For quantitation purposes, the response factor of the drug substance may be  
472 used for impurities without a reference standard. In cases where the response  
473 factors are not close, this practice may still be acceptable, provided a  
474 correction factor is applied or the impurities are, in fact, being overestimated.  
475 Acceptance criteria and analytical procedures used to estimate identified or  
476 unidentified impurities often are based on analytical assumptions (e.g.,  
477 equivalent detector response). Assumptions should be discussed and justified.

478  
479 ii. Drug Substance

480  
481 Data should be submitted showing the separation and detection of impurities  
482 using spiked or stress samples. Complete impurity profiles as graphic output  
483 (e.g., chromatograms) and raw data (e.g, integrated peak areas) of  
484 representative batches should be submitted in the sections on analytical  
485 procedures and controls for the drug substance. For ANDAs and related  
486 submissions, appropriate information for the batches used in the biobatch or  
487 submission batch should be provided. All responses (e.g., peaks) should be  
488 labeled.

489  
490 The analytical procedure used should be capable of differentiating changes, if  
491 any, between past and present batches. The quantitation limit and the type of  
492 organic impurity (e.g., degradant, process impurity) should be stated. The  
493 analytical procedure number, batch number, manufacturing date and site, and  
494 date of analysis should be provided.

495  
496 iii. Drug Product

497  
498 Information such as instrument output (e.g., chromatograms) and raw data  
499 (e.g., integrated peak areas) from representative batches under long-term and  
500 accelerated stability conditions, and stressed samples should be submitted in  
501 the sections on analytical procedures and controls of the drug product. For  
502 ANDAs and related submissions, appropriate information for the biobatch or  
503 submission batch should be provided. References to the raw data (e.g.,  
504 chromatograms) should be included in the stability section of the application.

505  
506 At a minimum, the submission should include instrument output and raw data  
507 for release testing and at the latest available time point for the same batch. All  
508 responses (e.g., peaks) should be labeled and identified. In addition, the  
509 analytical procedure number, batch number of the drug product,  
510 manufacturing date, date of analysis, source and batch number of drug  
511 substance, manufacturing site, and container/closure information should be  
512 provided. The analytical procedures used should be capable of differentiating  
513 changes, if any, between past and present batches. The quantitation limit and  
514 the type (e.g., degradant, leachables from packaging) should be reported.  
515 Multiple methodologies can be used.

516  
517 If process impurities from the drug substance and excipients with their related  
518 impurities are not reported in the impurities analytical procedure, the potential  
519 locations/identifier (e.g., RT, RRT) of these compounds should be described  
520 and listed in the analytical procedure.

521  
522 3. *Recommended Validation Characteristics for Types of Tests*

523  
524 Table 1 is a summary of the validation characteristics that should be addressed during  
525 validation of different types of analytical procedures. The same methodology can be  
526 used for several purposes. The validation information should support the intended  
527 purpose of the test. For example, if Raman spectroscopy is the methodology selected  
528 to quantitate polymorphic forms as impurities, or chiral HPLC for enantiomeric  
529 impurities, the recommended validation characteristics in Table 1 under *quantitative*  
530 *testing for impurities* would apply. However, if Raman spectroscopy or chiral  
531 HPLC are used for the purpose of identification or as specific tests, the recommended  
532 validation characteristics listed for those types of tests would apply.

