Commentary

USP 38–NF 33, Second Supplement

June 1, 2015

In accordance with USP's Rules and Procedures of the 2010-2015 Council of Experts ("Rules") and except as provided in Section 7.02 Accelerated Revision Processes, USP publishes proposed revisions to the *United States Pharmacopeia and the National Formulary* (*USP–NF*) for public review and comment in the *Pharmacopeial Forum (PF),* USP's free bimonthly journal for public notice and comment. After comments are considered and incorporated as the Expert Committee deems appropriate, the proposal may advance to official status or be republished in *PF* for further notice and comment, in accordance with the Rules. In cases when proposals advance to official status without republication in *PF,* a summary of comments received and the appropriate Expert Committee's responses are published in the Revisions and Commentary section of the USP Web site at the time the official revision is published.

The *Commentary* is not part of the official text and is not intended to be enforceable by regulatory authorities. Rather, it explains the basis of Expert Committees' responses to public comments on proposed revisions. If there is a difference between the contents of the Commentary and the official text, the official text prevails. In case of a dispute or question of interpretation, the language of the official text, alone and independent of the Commentary, shall prevail.

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Comments were received for the following, when they were proposed in Pharmacopeial Forum

General Chapters

<111> Design and Analysis of Biological Assays
<212> Oligosaccharide Analysis
<232> Elemental Impurities—Limits
<233> Elemental Impurities—Procedures
<755> Minimum Fill
<1025> Pancreatin
<1132>Residual Host Cell Protein Measurement in Biopharmaceuticals
<1223> Validation of Alternative Microbiological Methods
<1223.1> Validation of Alternative Methods to Antibiotic Microbial Assays

Monographs:

- American Ginseng
- Amlodipine and Valsartan Tablets
- Asian Ginseng
- Aspartame
- Aspartic acid
- Bacopa
- Beclomethasone Dipropionate Compounded Oral Solution
- Benzocaine Topical Solution
- Black Cohosh
- Black Pepper
- *Boswellia serrata*
- Bupropion Hydrochloride
- Butylated Hydroxytoluene
- Carbachol
- Carbamazepine
- Carbamazepine Oral Suspension
- Carboprost Tromethamine
- Cat's Claw
- *Centella asiatica*
- Cisapride Compounded Injection, Veterinary
- Crypthecodinium Cohnii Oil Capsules
- Cyclosporine Compounded Ophthalmic Solution, Veterinary
- Desmopressin Acetate
- Doxorubicin Hydrochloride
- Doxorubicin Hydrochloride for Injection
- Doxorubicin Hydrochloride Injection
- Eleuthero
- Enrofloxacin Compounded Oral Suspension, Veterinary
- Ezetimibe
- Ezetimibe Tablets
- Fenugreek Seed
- Fenugreek Seed Powder
- Fenugreek Seed Powdered Extract
- Forskohlii
- *Garcinia cambogia*
- *Garcinia indica*
- Ginkgo
- Guggul
- Gymnema
- Isoflurane
- Lactulose Concentrate
- Lamivudine Tablets
- Lamotrigine Extended-Release Tablets
- Levocetirizine Dihydrochloride Tablets
- Loperamide Hydrochloride Tablets
- Malabar-Nut-Tree, Leaf
- Mercaptopurine
- Methimazole
- Mycophenolate Mofetil Capsules
- Mycophenolate Mofetil Tablets
- Nicotine

- Nicotine Polacrilex
- Northern Schisandra Fruit
- Northern Schisandra Fruit Powder
- Oleyl Alcohol
- Phenylephrine Bitartrate
- Phenylephrine Hydrochloride
- Phenytoin
- *Phyllanthus amarus*
- Powdered American Ginseng
- Powdered Asian Ginseng
- Powdered Ashwagandha Root
- Powdered Bacopa
- Powdered Black Cohosh
- Powdered Black Pepper
- *Powdered Centella asiatica*
- Powdered Chaste Tree
- Powdered Eleuthero
- Powdered Forskohlii
- Powdered *Garcinia cambogia*
- Powdered *Garcinia indica*
- Powdered Goldenseal
- Powdered Gymnema
- Powdered Horse Chestnut
- Powdered Licorice
- Powdered Malabar-Nut-Tree, Leaf
- Powdered *Phyllanthus amarus*
- Powdered St. John's Wort
- Powdered Turmeric
- Propafenone Hydrochloride Extended-Release Capsules
- Rizatriptan Benzoate Orally Disintegrating Tablets
- Rizatriptan Benzoate Tablets
- Scaffold Silk Fibroin
- Schizochytrium Oil Capsules
- Spirulina
- Spirulina Tablets
- St. John's Wort
- Timolol Maleate
- Tolterodine Tartrate
- Turmeric

No Comments received for the following, when they were proposed in Pharmacopeial Forum

General Chapters

<31> Volumetric Apparatus
<561>Articles of Botanical Origin
<565>Botanical Extracts

Monographs:

- Andrographis
- Anethole
- Ashwagandha Root
- Azatadine Maleate
- Aztec Marigold Zeaxanthin Extract
- Benzocaine Ointment
- Benzocaine Topical Aerosol
- Cabergoline
- Calcium and Vitamin D with Minerals Tablets
- Calcium with Vitamin D Tablets
- Capsules Containing at least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Phenylpropanolamine
- Capsules Containing at least Three of the Following—Acetaminophen and Salts of Chlorpheniramine,
- Dextromethorphan, and Phenylpropanolamine
- Carbamazepine Tablets
- Cetostearyl Alcohol
- Cetyl Alcohol
- Chase Tree
- Chlorpheniramine Maleate and Phenylpropanolamine Hydrochloride Extended-Release Capsules
- Chlorpheniramine Maleate and Phenylpropanolamine Hydrochloride Extended-Release Tablets
- Cisapride Compounded Oral Suspension, Veterinary
- Clemastine Fumarate
- Clofazimine
- Clofazimine Capsules
- Clomiphene Citrate
- Copper Gluconate
- Corticotropin for Injection,
- Corticotropin Injection
- Crypthecodinium Cohnii Oil
- Dexamethasone Sodium Phosphate
- Dried Ferrous Sulfate
- Econazole Nitrate
- Evening Primose Oil Capsules
- Extended Insulin Human Zinc Suspension
- Famciclovir Compounded Oral Suspension
- Feverfew
- Ferrous Fumarate
- Garlic
- Ginger
- Goldenseal
- Ferrous Gluconate
- Ferrous Sulfate
- Fish Oil Containing Omega-3 Acids
- Fish Oil Containing Omega-3 Acids Capsules
- Fluoxetine Delayed-Release Capsules
- Fluoxetine Tablets
- Gemfibrozil Tablets
- Gonadorelin for Injection
- Gonadorelin Hydrochloride
- Graftskin Graftskin (Future: Construct Human Keratinocytes and
- Fibroblasts in Bovine Collagen Scaffold)
- Guanadrel Sulfate
- Guanadrel Sulfate Tablets
- Halobetasol Propionate
- Hawthorn Leaf with Flower
- Horse Chestnut
- Human Insulin Isophane Suspension and Human Insulin Injection
- Hydrocodone Bitartrate and Hometropine Methylbromide Tablets
- Insulin Human
- Insulin Human Injection
- Insulin Human Zinc Suspension
- Insulin Lispro
- Insulin Lispro Injection
- Iron Dextran Injection
- Isophane Insulin Human Suspension
- Isopropyl Palmitate
- Ketoconazole
- Lactulose Solution
- Licorice
- Lopinavir and Ritonavir Oral Solution

- Lypressin Nasal Solution
- Magnesium Salicylate
- Magnesium Salicylate Tablets
- Manganese Gluconate
- Marbofloxacin Compounded Oral Suspension, Veterinary
- Methyl Salicylate
- Methyltestosterone
- Milk Thistle
- Minerals Capsules
- Minerals Tablets
- Myristyl Alcohol
- Myrrh
- Nadolol
- Octyldodecanol
- Oil-Soluble Vitamins Capsules
- Oil-Soluble Vitamins Tablets
- Oil-Soluble Vitamins with Minerals Capsules
- Oil-Soluble Vitamins with Minerals Tablets
- Olive Oil
- Omega-3 Acids Triglycerides
- Oral Solution Containing at least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Phenylpropanolamine
- Phenylpropanolamine Bitartrate
- Phenylpropanolamine Hydrochloride
- Phenylpropanolamine Hydrochloride Capsules
- Phenylpropanolamine Hydrochloride Extended-Release Capsules
- Phenylpropanolamine Hydrochloride Extended-Release Tablets
- Phenylpropanolamine Hydrochloride Oral Solution
- Phenylpropanolamine Hydrochloride Tablets
- Psyllium Husk
- Pygeum
- Powdered Andrograpis
- Powdered Cat's Claw
- Powdered Feverfew
- Powdered Garlic
- Powdered Ginger
- Powdered Hawthorn Leaf with Flower
- Powdered Milk Thistle
- Powdered Saw Palmeto
- Repository Corticotropin Injection
- Ritonavir Oral Solution
- Saw Palmeto
- Scaffold Porcine Bladder
- Schizochytrium Oil
- Sennosides
- Sertraline Hydrochloride Tablets
- Small Intestinal Submucosa Wound Matrix (Future: Scaffold Porcine Small Intestinal Submucosa)
- Soybean Oil
- Stearyl Alcohol
- Tablets Containing at least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Phenylpropanolamine
- Thimerosal
- Trehalose
- Water-Soluble Vitamins with Minerals Oral Solution
- Wheat Bran
- Zein
- Zinc Gluconate

General Chapter/Section(s): <111> Design and Analysis of Biological Assays/ Multiple Sections
Expert Committee: Statistics
No. of Commenters: 6

General Comments
Comment Summary #1: The commenter requested that the General Chapter be numbered above 1000 so that alternative statistics can be used and to ensure that monographs that reference this General Chapter are suitably analyzed.
Response: Comment not incorporated. General Chapter <111> has been cited by the same monographs for decades. At this time, monographs that referenced <111> were either revised to no longer require a citation of <111> or are continuing to be supported by <111>.
Comment Summary #2: The commenter indicated that the title of the General Chapter did not reflect the scope of the General Chapter and suggested incorporating the General Chapter content into General Chapter <1034> *Analysis of Biological Assays*.
Response: Comment not incorporated. To minimize disruption to monograph references and stakeholders' SOPs the Expert Committee decided to maintain the current <111> title. The content that is cited by monographs was maintained in the <111> revision proposal and updated. Much of the old <111> content that is not required in a below 1000 General Chapter for citation by monographs was distributed among the informational bioassay General Chapter series <1030> *Biological Assay Chapters—Overview and Glossary*, <1032> *Design and Development of Biological Assays,* <1033> *Biological Assay Validation*, and <1034> *Analysis of Biological Assays*.
Comment Summary #3: The commenter stated that the previous <111> General Chapter introduced cell-based bioassays and analysis and recommended a short section stating mandatory requirements for cell based assays.
Response: Comment not incorporated. This material is beyond the scope of <111> and cannot be generalized in a General Chapter such as <111>. Sufficient information already exists in the above-1000 bioassay General Chapters.
Comment Summary #4: The commenter requested that the General Chapter retain Table 9.
Response: Comment not incorporated. The Table content is no longer cited by any monographs and some variables are for tests that are no longer recommended (e.g., F tests). Users should consult appropriate statistical references if needed.

Rejection of Outlying or Aberrant Observations
Comment Summary #5: The commenter indicated that outliers should be covered in the <1030> to <1034> bioassay General Chapter series.
Response: Comment not incorporated. See response to Comment Summary #1.
Comment Summary #6: The commenter noted that the outlier methods only address a single outlier in each tail and suggested including a recommendation for cases in which there are more than one outlier in each tail.
Response: Comment partially incorporated. A sentence was added stating that methods that address multiple outliers may be needed: "*Alternative outlier methods are available that are intended for use on data sets that may contain multiple outliers and for detection of outliers associated with the bioassay design or model*." This complex topic is beyond the scope of

<111>, but the Expert Committee will consider this topic for future compendial revisions or new General Chapters.
Comment Summary #7: The commenter suggested adding the following sentence, “Alternative statistical approaches for outlier detection may be used if well-justified by a statistician.”
Response: Comment partially incorporated. The sentence was added, but requirement for explicit justification by a statistician was omitted: “*Alternative statistically sound approaches to outlier detection may be used*.”
Comment Summary #8: The commenter requested some examples or references to explain the statement, “data have a nearly normal distribution.”
Response: Comment partially incorporated. The comment is beyond the scope of <111>. The Expert Committee will consider this suggestion for future General Chapters. Many references exist in the literature and General Chapter <1032> also discusses these issues. In response to the comment, the sentence was edited for better clarity as follows, “data have an approximately ~~nearly~~ normal distribution.”
Comment Summary #9: The commenter suggested revising General Chapter <111> to be more consistent with the outlier guidance in General Chapter <81> *Antibiotics-Microbial Assays* which makes specific recommendations regarding frequency of outliers.
Response: Comment not incorporated. General Chapters <111> and <81> do not cross-reference each other; however, the comment was forwarded to the Expert Committee responsible for <81> for their consideration in future revisions.
Comment Summary #10: Two commenters requested better clarity of the subscripts and minus signs in Table 1.
Response: Comment incorporated.
Comment Summary #11: Two commenters suggested changing certain Table 2 values to be consistent with Table A2-1 values in General Chapter <81>.
Response: Comment not incorporated. General Chapters <111> and <81> do not cross-reference each other; however, the comment was forwarded to the Expert Committee responsible for <81> for their consideration in future revisions.
Comment Summary #12: The commenter suggested that General Chapter <111> should have the same flexibility as General Chapter <1010> in making the distinction between using the Dixon’s or Grubb’s test to examine for outlying values of N larger than13. The proposed General Chapter specifies “For N larger than 13, use Criterion 2 (Grubbs, Extreme Studentized Deviate Test).”
Response: Comment not incorporated. Being a standard practice and not contradicted by the associated text in <1010> *Analytical Data—Interpretation and Treatment*, the recommendation made in <111> will remain. The Statistics Expert Committee will consider these matters for future General Chapters or revisions of existing General Chapters.
Comment Summary #13: The commenter stated that Dixon’s and Grubb’s tests in the proposed General Chapter are too restrictive and may not be the best choice, and asked to allow alternative approaches (e.g., Lund’s etc.).
Response: Comment incorporated. The sentence, ““*Alternative statistically sound approaches to outlier detection may be used*” was added. In addition, *General Notices* 6.30 allows the use of alternative methods and/or procedures if demonstrated to yield equivalent or better results.

The Confidence Interval and Limits of Potency
Comment Summary #14: The commenter suggested adding the text,

"The confidence interval for the estimate of relative potency may be estimated directly as a parameter of a global model with appropriate formulation. In this case, this confidence interval obtained directly from the non-linear regression procedure as the confidence interval of a model coefficient for relative potency may be estimated directly as a parameter of the fitted global."

Response: Comment not incorporated. This topic is beyond the scope of General Chapter <111>. Some information can be found on this topic in the General Chapters <1032>, <1033>, and <1034>.

Comment Summary #15: The commenter suggested adding the equation for "*g*" that is included in the "g" in the confidence interval equation within this section.

Response: Comments not incorporated. This is not necessary because the equation for this "*g*" already exists in the General Chapter.

Combination of Independent Assays

Comment Summary #16: The commenter requested that the section be implemented in General Chapters <1032>, <1033>, and <1034>.

Response: Comment not incorporated. Many monographs use this method and thus it is suitable content for the General Chapter.

Comment Summary #17: The commenter requested that the geometric mean calculation with GSD% be implemented in the General Chapter.

Response: Comment not incorporated. General Chapter <111>'s formulae all use log-transformed data; therefore, it is not necessary to add GSD.

Comment Summary #18: The commenter requested guidance for the appropriate measure of variability (%RSD, %GSD, etc.).

Response: Comment not incorporated. General Chapter <111> uses confidence intervals to determine suitability therefore these other measures are not necessary. General Chapter <1033> provides additional guidance.