533  
534  
535**Table 1. Recommended Validation Characteristics of the Various Types of Tests.**

Type of Tests / Characteristics	Identification	Testing for Impurities		Assay Dissolution (Measurement Only), Content/Potency	Specific Tests
		Quantitative	Limit		
Accuracy	-	+	-	+	+ <sup>4</sup>
Precision-Repeatability	-	+	-	+	+ <sup>4</sup>
Precision-Intermediate Precision	-	+ <sup>1</sup>	-	+ <sup>1</sup>	+ <sup>4</sup>
Specificity	+ <sup>2</sup>	+	+	+ <sup>5</sup>	+ <sup>4</sup>
Detection Limit	-	- <sup>3</sup>	+	-	-
Quantitation Limit	-	+	-	-	-
Linearity	-	+	-	+	-
Range	-	+	-	+	-
Robustness	-	+	- <sup>3</sup>	+	+ <sup>4</sup>

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## NOTE:

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

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## a. Identification

Identification analytical procedures may include tests such as IR, differential scanning calorimetry (DSC), X-ray diffraction (XRD), UV, and HPLC retention time. A specific identification test should be included for the active ingredient whenever possible. In cases where a nonspecific identification analytical procedure is proposed for the active ingredient, two independent analytical procedures are generally sufficient, if justified. For other identification tests (e.g., a chiral HPLC retention time as confirmation for the presence of an enantiomer, chloride test for a counterion) a single test is acceptable. This concept of the number of identification tests is

557 applicable to both the drug substance and drug product.

558  
559 b. Impurities

560  
561 The validation characteristics under *quantitative testing for impurities*, as described  
562 in Table 1, apply, regardless of which methodology is used to quantitate impurities. If  
563 the same analytical procedure is proposed as a limit test, validation characteristics  
564 under *limit testing for impurities* will apply.

565  
566 c. Assay

567  
568 Assay includes the content of the active ingredient, preservative (if used), and  
569 measurement of content in dissolution and content uniformity samples.

570  
571 d. Specific Tests

572  
573 Specific tests to control the drug substance, excipient, or drug product can include  
574 tests such as particle size analysis, droplet distribution, spray pattern, dissolution  
575 (excludes measurement), optical rotation, and methodologies such as DSC, XRD, and  
576 Raman spectroscopy. The validation characteristics may differ for the various  
577 analytical procedures. For example, accuracy, repeatability, intermediate precision  
578 and robustness should be evaluated for molecular size distribution gel permeation  
579 chromatography (GPC).

580  
581 **B. Compendial Analytical Procedures**

582  
583 The suitability of a compendial analytical procedure must be verified under actual conditions of  
584 use (21 CFR 211.194(a)(2)). Information to demonstrate that USP/NF analytical procedures  
585 are suitable for the drug product or drug substance should be included in the submission.  
586 Information on the specificity, intermediate precision, and stability of the sample solution  
587 should be included. Compendial assay analytical procedures may not be stability-indicating,  
588 and this should be considered when developing the specification (see section III.C). For  
589 compendial items, additional analytical procedures, such as impurities or osmolality, may be  
590 requested to support the quality of the drug product or drug substance. These additional  
591 analytical procedures should be validated (see section VII.A).

592  
593  
594 **VIII. STATISTICAL ANALYSIS**

595  
596 **A. General**

597  
598 Methods validation includes an assessment of the adequacy of the analytical procedure.  
599 Statistical analysis (e.g., linear regression analysis, relative standard deviation) of methods

600 validation data is often used to demonstrate the validity of the method. The statistical  
601 procedures for the analysis of the validation data should be determined prior to the start of any  
602 validation study. The procedure followed, including the amount of data to collect and the  
603 criteria used in determining the acceptability of the analytical procedure, should be specified.  
604

605 The raw methods validation data and statistical procedures used to analyze the raw data  
606 should be provided and discussed in the sections on analytical procedures and controls. All  
607 statistical procedures used in the analysis of the data should be based on sound principles and  
608 be suitable for evaluating the dataset.  
609

## 610 **B. Comparative Studies**

611 Comparative studies are performed to evaluate intermediate precision (e.g., different  
612 equipment, analysts, days). Comparative studies are also used to evaluate *between*  
613 *laboratory* variability (i.e., reproducibility) when an analytical procedure is used in more than  
614 one laboratory or to compare and evaluate the precision and accuracy of two analytical  
615 procedures (e.g., regulatory analytical procedure and an alternative analytical procedure).  
616 When comparative studies are performed, homogeneous samples from the same batch should  
617 be used, if feasible. Comparative results should be statistically analyzed and discussed and  
618 any bias explained.  
619