Comment Summary #19: The commenter stated that it was not clear why the statement "Use Method 1 unless otherwise directed by the pertinent monograph or General Chapter," was made.

Response: Comment incorporated. The sentence was deleted.

Comment Summary #20: The commenter requested additional background on Method 2.

Response: Comment not incorporated. There is sufficient background for its use in the General Chapter and additional information would be out of scope for a General Chapter numbered below 1000. Readers seeking additional guidance should consult the literature (e.g., General Chapter 14 of Finney, D.J. (1964), *Statistical Method in Biological Assays*. 2nd edition, Charles Griffin & Co. Ltd., London).

Comment Summary #21: The commenter suggested the following text revision, "For each assay, *i*, obtain the confidence interval for the log potency or log relative potency. Then compute value L_i by subtracting the *ith* lower confidence limit from the *ith* upper confidence limit."

Response: Comment incorporated.

Comment Summary #22: Two commenters suggested labeling a particular confidence interval equation to clarify its intended use.

Response: Comment incorporated. The number "1" was added next to the equation and it was cited it appropriately in the text below.

Expert Committee-initiated Change #1: The following text was edited for greater clarity, "~~On the average~~ At a confidence interval of 99%, a valid observation will be rejected once in 100 trials (when the suspected outlier can occur at only one end) or once in 50 trials (when the suspected outlier can occur at either end), provided that relatively few, if any, responses within the group are identical."
Expert Committee-initiated Change #2: The following text was edited for greater clarity, "Compute the relative gap by using ~~*Table 2. Test for Outlier Measurements* and~~ the formulas in Table 1 below."
Expert Committee-initiated Change #3: The following text was edited for greater clarity, "In samples from a normal population, at a confidence interval of 99%, gaps equal to or larger than…"
Expert Committee-initiated Change #4: Five values in Table 2 were updated in the hundredth decimal place to reflect the most recent data: 0.780~~1~~, 0.683~~1~~, 0.635~~4~~, 0.679~~4~~, 0.642~~3~~, and 0.615~~7~~.

General Chapter/Section(s): <212> Oligosaccharide Analysis/Multiple Sections
Expert Committee: General Chapters—Biological Analysis
No. of Commenters: 3

General Comments
Comment Summary #1: The commenter suggested including specific procedures/methods for proteins with more complicated glycosylation structures, because the current version appears to be more suitable for recombinant monoclonal antibody molecules analysis. Well characterized glycan standards derived from glycoproteins with highly complex glycans could be an alternative reference standard.
Response: Comment not incorporated. The General Chapter is under revision to add a high-pH anion exchange chromatography (HPAEC) procedure for analysis of more complex charged *N*-linked oligosaccharides.
Comment Summary #2: The commenter suggested including a description for allowing orthogonal methods being applied to *N*-glycan characterization, because each separation has its own limitation. In principle, at least hydrophilic interaction chromatography (HILIC) and charged based separation should be used in conjunction. This is particularly important for highly glycosylated proteins.
Response: Comment not incorporated. Inclusion of this type of description is outside the scope of this General Chapter because <212> is an analytical method General Chapter for routine analysis.
Comment Summary #3: Two commenters recommended USP to consider updating and/or including newer technologies in the future (e.g., UPLC or instant AB labeling).
Response: Comment incorporated. Methods are subject to updating to match developing technology as long as they are validated.

Introduction
Comment Summary #4: The commenter suggested adding wording to explain that the current scope focuses on analysis of *N*-linked oligosaccharides released from glycosylated recombinant therapeutic proteins that contain relatively simple biantennary and high mannose chains, with no or low levels of sialylated, phosphorylated, or sulfated structures.

Response: Comment incorporated.
Comment Summary #5: The commenter suggested clarifying in which conditions method verification or validation is required and defining testing requirements for verification or validation.
Response: Comment incorporated. References to <1225> *Validation of Compendial Procedures* and <1226> *Verification of Compendial Procedures* were added to the General Chapter.
Comment Summary #6: The commenter suggested adding a note to the *Introduction* stating, "Methods could be modified to use other fluorescent probes, other separation columns and other procedures as deemed appropriate."
Response: Comment not incorporated. The option of using alternative methods and/or procedures is always open to stakeholders and is covered under *General Notices*, section 6.30 *Alternative and Harmonized Methods and Procedures*.
Comment Summary #7: The commenter suggested including the justification for not addressing electrochemical detection (pulsed amperometric detection or pulsed electrochemical detection) in this General Chapter, because this method is used in oligosaccharide analysis under a strong base elution.
Response: Comment not incorporated. The Expert Committee's decided not to include the electrochemical detection method, because this detection method is less sensitive and has a higher noise to signal ration. In addition, the prioritization of methods to be included in this General Chapter was based on the data from a large survey, followed by an international collaborative study on *N*-glycan analysis led by the USP Convention in 2008.
Comment Summary #8: The commenter suggested adding a note referencing where the method for electrochemical detection (pulsed amperometric detection or pulsed electrochemical detection) can be found.
Response: Comment incorporated. A reference to <1084> *Glycoprotein and Glycan Analysis—General Considerations* was added to the General Chapter.
Comment Summary #9: The commenter suggested clarifying that current recombinant glycosylated biological medicines in commerce and in clinical trials contain relatively simple biantennary and high mannose chains of neutral oligosaccharides.
Response: Comment not incorporated. The General Chapter is under revision to add a HPAEC procedure for analysis of more complex charged *N*-linked oligosaccharides.
Comment Summary #10: The commenter suggested adding text to clarify that other methods are as acceptable as the ones described in the General Chapter.
Response: Comment not incorporated. The option of using alternative methods and/or procedures is always open to stakeholders and is covered under *General Notices*, section 6.30 *Alternative and Harmonized Methods and Procedures*.
Comment Summary #11: The commenter suggested adding information in the *Introduction* section to help users make informative decisions when it comes to choosing which method to use, because this General Chapter provides three HPLC methods.
Response: Comment not incorporated. The first column in Table 1 provides distinctive information for each method. Example chromatograms for the associated Reference Standards will be provided in the USP Certificates of the corresponding reference standards to help the users select the method.

Comment Summary #12: The commenter suggested providing additional information on what partially sialylated panel of *N*-linked oligosaccahrides in USP Oligosaccharide System Suitability Mixture A RS contains.
Response: Comment not incorporated. The information can be found in the USP Certificate for this reference standard.
Comment Summary #13: The commenter recommends the following wording: **"**The procedures described in this General Chapter all provide qualitative analysis. The results from each of the procedure are reported as %glycan peak area equals the individual glycan peak area over the total glycan peak area."
Response: Comment partially incorporated. It is up to the manufacturers to define the data analysis; however, the statement, "Data analysis, quantitation, and lot release specifications, which are expected to be product specific, will be found in individual product monographs. These aspects are not covered in this General Chapter" was added to the General Chapter for clarification.

Analytical Procedures Overview
Comment Summary #14: The commenter suggested that the following scenarios should be clarified: 1) How the sample analysis should be performed, 2) Whether both reference standards are required to be analyzed to establish the system suitability followed by sample analysis, and 3) If for other non-mAb proteins, both reference standards are not suitable, what is the suggested reference standard that should be used for system suitability?
Response: Comment not incorporated. The system suitability requirement described in each analytical procedure offers the option to choose the reference standard most appropriate for the test sample. If there is a mixture of glycan types, using both reference standards may be appropriate. Furthermore, two additional reference standards that are suitable for assessing the system performance of the HPEAC procedure for analysis of highly complex glycans are under development.
Comment Summary #15: The commenter suggested adding "2 AB-oligosaccharides" to the HILIC and HPAEC procedure in the *Table* 1 with explanation of the type of samples that should be used.
Response: Comment incorporated.
Comment Summary #16: The commenter suggested providing example chromatographic separations of USP Oligosaccharide System Suitability Mixture A and B Reference Standards for each proposed glycan method.
Response: Comment not incorporated. The typical chromatograms are provided in the Certificate for the reference standards.
Comment Summary #17: The commenter suggested indicating *Normal phase, Chromatography*/*HILIC Procedure 1* in Table 1 results in better resolution compared to *HILIC Procedure 2*.
Response: Comment not incorporated. This statement is too specific to be included in the General Chapter. The user can decide which procedure is suitable for their application based on the condition of each procedure.
Comment Summary #18: The commenter suggested adding another bullet for *Normal phase, Chromatography*/*HILIC Procedure 1* in Table 1 to read "Potential for simple in-line identification of oligosaccharides using mass spectrometry."