## 620 **C. Statistics**

621 For information on statistical techniques used in making comparisons, as well as other general  
622 information on the interpretation and treatment of analytical data, appropriate literature or texts  
623 should be consulted (see references) .  
624  
625  
626  
627

## 628 **IX. REVALIDATION**

629 When sponsors make changes in the analytical procedure, drug substance (e.g., route of synthesis), or  
630 drug product (e.g., composition), the changes may necessitate revalidation of the analytical  
631 procedures. Revalidation should be performed to ensure that the analytical procedure maintains its  
632 characteristics (e.g., specificity) and to demonstrate that the analytical procedure continues to ensure  
633 the identity, strength, quality, purity, and potency of the drug substance and drug product, and the  
634 bioavailability of the drug product. The degree of revalidation depends on the nature of the change.  
635 When a different regulatory analytical procedure is substituted (e.g., HPLC for titration), the new  
636 procedure should be validated (see section VII).  
637  
638

639 If during each use an analytical procedure can meet the established system suitability requirements only  
640 with repeated adjustments to the operating conditions stated in the analytical procedure, the analytical  
641 procedure should be reevaluated, amended, and revalidated, as appropriate.  
642

643 FDA intends to provide guidance in the future on postapproval changes in analytical procedures.  
644

645

646

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

647

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

651

652

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

653

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

657

658 For ANDA and NDA products, the archival copy and extra copies of the methods validation  
659 packages should be submitted with the application. For ANDAs and related supplemental  
660 applications, one archival copy and two extra copies of the methods validation package  
661 should be submitted. For NDAs and related supplemental applications, one archival copy and  
662 three extra copies should be submitted. For BLAs and PLAs, a separate methods validation  
663 package need not be submitted. Information similar to that specified here should be included  
664 in the BLA or PLA submission.

665

666 The methods validation package should include:

667

668

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

669

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

673

674

#### *2. Analytical Procedures*

675

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

679

680

#### *3. Validation Data*

681

682 Appropriate validation data to support the analytical procedures should be submitted.

683

684 Individual values as well as summary tables should be provided. Representative  
685 instrument output and raw data and information regarding stress studies should be  
included (see section VII).

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4. *Results*

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

5. *Composition*

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

6. *Specifications*

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

7. *Material Safety Data Sheets*

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

**B. Selection and Shipment of Samples**

On request from CDER, an NDA or ANDA applicant must submit samples of drug product, drug substance, noncompensial reference standards, and blanks, so that the suitability of the applicant's drug substance and drug product analytical procedures can be evaluated by FDA laboratories (21 CFR 314.50(e) and 314.94(a)(10)). For BLAs and PLAs, representative samples of the product must be submitted, and summaries of the results of tests performed on the lots represented by the submitted sample must be provided (21 CFR 601.2(a) and 601.2(c)(1)(vi)).

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

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

729 Unless specified differently by the reviewer, samples from any batch, preferably samples from  
730 an aged batch, may be selected for NDAs and NDA supplemental applications. The  
731 submitted drug product samples should be from a batch made with the proposed market  
732 formulation. For ANDAs and appropriate supplements, a sample of the finished product from  
733 a batch being used to support approval of the submission should be used. If a sample is  
734 selected from a batch not described in the application, an amendment containing a copy of the  
735 batch record and certificate of analysis should be provided to the ANDA. For supplements  
736 that do not require submission and review of an exhibit batch record and associated data, any  
737 commercial batch may be submitted. For biological products, samples from several  
738 consecutively manufactured batches should be submitted.

739  
740 The drug product should be supplied in its original packaging. Bulk substances (e.g., drug  
741 substances, impurities, excipients) should be stored in opaque nonreactive containers. To  
742 prevent breakage during shipping, the samples should be adequately packaged in a sturdy  
743 container. Samples shipped from outside the United States should contain the appropriate  
744 customs forms to reduce delay in delivery.

745  
746 If special storage precautions (e.g., freezing, use of an inert gas blanket) are required to  
747 protect sample integrity, arrangements should be made in advance with the validating  
748 laboratory for scheduled direct delivery. If a sample is toxic or potentially hazardous, the  
749 container should be prominently labeled with an appropriate warning and precautionary  
750 handling instructions.

751  
752 **C. Responsibilities of the Various Parties**

753  
754 *1. Applicant*

755  
756 In the sections of the application on analytical procedures and controls, the applicant  
757 should provide a name, address, telephone number, and facsimile number so that  
758 samples can be requested. If this information is not provided, the contact person and  
759 address listed in the NDA, ANDA, BLA, or PLA submission will be used.

760  
761 The methods validation packages should be compiled and submitted with the NDA or  
762 ANDA submission. For BLAs and PLAs, a separate methods validation package  
763 need not be submitted.

764  
765 When an FDA laboratory contacts the applicant for samples, the applicant should  
766 provide FDA laboratories with the samples within 10 working days. With the  
767 exception of sample delivery arrangements, all communications concerning validation  
768 at the FDA laboratories should be made through or with the knowledge of the review  
769 chemist for CDER applications, or the BLA/PLA committee chair for CBER  
770 applications.

771

772 2. *Review Chemist*

773  
774 The review chemist will review the application to determine that the analytical  
775 procedures are adequate to ensure the identity, strength, quality, purity, and potency  
776 of the drug substance and/or drug product. Any changes in the methods resulting from  
777 the review of the application may require resubmission of the methods validation  
778 package. The review chemist, in coordination with the appropriate FDA laboratories,  
779 will decide which analytical procedures are to be validated. Comments from the FDA  
780 laboratories, if any, will be forwarded by the review chemist to the applicant on  
781 completion of the studies by the laboratories.

782  
783 3. *FDA Laboratory*

784  
785 An FDA laboratory will contact applicants with instructions on the submission of  
786 samples and the addresses to which samples should be mailed. The laboratory will  
787 test the samples according to the submitted analytical procedures to determine  
788 whether the analytical procedures are acceptable for quality control and suitable for  
789 regulatory purposes. Results and comments will be forwarded to the review chemist  
790 on completion of the studies.

791  
792 4. *Investigator*

793  
794 The investigator inspects the analytical laboratory testing sites where the release and  
795 stability testing are performed to ensure that the analytical procedures are performed  
796 in compliance with CGMP/GLP.

797  
798  
799 **XI. METHODOLOGY**

800  
801 Sections II through IX provide general information on the submission of analytical procedures and  
802 methods validation information, including validation characteristics. Additional information on certain  
803 methodologies is provided below.

804  
805 **A. High-Pressure Liquid Chromatography (HPLC)**

806  
807 The widespread use of HPLC analytical procedures and the multitude of commercial sources  
808 of columns and packings frequently have created problems in assessing comparability. Many  
809 of the following points may also apply to other chromatographic analytical procedures.

810  
811 1. *Column*

812  
813 The following characteristics are useful for defining a particular column and, if known,  
814 should be included in the analytical procedure description. If method development has

815 indicated that columns from only one commercial source are suitable, this information  
816 should be included as part of the analytical procedure. If more than one column is  
817 suitable, a listing of columns found to be equivalent should be included.