Response: Comment not incorporated. This comment is beyond the scope of this General Chapter, which only describes analytical procedures

Comment Summary #19: The commenter suggested adding a statement to Table 1 regarding the advantage of HPLC analysis that oligosaccharides may be easily identified during assay development or validation using in-line mass spectrometry because this table contained a description that capillary electrophoresis has enhanced separation efficiency.

Response: Comment not incorporated. The suggested language is beyond the scope of this General Chapter and is more appropriate for the associated informational General Chapter <1084> Glycoprotein and Glycan Analysis. In addition, the description of "enhanced separation efficiency" for capillary electrophoresis is removed.

Sample Preparation

Comment Summary #20: The commenter recommended revising the section on sample clean-up by incorporating the provided language, because detergent, such as polysorbate 20 or polysorbate 80, is not easily removed, and furthermore, significant protein loss is common during purification.

Response: Comment incorporated.

Comment Summary #21: The commenter suggested that dialysis be performed against buffer instead of water, noted that other methods are capable of operating with typical level of excipients/salts in therapeutic protein formulations, and that the step for sample clean-up may not be needed.

Response: Comment incorporated. The option of dialysis against buffer is included, and the section is revised to allow the flexibility for the users to decide whether sample clean-up step is needed.

Comment Summary #22: The commenter suggested adding additional specifics, because the section of sample clean-up seems very broad.

Response: Comment not incorporated. The purpose of this section is to provide general guidelines and avoid providing overly descriptive methods.

Comment Summary #23: The commenter suggested clarifying when procedure 1 is advantageous to use over procedure 2.

Response: Comment not incorporated. Differences between the conditions for Procedure 1 and Procedure 2 are summarized in the *Table* 1.The users can decide which procedure is more appropriate based on the information in the *Table* 1 and the detailed conditions described in section of *Separation and Identification of Oligosaccharides*.

Sample Preparation, Digestion with PNGase F

Comment Summary #24: The commenter suggested including a sample reaction blank, prepared from the buffer matrix of the glycoprotein sample to be carried through the process to confirm the specificity of the method.

Response: Comment incorporated.

Sample Preparation, Digestion with PNGase F, Method 1

Comment Summary #25: The commenter suggested adding the following Note to the end of Method 1: "lower molecular weight cutoff ultrafiltration membranes may be used for proteins smaller than 150 KDa."

Response: Comment incorporated.

Comment Summary #26: The commenter suggested including ethanol precipitation method as protein clean-up method alternative.
Response: Comment not incorporated. The Expert Committee will consider further revisions to the General Chapter upon receipt of a validated method.

Sample Preparation, 2-Aminobenzamide (2-AB) Labeling for Liquid Chromatographic Separation
Comment Summary #27: The commenter recommended adding the following information to the *Labeling Solution*: "Use the *Labeling solution* within 1 h of its preparation. Protect the solution from light exposure."
Response: Comment incorporated.
Comment Summary #28: The commenter suggested adding the following statement in Method 2: “During method verification, it should be confirmed that no oligosaccharide species are lost during the 2-AB removal step by comparing the chromatographic profiles of an unextracted reaction blank, an un-extracted sample, and an extracted sample.”
Response: Comment incorporated. A Note containing this statement was added to the General Chapter.

Sample Preparation, 8-Aminopyrene-1,3,6-trisulfonic acid (APTS) Labeling for CE Separation
Comment Summary #29: The commenter suggested adding clean-up steps after APTS labeling, because excess APTS could cause high background signals from labeling reagents when analyzed by CE.
Response: Comment not incorporated. The method provided was validated without clean-up steps. The Expert Committee will consider further revisions to the General Chapter upon receipt of a validated method.

Separation and Identification of Oligosaccharides
Comment Summary #30: The commenter suggested including a procedure for the Strong and Weak Needle Washes and Seal Washes for Normal phase/HILIC procedures.
Response: Comment not incorporated. A procedure for instrumentation maintenance is not typically provided, and it is up to the individual on how to manage the instrumentation.

Comment Summary #31: The commenter suggested adding the following statement in *Analysis*, *"*When peaks detected from samples that are not corresponding to the glycan peaks from the reference standard, it is recommended using on-line HILIC-MS analysis to determine the possible oligosaccharide structures.” In cases when peaks are detected but do not correspond to any glycans used in the reference standard, this peak will not be identified. This is important because many glycan structures are often present at low amount.
Response: Comment not incorporated. These reference standards are developed to assess system suitability as described in the *Introduction*. Furthermore, including alternative approach for identification and characterization is beyond the scope of this General Chapter.
Comment Summary #32: The commenter suggested adding a range for each RRT (e.g., target RRT is 0.47±0.02). This will compensate any LC system variation from different labs. This becomes critical for G1Fa and G1Fb which elute very close to each other. Also, providing the identity of G1Fa and G1Fb would be helpful.

Response: Comment not incorporated. The addition of the word “approximate” to the RRT table and a revision of text to include the word “approximate” will be proposed in PF 41(5), to cover the possible variation from laboratories. The information of G1Fa and G1Fb is provided in the USP Certificate for the reference standard.
Comment Summary #33: The commenter suggested the use of a Dextran Calibration Ladder to aid in determining glycan peak identity according to Glucose Units (GU).
Response: Comment not incorporated. The Expert Committee will consider further revisions to the General Chapter upon receipt of a validated method or supporting data using this material.
Comment Summary #34: The commenter recommended using both a reference standard and a control when labeling for fluorescent detection. The reference standard (with 2-AB labeling for fluorescent detection) is used to establish chromatographic system suitability. The control is carried through the sample preparation process (glycan release, labeling and clean-up) and assures sample preparation integrity.
Response: Comment incorporated.
Comment Summary #35: The commenter suggested defining major peaks by setting criteria for this term (e.g. >1 %) or clearly indicating what is considered as "major peaks" in *Analysis*, and also providing guidance on how to deal with non "major " peaks. This will allow users to identify the appropriate peaks for integration for the evaluation of the specificity. This practice will also exclude potentially critical structures which are present at very low amount.
Response: Comment partially incorporated. This General Chapter was revised to reference <621> *Chromatography* on how to integrate the peaks and on how to define % of peak. In addition, revising the *Introduction* by using more descriptive language on referencing <1084> *Glycoprotein and Glycan Analysis—General Considerations* to clarify that product specifications reside in the monographs, and not in this procedural General Chapter.

Separation and Identification of Oligosaccharides, Normal Phase Chromatography/HILIC, Procedure 1
Comment Summary #36: The commenter suggested changing the autosampler temperature from 20° to 2°–8° in the *Normal Phase Chromatography/Procedure 1* to ensure the sample stability during analysis.
Response: Comment incorporated.
Comment Summary #37: The commenter recommended changing the *Blank solution* composition from acetonitrile to buffer matrix of sample that is carried through the totality of the sample preparation procedure, to confirm the presence of any reagent-related peaks.
Response: Comment incorporated.
Comment Summary #38: The commenter suggested changing the blank solution composition from acetonitrile to buffer matrix of glycoprotein sample, because the suitability requirement from *Blank solution* is “No peak in the chromatogram of the *Blank solution* within the retention time window at 5–113 min.” This would only be true provided that the blank solution is representative of the process and not just pure acetonitrile, which would not yield any fluorescent peaks.
Response: Comment incorporated.
Comment Summary #39: The commenter suggested adding the word “approximate” to the column title for Table 3 and Table 4 because the relative retention times listed in these tables are only approximate.
Response: Comment not incorporated. The addition of the word “approximate” to the RRT table and a revision of text to include the word “approximate” will be proposed in PF 41(5), to cover

the possible variation from laboratories. The information of G1Fa and G1Fb is provided in the USP Certificate for the reference standard.