818

819 a. Column Parameters

820

821 ! Material: glass, stainless steel, plastic

822 ! Dimensions: length, inner diameter

823 ! Frit size

824 ! Filter type

825 ! Precolumn and/or guard column type, if used

826

827 b. Packing Material

828

829 ! Particle type: size, shape, pore diameter

830 ! Surface modification (e.g., bonded surface type, surface coverage, percent  
831 carbon, additional silylation)

832 ! Recommended pH range for column use

833

834 2. *System Suitability Testing*

835

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

841

842 ! Tailing factor

843 ! Relative retention

844 ! Resolution

845 ! Relative standard deviation (RSD)

846 ! Capacity factor

847 ! Number of theoretical plates

848

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

853

854 If an internal standard is used, the minimum acceptable resolution between the  
855 internal standard and one or more active ingredients should be specified. If the  
856 analytical procedure is used to control the level of impurities, the minimum resolution  
857 between the active ingredient and the closest eluting impurity, or the two peaks

858 eluting closest to each other, should be given.

859

860 3. *Operating Parameters*

861

862 The sequence of injection of blanks, system suitability standards, other standards,  
863 and samples should be defined. Flow rates, temperatures, and gradients should be  
864 described.

865

866 Complete details should be provided for the preparation of the mobile phase,  
867 including the order of addition of the reagents and the methods of degassing and  
868 filtration. The effect of adjustments in mobile phase composition on retention times  
869 should be included in the analytical procedure. The rationale for the use of  
870 precolumns and/or guard columns should be provided and justified. Any special  
871 requirements, such as the use of inert tubing or injection valves, should be specified.

872

873 **B. Gas Chromatography (GC)**

874

875 At a minimum, the following parameters should be included in the description of a GC  
876 procedure. Additional parameters should be specified if required by the analytical procedure.

877 If method development has indicated that columns from only one commercial source are  
878 suitable, this information should be included as part of the analytical procedure. If more than  
879 one column is suitable, a listing of columns found to be equivalent should be included.

880

881 1. *Column*

882

883 ! Column dimensions: length, internal diameter, external diameter

884 ! Stationary phase

885 ! Column material (e.g., silica, glass, stainless steel)

886 ! Column conditioning procedure

887

888 2. *Operating Parameters*

889

890 ! Gases: purity, flow rate, pressure

891 ! Temperatures: column, injector, detector (including temperature program, if  
892 used)

893 ! Injection (e.g., split, splitless, on-column)

894 ! Detector

895 ! Typical retention time and total run time

896

897 3. *System Suitability Testing*

898

899 Appropriate system suitability criteria should be defined and included in all analytical  
900 procedures.

901  
902 If an internal standard is used, the minimum acceptable resolution between the internal  
903 standard and one or more active ingredient should be specified. If the analytical  
904 procedure is used to control the level of impurities, the minimum resolution between  
905 the active ingredient and the closest eluting impurity, or the two peaks eluting closest  
906 to each other, should be given.

907  
908 The RSD is normally performed at the beginning of the run. However, for assays with  
909 lengthy run times or as otherwise justified by the applicant, the reported average may  
910 be taken from injections at the beginning and end of the run, or beginning, middle, and  
911 end of the run.

912  
913 **C. Spectrophotometry, Spectroscopy, Spectrometry and Related Physical**  
914 **Methodologies**

915  
916 These analytical procedures include, but are not limited to, IR spectrophotometry, near IR  
917 spectrophotometry (NIR), UV/visible spectrophotometry (UV/Vis), atomic emission and  
918 atomic absorption, NMR, Raman spectroscopy, MS, and XRD.

919  
920 Spectrometric analytical procedures may not be stability-indicating. The bias of the analytical  
921 procedure should be evaluated by comparing it with a chromatographic procedure, where  
922 appropriate. When manually operated equipment is used, the description of the analytical  
923 procedure should include an acceptance criterion for the amount of time that may elapse  
924 between sampling and reading. Appropriate system suitability and/or calibration testing is  
925 recommended. Validation criteria should include specificity (demonstrating no interference of  
926 placebo), linearity, repeatability, intermediate precision, and robustness.

927  
928 **D. Capillary Electrophoresis (CE)**

929  
930 At a minimum, the parameters listed below should be specified for a capillary electrophoretic  
931 analytical procedure. Additional parameters may be included as required by the procedure.  
932 If method development has indicated that capillaries from only one commercial source are  
933 suitable, this information should be included as part of the analytical procedure. If more than  
934 one capillary is suitable, a listing of capillaries found to be equivalent should be included.

935  
936 1. *Capillary*

937  
938 ! Capillary dimensions: length, length to detector, internal diameter, external  
939 diameter

940 ! Capillary material

941 ! Capillary internal coating (if any)

942  
943 2. *Operating Parameters*

- 944
- 945 ! Capillary preparation procedure: procedure to be followed before the first
- 946 use, before the first run of the day, before each run (e.g., flush with 100
- 947 millimolar sodium hydroxide, flush with running buffer)
- 948 ! Running buffer: composition, including a detailed preparation procedure with
- 949 the order of addition of the components
- 950 ! Injection: mode (e.g., electrokinetic, hydrodynamic), parameters (e.g.,
- 951 voltage, pressure, time)
- 952 ! Detector
- 953 ! Typical migration time and total run time
- 954 ! Model of CE equipment used
- 955 ! Voltage (if constant voltage)
- 956 ! Current (if constant current)
- 957 ! Polarity (e.g., polarity of electrode by detector)
- 958

### 959 3. *System Suitability Testing*

960

961 Each analytical procedure should include the appropriate system suitability tests

962 defining the critical characteristics of that system. Other parameters may be included

963 at the discretion of the applicant.

964

965 If an internal standard is used, the minimum acceptable resolution between the internal

966 standard and one or more active ingredient should be specified. If the analytical

967 procedure is used to control the level of impurities, the minimum resolution between

968 the active ingredient and the closest eluting impurity, or the two peaks eluting closest

969 to each other, should be given.

970

### 971 **E. Optical Rotation**

972

973 Optical rotation is used for the measurement of stereochemical purity. Visual polarimeters rely

974 on a monochromatic source, which traditionally was sodium D, but has expanded to virtually

975 any wavelength.

976

977 If measurements are to be made at a wavelength other than sodium D, an explanation for

978 selecting the wavelength should be given, along with a comparison of the specific rotation at

979 sodium D and the wavelength to be used. Circular dichroism (CD) spectra may suffice for this

980 purpose. In addition to the provisions of USP <781>, procedures for measurement of

981 specific rotation should include the solvent, concentration, and, for aqueous solutions, the pH

982 to which the solution should be adjusted. The conditions and equipment should be shown to

983 be suitable to confirm the stereochemical identity of a racemate or an enantiomer.

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985 The enantiomeric purity can be expressed as *enantiomeric excess* (e.e.), using the following

986 formula as an example:

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$$\text{e.e.} = 100\% * \frac{\{M\} - [m]}{\{M\} + [m]}$$

where [M] and [m] are the concentrations of the major and minor enantiomers, respectively. This yields values of zero for a racemate and 100 percent for a pure enantiomer. An intermediate concentration gives intermediate values; for example, 97:3 would give an e.e. of 94 percent.

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

## F. Methodologies Relating to Particle Size Analysis

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

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

### 1. Particle Size Methods

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

- a. Nonfractionation methods that evaluate an entire population of particles
  - ! Microscopy (optical, electron)
  - ! Light scattering (dynamic, photon correlation, laser diffraction)
  - ! Electrozone sensing
  - ! Photozone sensing
- b. Fractionation methods that use physical techniques to separate particles on the basis of size
  - ! Sieving
  - ! Cascade impactor
  - ! Sedimentation
  - ! Size exclusion chromatography

### 2. Calibration and Validation Characteristics

1030 To ensure proper instrument operation, the system should be calibrated according to  
1031 the manufacturer's and/or the laboratory's specification, as appropriate.