Separation and Identification of Oligosaccharides, Normal Phase Chromatography/HILIC, Procedure 2
Comment Summary #40: The commenter asked the purpose of the wavelength change during the HPLC run.
Response: Comment not incorporated. The wavelength shift early in the method was implemented to aid with automated integration by avoiding detection of unreacted 2-AB reagent (and possibly other contaminants) and the need to subtract or ignore the area counts that would be associated with them.

Separation and Identification of Oligosaccharides, Capillary Electrophoresis
Comment Summary #41: The commenter requested the reason for providing relative retention times for three Man-7 structures in *Table 13*.
Response: Comment not incorporated. There were isomers for Man-7.

APPENDIX 1
Comment Summary #42: The commenter recommended that the naming align with accepted IUPAC and/or Dublin/Oxford, or CFG for *Table 14 Glycan Description*.
Response: Comment incorporated. IUPAC description was added to *Table 14*.

General Chapter/Section(s): <232> Elemental Impurities—Limits
Expert Committee: General Chapters—Chemical Analysis
No. of Commenters: 17

General Comments
Comment Summary #1: The commenter requested to delay the implementation date of the General Chapter until the harmonized PDE limits are reached.
Response: Comment incorporated. The implementation date of the General Chapter was changed to January 1, 2018.
Comment Summary #2: The commenter suggested that the timeline for implementation be reconsidered in relation to the availability of associated reference standards.
Response: Comment not incorporated**.** USP will not be developing elemental impurities reference standards at this time.
Comment Summary #3: The commenter suggested harmonizing USP requirements with those of the future *ICH Q3D Guideline for Elemental Impurities*. Manufacturers and suppliers should not be expected to implement the standards multiple times – once for USP, then when ICH is adopted in each of the 3 regions, and then again in response to revisions of USP to match ICH.
Response: Comment partially incorporated. The General Chapter is harmonized with *ICH Q3D* to the extent possible. *ICH Q3D* elements currently not included in General Chapter <232> will be included in an above 1000 informational general chapter in the near future.
Comment Summary #4: The commenter recommended the need for a harmonized approach to specifications between the General Chapter <232> and *ICH Q3D* requirements. The commenter requests that USP and ICH reach a consensus on the limits set in the final documents.

Response: Comment incorporated. Elements not listed in General Chapter <232> will be addressed in a future informational General Chapter.

Comment Summary #5: The commenter indicated that the USP proposed limits keep changing for selected elements, and in limited cases, the elements themselves have changed. The speciation of the elements Arsenic and Mercury (i.e., contrast inorganic versus organic forms), the differential treatment for Chromium, and the deletion of Manganese have complicated the process to perform the appropriate development work and required validation work needed for our laboratory operations. USP has not harmonized the specifications for the 15 elements that are listed in General Chapter <232> to the most recent publication of *ICH Q3D Guideline for Elemental Impurities*. The elements with different specifications for parenteral dosage forms**,** comparing General Chapter <232> to *ICH Q3D*, include Cadmium, Mercury, Molybdenum and Chromium. This complicates the validation and qualification requirements for products intended for US and European distribution.

Response: Comment incorporated. The General Chapter was revised to harmonize the specifications for Cadmium, Mercury, Molybdenum and Chromium with *ICH Q3D*.

Comment Summary #6: The commenter indicated that excipient manufacturers will be particularly impacted by the aggressive timeline that is planned for implementation. As communicated in much of the literature, suppliers of the active pharmaceutical ingredients may have somewhat less difficulty in the actual implementation, but without a steady source of supplied excipients that meet the USP compendial requirements this will directly impact the ability of drug manufacturers to supply the market. Excipient suppliers of such common ingredients as simple inorganic salts that are mined or obtained from natural processes do not have the immediate hands-on resources available to provide all the development resources needed to implement General Chapter <232> and General Chapter <233> in a short time frame.

Response: Comment incorporated. The implementation date was revised to be January 1, 2018. General Chapter <232> clearly states that the onus is on the drug product manufacturer for compliance, not on the excipient manufacturers. Additionally, the summation option permits taking into consideration the amount of a given excipient in a given drug product.

Comment Summary #7: The commenter asked whether the end user is required to conduct any testing if the supplier provides a statement that there are no elemental impurities, and there is control on the supplier's manufacturing process (i.e. studies demonstrate compliance to the limits).

Response: Comment not incorporated. Each manufacturer must establish their own risk-based approach and determine the need for testing, based on their own assessment criteria. This may be done in conjunction with discussions with the regulatory agency. It is the responsibility of manufacturer is to ensure regulatory compliance.

Comment Summary #8: The commenter indicated that the stage 2 draft of *ICH Q3D* provides an important provision for performing risk assessments – a 30% threshold for applying additional controls. USP should include this provision in General Chapter <232> to provide useful instructions on risk analysis and to establish consistency with *ICH Q3D*.

Response: Comment not incorporated. USP sets standards and cannot establish regulatory requirements. Users may employ any appropriate guideline such as ICH. It is the responsibility of each manufacturer to best determine how to demonstrate compliance in coordination with regulatory agencies. The risk-based approach offers many opportunities, including but not limited to the 30% threshold described by ICH. For these reasons, the 30% threshold is not included in General Chapter <232>.

Comment Summary #9: The commenter suggested adding additional information and language on risk assessments, perhaps its own section, to further harmonization the General Chapter with ICH and clarify the expectation and intent.
Response: Comment not incorporated. The USP is responsible for providing a standard that may be used to demonstrate compliance of a drug product. Approaches for performing risk assessment are beyond the scope of the USP standard. Users may employ any appropriate guideline such as *ICH Q3D*.
Comment Summary 10: The commenter suggested changing the title of the General Chapter to "Elemental Impurities—Toxicological Considerations and Limits," because the current title is misleading. In addition to a brief mention of the actual limits for the elemental impurities, this General Chapter goes into greater detail on the toxicological considerations of the impurities that could be present.
Response: Comment not incorporated**.** The Expert Committee determined that the title should not be changed, because the full discussion regarding the toxicological considerations is not contained in its entirety in General Chapter <232>, but is also found in stimuli to the revision process articles.
Comment Summary #11: The commenter recommended not proceeding with further official changes until *ICH Q3D* Step 4 is finalized.
Response: Comment incorporated. General Chapter <232> has been harmonized with ICH Q3D to the extent possible.

Introduction
Comment Summary #12: The commenter requested aligning the General Chapter with *ICH Q3D* by indicating that veterinary and conventional vaccines are out of the scope of the General Chapter <232> and it is not just the limits specified in General Chapter <232> that are out of scope for veterinary and conventional vaccines. The following wording was proposed, "This General Chapter does not apply to conventional vaccines and articles intended only for veterinary use*."*
Response: Comment incorporated.
Comment Summary #13: The commenter suggested that the "for cause" case approach be considered, i.e. testing only the elements used in synthesis/preparation (as in the EMA guide on residual metals, reagents and catalysts and also in the EDQM primary approach). The commenter also inquired on USP's justification for expanding the scope for a general screening of elements and not for a screening based on cause.
Response: Comment not incorporated**.** USP has detailed the rationale for assessment for potential inadvertent contaminants in numerous public presentations, workshops, etc. Also, see response to comment #8.
Comment Summary #14: The commenter requested more information on how much USP agrees on ICH risk-based approach control strategy and noted that USP did not go into the details of its perspective on the risk-based approach control strategy.
Response: Comment not incorporated. USP's responsibility, unlike ICH, is to provide a standard, rather than a guideline. See responses to the comments #s 8, 9, and 13.
Comment Summary #15: The commenter requested the following sentence be rewritten to clarify expectations for Drug Product Manufacturers, because there are no reporting thresholds or limits associated with Elemental Impurities for drug substance or excipients:

"The limits presented in this General Chapter do not apply to excipients and drug substances, except where specified in this General Chapter or in the individual monographs. However, elemental impurity levels present in drug substances and excipients must be known, documented, and made available upon request."