1032  
1033 The methods validation usually involves evaluation of intermediate precision and  
1034 robustness. Assurance should be provided that the data generated are reproducible  
1035 and control the product's quality. See additional information in sections V and VII.

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1037 **G. Dissolution**

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1039 The equipment used for dissolution is covered by USP <711> or USP <724>. The  
1040 dissolution procedure description and validation should include the following.

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1042 *1. Dissolution Medium*

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1044 A brief discussion of the reasons for selecting the medium.

1045  
1046 *2. Procedure*

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1048 A dissolution test consists of a dissolution procedure and method of analysis  
1049 (automated on-line analysis or manual sampling followed by HPLC analysis). The  
1050 written procedure should cover the following items:

- 1051
- 1052 ! Apparatus
  - 1053 ! Preparation of standard
  - 1054 ! Preparation of sample
  - 1055 ! Method of analysis (e.g., UV, HPLC)
  - 1056 ! Sampling procedure (e.g., intervals, filtration, handling of samples, dilutions)
  - 1057
  - 1058 ! Calculations
  - 1059 ! Acceptance criteria

1060  
1061 Regardless of the method of analysis, system suitability criteria should be described.  
1062 Blank and standard solution spectra or chromatograms should be included.

1063  
1064 *3. Validation Characteristics*

1065  
1066 Both the dissolution procedure and the method of analysis should be validated.

1067  
1068 The time needed for the completion of the sample analysis should be stated in the  
1069 procedure. Data should be submitted to support the stability of the dissolution sample  
1070 during the procedure. If filters are used on-line or during sample preparation,  
1071 appropriate recovery studies should be performed and documented and any bias  
1072 should be addressed.

1073 **H. Other Instrumentation**

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*1. Noncommercial Instrumentation*

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

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

*2. Automated Analytical Procedures*

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

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

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1098  
1099 The information relating to analytical procedures and methods validation that should be submitted in  
1100 NDAs, ANDAs, BLAs, and PLAs is identified below with a cross-reference to the section of this  
1101 guidance that provides recommendations and/or discussion on the topics.

1102  
1103 Information that should be included in the analytical procedures and controls sections

- 1104
- 1105 ! Reference standard information Section IV
  - 1106 ● Analytical procedures Section III, VI
  - 1107 ● Validation data Section VII
  - 1108 ● Stress studies Section VII.A.2.c
  - 1109 ● Instrument output/raw data for impurities Section VII.A.2.b
  - 1110 ● Statistical analysis Section VIII
  - 1111 ● Revalidation, as needed Section IX

1112  
1113 Information that should be included in the methods validation package<sup>5</sup>

- 1114
- 1115 ● Contents of the MV Package Section XI
  - 1116 ● Representative instrument output/data for stress studies Section VII.A.2.c
  - 1117 ! Representative instrument output and raw data for initial  
1118 and oldest sample of a batch Section VII.A.2.b

1119  
1120 Information that should be included in the stability section

- 1121
- 1122 ! Stress study designs and results Section VII.A.2.b
  - 1123 ! Reference (volume and page number of submission)  
1124 to instrument output and raw data submitted to the section  
1125 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.

**ATTACHMENT B**

**METHODS VALIDATION PROBLEMS AND DELAY**

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

- ! Failure to provide a sample of a critical impurity, degradation product, internal standard, or novel reagent
- ! Failure to submit well-characterized reference standards for noncompendial drugs
- ! Failure to provide sufficient detail or use of unacceptable analytical procedures. For example:
  - C Use of arbitrary arithmetic corrections
  - C Failure to provide system suitability tests
  - C 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
- ! Failure to submit complete or legible data. For example:
  - C Failure to label instrument output to indicate sample identity
  - C Failure to label the axes
- ! Inappropriate shipping procedures. For example:
  - C Failure to properly label samples
  - C Failure to package samples in accordance with product storage conditions
  - C Inadequate shipping forms (e.g., missing customs form for samples from outside the United States)
- ! Failure to describe proper storage conditions on shipping containers

1159 **REFERENCES**

1160

1161 **FDA Documents<sup>6</sup>**

1162

1163 Guidance for Industry: *ANDAs: Impurities in Drug Products* (Draft, December 1998).

1164

1165 Guidance for Industry: *ANDAs: Impurities in Drug Substances* (February 2000).

1166

1167 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).

1168

1169 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).

1170

1171 Guidance for Industry: *Investigating Out of Specification (OOS) Test Results for Pharmaceutical Production* (Draft, September 1998).

1172

1173 Guidance for Industry: *Stability Testing of Drug Substances and Drug Products* (Draft, June 1998).

1174

1175 Guidance for Industry: *Submission of Chemistry, Manufacturing, and Controls Information for Synthetic Peptide Substances* (November 1994).

1176

1177 Guidance for Industry: *Submitting Documentation for the Stability of Human Drugs and Biologics* (February 1987).

1178

1179 Reviewer Guidance: *Validation of Chromatographic Methods* (November 1994).

1180

1181 FDA CDER MAPP 5221.1 *Requesting Methods Validation for ANDAs* (November 1998).

1182

1183 **International Conference on Harmonization Guidances**

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1185 ICH *Q1A: Stability Testing of New Drug Substances and Products* (November 1994)

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1187 ICH *Q1B: Photostability Testing of New Drug Substances and Products* (November 1996)

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1189 ICH *Q1C: Stability Testing for New Dosage Forms* (May 1997)

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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.

- 1198 ICH Q2A: *Text on Validation of Analytical Procedures* (March 1995)  
1199  
1200 ICH Q2B: *Validation of Analytical Procedures: Methodology* (May 1997)  
1201  
1202 ICH Q3A: *Impurities in New Drug Substances* (January 1996)  
1203  
1204 ICH Q3B: *Impurities in New Drug Products* (May 1997)  
1205  
1206 ICH Q3C: *Impurities: Residual Solvents* (December 1997)  
1207  
1208 ICH Q5C: *Quality of Biotechnological Products: Stability Testing of*  
1209 *Biotechnological/Biological Products* (July 1996)  
1210  
1211 ICH Q6A: *Specifications: Test Procedures and Acceptance Criteria for New Drug Substances*  
1212 *and New Drug Products: Chemical Substances* (Draft (Step 2) November 1997)  
1213  
1214 ICH Q6B: *Specifications: Test Procedures and Acceptance Criteria for*  
1215 *Biotechnological/Biological Products* (March 1999)  
1216  
1217 **U.S. Pharmacopeia/National Formulary**  
1218  
1219 Chapter <621> Chromatography; US Pharmacopeia 23, United States Pharmacopeial Convention,  
1220 Inc., Rockville MD: 1994  
1221  
1222 Chapter <781> Optical Rotation, US Pharmacopeia 23, United States Pharmacopeial Convention,  
1223 Inc., Rockville, MD: 1994  
1224  
1225 Chapter <1225> Validation of Compendial Methods; US Pharmacopeia 23, United States  
1226 Pharmacopeial Convention, Inc., Rockville MD: 1994  
1227  
1228 Interpretation and Treatment of Analytical Data; USP Pharmacopeial Forum, United States  
1229 Pharmacopeial Convention, Inc., Rockville MD: 1994, Volume 24, Number 5, pp. 7051 - 7056  
1230  
1231 **Other**  
1232  
1233 Miller, J.C., J.N. Miller, and E. Horwood, *Statistics for Analytical Chemistry*, 3rd edition, Prentice  
1234 Hall, 1993.  
1235  
1236 Saunders, B.D., and R.G. Trapp, *Basic and Clinical Biostatistics*, 2nd edition, Appleton and Lange,  
1237 1994.

## GLOSSARY

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

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

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

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

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

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

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

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

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

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

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

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