The commenter also recommended that the USP align with ICH Q3D, and add a specific reference to the concept of "Risk Assessment" and utilizing the 30% POE control threshold as a minimum reporting requirement. The commenter proposed the revised wording:

"The limits presented in this General Chapter do not apply to excipients and drug substances, except where specified in this General Chapter or in the individual monographs. However, elemental impurity levels present in drug substances and excipients must be known when needed to support the risk assessment and/or summation option. The minimum control threshold is defined as 30% of the PDE (Permissible Daily Exposure). This should be applied to the drug product, drug substance, and/o*r* excipients depending upon the approach used to demonstrate compliance."

Response: Comment not incorporated. USP sets standards and cannot establish regulatory requirements. Users may employ any appropriate guideline such as *ICH Q3D*. It is the responsibility of each manufacturer to best determine how to demonstrate compliance in coordination with regulatory agencies. The risk-based approach offers many opportunities, including but not limited to the 30% threshold described by *ICH Q3D*. For these reasons, the 30% threshold is not included in <232>.

Comment Summary #16: The commenter suggested revising the following sentence, "Due to the ubiquitous nature of arsenic, cadmium, lead and mercury, they (at the minimum) must be considered in the risk-based control strategy," to state *"Due to the ubiquitous nature of arsenic, cadmium, lead and mercury, they (at the minimum) must be considered in the risk ~~based control strategy~~ assessment," b*ecause the current statement could be misinterpreted to mean that routine testing is always required for arsenic, cadmium, lead and mercury.

Response: Comment partially incorporated. Changes to the section may be addressed by the Advisory Panel in the future. The word "control" has been removed.

Comment Summary #17: The commenter suggested revising the following sentence, "Elemental impurity levels present in drug substances and excipients must be known, documented and made available upon request," to state, "The introduction of elemental impurities in drug substances and excipients must be controlled and, where present concentrations should be documented and made available on request*,"* because the current sentence could be misinterpreted to mean that drug substances and excipients must be tested for all elements listed in General Chapter <232>.

Response: Comment not incorporated. General Chapter <232> encourages the use of a risk-based approach to assess product compliance. It is not necessary to perform routine testing if a risk-based approach is used. The statement will remain in the General Chapter.

Comment Summary #18: The commenter suggested adding the following statement, "Alternatively, a risk assessment concludes that elemental impurity levels are below applicable limits in Table 1,*"* to clarify that a risk assessment strategy could be sufficient for drug substances and excipients instead of analytical results. The current sentence, "The limits

presented in this General Chapter do not apply to excipients and drug substances, except where specified in this General Chapter or in the individual monographs. However, elemental impurity levels present in drug substances and excipients must be known, documented and made available upon request," seems to imply that the elemental impurities must be measured, which would contradicts the risk-based control strategy mentioned in the paragraph one of the *Introduction*. The commenter also inquired as to the level of documentation required for drug substances and excipients.

Response: Comment not incorporated. General Chapter <232> encourages the use of a risk-based approach to assess product compliance. It is not necessary to perform routine testing if a risk-based approach is used. USP cannot provide guidance on this topic, because it is a regulatory issue and beyond the scope of this General Chapter.

Comment Summary #19: The commenter recommended modifying the *Introduction* to provide for the exclusion of inhalation anesthetic products.

Response: Comment not incorporated. The General Chapter is harmonized with *ICH Q3D*; therefore, the exclusion would be a deviation. This comment will be forwarded to the USP Small Molecules 4 Expert Committees for their consideration.

Routes of Exposure

Comment Summary #20: The commenter indicated that General Chapter <232> should not arbitrarily assign the same PDEs to mucosal and topical drugs as for oral and parenteral products, respectively, as proposed in *PF* 40(2). The major guiding principle of USP's new requirements for metal impurities was to base limits on patient safety, but the lack of data makes this impossible for mucosal and topical drugs. Without data allowing general conclusions on these product types to be reached, assignment of PDEs should be made based on the characteristics and data on the individual pharmaceutical product. The commenter suggested that the text be revised as proposed in their letter.

Response: Comment not incorporated. USP is now harmonized with *ICH Q3D* on this topic.

Comment Summary #21: The commenter suggested that evaluation of dermal products be assessed on a case-by-case basis, because of the complexities associated with determining dermal exposure and any associated systemic toxicity stemming from dermal exposure, combined with other factors such as the difficulties in defining a dose. Systemic exposure to actives applied dermally is significantly lower than levels obtained through oral administration, even formulations deliberately designed to maximize absorption through the skin. Crucially there is no evidence to support the supposition that application to broken skin will result in exposure akin to oral exposure, either for the active or for any elemental impurity present.

Response: Comment not incorporated. The Expert Committee took into account available data and reasonable approaches when determining how to address mucosal and topical drugs. Consideration was given to toxicokinetics, nanoparticles and absorption via broken skin. In addition, his approach is harmonized with *ICH Q3D*.

Comment Summary #22: The commenter requested removing all language directing for the use of oral PDE's for topical products in General Chapter <232>. This approach (using oral exposure scenarios for topical exposure scenarios) is unscientific and ignores the natural barrier properties of the skin. In addition, the digestive properties that occur in the gut are not available on or below the dermal surface. Exposure to the skin naturally blocks most if not all substances and impurities from entering the body. In addition, substances or impurities entering the body through the skin, should that occur, are not expected to be subjected to acid digestion.

Response: Comment not incorporated. The Expert Committee took into account available data and reasonable approaches when determining how to address mucosal and topical drugs. Consideration was given to toxicokinetics, nanoparticles and absorption via broken skin. In addition, this approach is harmonized with *ICH Q3D*.

Analytical Testing

Comment Summary #24: The commenter requested removing the following statement, "When testing is done to demonstrate compliance, proceed as directed in General Chapter Elemental Impurities—Procedures <233> and minimally include arsenic, cadmium, lead, and mercury in the Target Element evaluation." Although it may be sensible to perform qualification testing on these four metals, where such qualification testing is considered necessary, it should not be required to perform routine tests for these four metals just because a routine test for a known metal impurity is performed.

Response: Comment not incorporated. General Chapter <232> encourages the use of a risk-based approach to assess product compliance. It is not necessary to perform routine testing if a risk-based approach is used. The statement will remain in the General Chapter.

Comment Summary #25: The commenter suggested to re-include the option to demonstrate control by process validation/impurity tracking, because the language in the published proposal implies that it is not sufficient to validate a manufacturing process for control of elemental impurities, but that a minimum of process-monitoring is required to justify the absence of routine testing for the drug substance, excipients or drug product.

Response: Comment not incorporated. USP encourages a risk-based approach and each manufacturer must determine how best to comply under this approach.

Drug Products. Large-Volume Parenterals

Comment Summary #26: The commenter requested clarification of the sentence, "When the daily dose of an injection is greater than 100 mL [large-volume parenteral (LVP)]..." This statement does not definitively define LVP or specify if it is only when a unit dose container is greater than 100mL or the combination of multiple units for a single infusion can be greater than 100mL that LVP are not considered in *ICH Q3D*.

Response: Comment not incorporated. USP definition of large volume parenteral resides in General Chapter *<1> Injections*

Comment Summary #9: The commenter requested clarifying the statement, "…amount of elemental impurities present in the drug product ~~must~~ may, [*USP 38–NF 33, First Supplement*] be controlled through the individual components used to produce the product component option." The commenter indicated that changing word "must" to "may" in this context does not make the intention of the statement clear and questioned when would it be allowed and why could it not be an option for all doses?

Response: Comment incorporated.

Drug Products. Options for Demonstrating Compliance, Drug Product Analysis Option

Comment Summary #27: The commenter suggested that General Chapter <232> should specify that water used in manufacturing, which complies with the relevant USP monograph, meets the expectations for elemental impurities. Developing analytical procedures capable of controlling elemental impurities down to the levels in General Chapter <232> is difficult or impossible, even for many non-parenteral products.

Response: Comment not incorporated. If using the drug product option, the drug product must comply with the requirements of the General Chapter, if using the component option (for example if the finished product ingredients includes water), then it must be considered in the summation for compliance of the final product, similar to text in General Chapter <467> *Residual Solvents*.

Comment Summary #28: The commenter requested that the last sentence of the section be revised to read, "Before products can be evaluated using this option, the manufacturer must ensure that additional impurities cannot be inadvertently added through the manufacturing process (for all dosage forms) or via the container closure system (the contribution of the container closure system can be disregarded for solid oral dosage forms) over the shelf life of the product," because it is stated in the ICH guideline, that the container closure system for a solid oral dosage form of a product contributes a minimal amount of elemental impurities and can be disregarded for those dosage forms

Response: Comment not incorporated**.** The ICH guideline does not completely rule out the possibility of contributions from the container closure system for solid oral dosage forms, as evident by the listing of elements requiring risk assessment, even if they are not included during the manufacture of the product for solid oral dosage forms

Drug Products. Summation Option

Comment Summary #29: The commenter requested clarifying the sentence, "Before products can be evaluated using this option, the manufacturer must ensure (ERR 1-Oct-2013) that additional elemental impurities cannot be inadvertently added through the manufacturing process…" The commenter also questioned as to how something would be "inadvertently added."

Response: Comment not incorporated. Unlike solvents or other chemicals, metals are ubiquitous in our daily environment. They need not originate from a specific manufacturer's process, but may also originate from processes used by suppliers, etc. Inadvertent contamination can occur for a variety of reasons, which are too numerous to enumerate in this commentary.

Comment Summary #30: The commenter suggested clarifying the expectations for packaging components (i.e. bottles, caps, cotton, desiccants, etc.) and how the General Chapters apply to colors, dyes, flavors, coating materials, capsules, cleaners, and sanitizers.

Response: Comment not incorporated. The final drug product must comply with the requirements of General Chapter <232>. If dyes, flavors, coatings, capsules are used in the product, then they must be included when assessing compliance, either using the summation option or the drug product option. Cleaners and sanitizers are not normally included in the drug product. A risk-based assessment may be used (and is encouraged) especially for packaging components, but also in general.

Comment Summary #31: The commenter suggested revising the statement, "Separately add the amounts of each elemental impurity (in µg/g) present in each of the components of the drug product," to state, **"**Separately add the amounts of each measured elemental impurity (in µg/g) present in each of the components (active ingredients, drug substances and excipients) of the drug product" This will allow the *Summation Option* to stand alone as an exercise for addressing the determination of elemental impurities in drug products, rather than be confounded with all the text for *Table 2*.

Response: Comment not incorporated. The “measured” text is implied.

Drug Products. Table 1

Comment Summary #32: The commenter suggested indicating that the inhalation PDE for chromium and the footnote "Not a safety concern" for oral and parenteral exposure to chromium are based upon data for Cr (III) (and maybe Cr (0)), and that different limits may be needed for the more toxic/carcinogenic Cr (IV) compounds.

Response: Comment not incorporated. See response to comment #29 which indicates that USP and ICH are now harmonized.

Comment Summary #33: The commenter indicated that the proposed changes in *PF* 40(2) have given rise to new implementation concerns as every time a PDE changes (specifically decreases), there is the potential for existing drug products to be affected. The cadmium content of various suppliers of calcium carbonate will push some antacid formulations above the newly proposed cadmium PDE (oral exposure), based on their formulation and dosing recommendations. A delay would provide additional time for toxicology assessments to be completed and revision petitions filed, reviewed, published in the *Pharmacopeial Forum* for comments, published in the *USP–NF,* and implemented. Without such a delay, there is the potential for antacid drug shortages In the United States.

Response: Comment incorporated.

Comment Summary #34: The commenter expressed concern that their current production of USP Potassium Chloride will not consistently meet the new lower limit for lead. Potassium chloride is produced from mining potash deposits and refining the mined ore through dissolution and recrystallization. Trace amounts of lead are inherent to potash deposits and unfortunately the levels of lead are variable throughout such deposits. Lead is not significantly reduced through the re-crystallization refinement process, because the lead is commonly in the soluble Pb+2 form.

Response: Comment not incorporated. The General Chapter is now harmonized with *ICH Q3D*. Please refer to your regulatory agency for specific concerns about a specific product. The Small Molecules 4 Expert Committee will also be notified as this comment may be best addressed by them.

Comment Summary #35: The commenter requested that USP provide harmonized limits for methyl mercury (applicable only to those articles with the potential to contain methyl mercury e.g. materials derived from fish).

Response: Comment not incorporated. Methyl mercury limit is addressed in <2232> *Elemental Contaminants in Dietary Supplements.*

Comment Summary #36: The commenter indicated that the request to adjust for lower body weight for pediatric specific formulations directly conflicts with *ICH Q3D* and should be removed.

Response: Comment incorporated.

Drug Substance and Excipients.

Comment Summary #37: The commenter requested replacing the text, “Default Concentration Limits” in Table 2 with “Examples of Concentration Limits” to prevent construing these concentrations with regulatory limits. The regulatory limits should be based on Permitted Daily Exposure (PDE) limits and not hypothetical concentration limits.

Response: Comment incorporated.

Comment Summary #38: The commenter recommended removing Table 2 and its associated language from the General Chapter, because it is not for drug substances and excipients. USP's inclusion of the language in footnote 1 (in this correspondence) and Table 2, potentially leads users into mistakenly concluding that USP has actually issued limits on drug substances and excipients.
Response: Comment not incorporated. USP has stated repeatedly that the final drug product must comply with the requirements of General Chapter <232>. This has been presented at numerous public venues, including, but not limited to: workshops (initiated by both industry groups and USP), USP annual meetings, presentations at various scientific conferences in responses to previous comments received. The Expert Committee determined that the example provided in Table 2 is valuable and should remain in the General Chapter.
Comment Summary #39: The commenter requested clarifying the following statement, "The concentration of elemental impurities in drug substances and excipients must be controlled and, where present level documented" or replacing with the following text, "'*Not present' means not more than 30% of the applicable limit."*
Response: Comment not incorporated**.** To the extent that USP is harmonized with *ICH Q3D*, we can make clear that one way to know this is to do a risk assessment and understand the variability and expected range of concentrations. USP sets standards and does not establish regulatory requirements. Users may employ any appropriate guideline such as *ICH Q3D*. It is the responsibility of each manufacturer to best determine how to demonstrate compliance, in coordination with regulatory agencies. The risk-based approach offers many opportunities, including but not limited to the 30% threshold described by ICH. For these reasons, the 30% threshold is not included in General Chapter<232>.
Comment Summary #40: The commenter requested aligning the limits in Table 2 with the limits presented in the Table A.2.2 of *ICH Q3D*.
Response: Comment incorporated**.** General Chapter <232> and *ICH Q3D* are harmonized to the extent possible. *ICH Q3D* elements currently not included in General Chapter <232> will be included in an above 1000 General Chapter in the near future.

Analytical Testing
Comment Summary #41: The commenter requested removing the following statement: "When testing is done to demonstrate compliance, proceed as directed in General Chapter Elemental Impurities—Procedures <233> and minimally include arsenic, cadmium, lead, and mercury in the Target Element evaluation." Although it may be sensible to perform qualification testing on these four metals, where such qualification testing is considered necessary, it should not be required to perform routine tests for these four metals just because a routine test for a known metal impurity is performed.
Response: Comment not incorporated. General Chapter <232> encourages the use of a risk-based approach to assess product compliance. It is not necessary to perform routine testing if a risk-based approach is used. The statement will remain in the General Chapter.
Comment Summary #42: The commenter suggested to re-include the option to demonstrate control by process validation/impurity tracking in the section on "Analytical Testing**"** because language in the published proposal implies that it is not sufficient to validate a manufacturing process for control of elemental impurities, but that a minimum of process-monitoring is required to justify the absence of routine testing for the drug substance, excipients or drug product.

Response: Comment not incorporated. USP encourages a risk-based approach, each manufacturer must determine how best to comply with regulatory requirements.
Comment Summary #43: The commenter suggested revising the statement, "When testing is done to demonstrate compliance...and minimally include arsenic, cadmium, lead and mercury in the target element evaluation," to remove the phrase, "and minimally include arsenic, cadmium, lead and mercury in the target element evaluation," because this requirement is not scientifically founded. Any testing should be in line with the risk assessment. Routine testing should be focused on those impurities identified as a concern. As, Cd, Hg and Pb must be part of the risk assessment, but not necessarily routine test schedules.
Response: Comment not incorporated. General Chapter <232> encourages the use of a risk-based approach to assess product compliance. It is not necessary to perform routine testing if a risk-based approach is used. The statement will remain in the General Chapter.
Comment Summary #44: The commenter suggested introducing the risk assessment approach and replacing the following sentence, "If, by process monitoring and supply-chain control, manufacturer can demonstrate the absence of impurities, then further testing may not be needed," with the proposed text, "Risk assessment or process monitoring and supply-chain control, manufacturer can demonstrate the absence of impurities, then further testing may not be needed."
Response: Comment not incorporated. To the extent that the General Chapter is harmonized with *ICH Q3D,* we can make clear that one way to know if testing is needed is to do a risk assessment and understand the variability and expected range of concentrations. USP sets standards and does not establish regulatory requirements. Users may employ any appropriate guideline such as *ICH Q3D*. It is the responsibility of each manufacturer to best determine how to demonstrate compliance, in coordination with regulatory agencies. The risk-based approach offers many opportunities, including but not limited to the 30% threshold described by ICH. For these reasons, the 30% threshold is not included in General Chapter <232>.

General Chapter/Section(s): <233> Elemental Impurities-Procedures
Expert Committee: General Chapter—Chemical Analysis
No. of Commenters: 9

General Comments
Comment Summary #1: The commenter requested to delay the implementation date of the General Chapter until the harmonized PDE limits are reached.
Response: Comment incorporated. The implementation date of the General Chapter was changed to January 1, 2018.
Comment Summary #2: The commenter recommended that the General Chapter be changed to an informational General Chapter, because it does not give specific, validated procedures. *Procedures 1 and 2* and are too general and do not provide enough information to be considered actionable compendial procedures. The validation of analytical methods is discussed elsewhere in General Chapter <1225> *Validation of Compendial Procedures* and *ICH Guideline Q2 (R1) Validation of Analytical Procedures: Text and Methodology*.
Response: Comment not incorporated. Any standard included in the compendia should have an analytical procedure and corresponding acceptance criteria. Because compliance with USP standards is required by law, it is important for USP to establish referee procedures to

conclusively demonstrate compliance. This particular standard is designed to cover all articles in the compendia, so the description of the procedure needs to be open to adjustment to accommodate all different analytical matrices. To define what constitutes an acceptable procedure, General Chapter <233> provide a series of validation/verification requirements along with acceptance criteria for method performance to determine whether the method, when applied to a particular matrix, is suitable for its intended use. The fact that <233> allows for this flexibility does not mean it should be numbered above 1000, as the General Chapter has always been intended to create mandatory requirements, made applicable to articles through references in <232> and individual monographs as appropriate. Properly followed, <233> provides all of the information needed to perform an analysis that is suitable for its intended use and provide a basis upon which compliance with the standard can be determined.

Comment Summary #3: The Commenter suggested that General Chapter <233> should make reference to General Chapter <730> *Plasma Spectrochemistry* in the newly added system suitability section to help clarify some of the missing information in <233>. Wording in General Chapters <233> and <730> should also be aligned.

Response: Comment incorporated. USP is in the process of revising <730>. Efforts will be made to align wording. General Chapter <730> provides general guidance, whereas <233> provides specific guidance for the determination of elemental impurities.

Comment #4: The commenter requested that ICP-OES and ICP-MS be spelled out.

Response: Comment incorporated. The first use the abbreviations were spelled out, with the abbreviation provided in parenthesis.

Comment Summary #5: The commenter requested adding clarification on the intended applicability of <233> to clinical/analytical development.

Response: Comment not incorporated. USP General Chapters pertain to marketed drug products. Companies must make their own decisions regarding the applicability of <233> to clinical/analytical development.

Comment Summary #6: The commenter indicated that the General Chapter should give the option of any open or closed vessel digestion procedure that yields acceptable results based on the validation acceptance criteria. We have validated open vessel digestion procedures for many different matrices and obtained acceptable results for all elements including volatile elements such as mercury.

Response: The General Chapter permits the development of your own method--including sample preparation procedure--should that be desired or available. The requirements of the General Chapter, in that case, are to make certain that the method meets the validation criteria of <233>. The procedures provided are for those who either do not have, or do not wish to develop their own procedure.

Comment Summary #7: The commenter suggested clarifying that the analysis for elements typically introduced as catalysts (particularly Pt, Pd, Ir, Os, Rh and Ru) is not required, if no such catalysts are used in the production of the material, and are therefore not likely to be present.

Response: Comment not incorporated. USP cannot dictate a specific risk-based approach. General Chapter <232> permits a risk-based approach. Part of that approach includes a full understanding of a given synthetic process. The inclusion/exclusion of elements would be part of a company's risk-based approach.

Comment Summary #8: The commenter indicated that there are still references to "verify" in the General Chapter and recommend changing these references to avoid confusion.

Response: Comment incorporated.

Compendial Procedures 1 and 2

Comment Summary #9: The commenter suggested removing the rinse time of 60s from *Procedures 1 and 2*. It is stated in the procedure that if samples are high in mineral content the system must be rinsed well "(60s)" before introducing the sample. The rinse time however must be optimized for each specific situation as it will vary depending on the specific sample introduction system, tubing length, rinse solutions, and sample type. The 60 second requirement therefore may be too short for some systems, and too long for others.

Response: Comment incorporated.

Comment Summary #10: The commenter suggested adding “ICP-OES” to the heading of the Procedure 1: ICP-AES section, because the General Chapter specifies prior to this section that ICP-OES can also be used wherever it is able to use ICP-AES.

Response: Comment incorporated. The terms ICP-OES and ICP-AES are generally accepted to refer to the same instrumental technique. Both terms are spelled out in the first reference to them, with abbreviations provided in parentheses. “AES” will be removed.

Comment Summary #11: The commenter indicated that the two analytical procedures (Procedure 1 and 2) within the General Chapter are not FDA approved methods and validation is required, therefore, add text to clearly state that the procedures are informational.

Response: Comment not incorporated. Please see response to comment #2.

Quantitative Procedures. Accuracy

Comment Summary #12: The commenter indicated that the accuracy range should not be changed to 50%-200%. There is no additional value-added in demonstrating recovery at 2 times the failure level versus 1.5 times the failure level. Spike-recovery ranges of 50-150% are considered standard practice. This proposed change also has the potential to invalidate work already completed.

Response: Comment incorporated.

Compendial Procedures. Sample Preparation

Comment Summary #13: The commenter pointed out that the note, “All liquid samples should be weighed,” is unnecessarily restrictive and allowance should be made for volumetric manipulations. Furthermore, there is a potential increase in uncertainty when relying on weight to prepare liquid samples, because formulations are prepared within a concentration tolerance range and the density is not established for each batch. In addition, when the maximum daily dose is based on the volume delivered to the patient (as is the case with parenteral products), having to prepare samples by weight results in unnecessary conversions, and may require the solution's density to convert from the measured value to the daily PDE.

Response: Comment not incorporated. All samples must be weighed, because some liquid samples may be difficult to accurately pipette (may need positive displacement pipettes, for example). If the density of a given sample is such that omitting a correction for it would result in a statistically significant analytical result, then it is advised that the density correction be performed.

Comment Summary #14: The commenter requested that the statement "Total metal extraction is the preferred sample preparation approach" should be modified to state "Total sample digestion is the preferred sample preparation approach,” because the preferred approach described for *the indirect solution* preparation is closed vessel microwave digestion.

Response: Comment not incorporated. The goal of the procedure is to solubilize the analytes of interest. It may not be necessary to fully digest a sample if the analytes of interest are fully extracted. Additionally, determining that a sample is totally, completely, 100% digested is sometimes difficult; therefore, the use of "total metal extraction" is correct. Additional laboratory confirmatory experiments may need to be performed.

Comment Summary #15: The commenter indicated that the wording used in the sentence justifying leachate extraction is too specific and would be better defined by simply stating that the justification should be based on bioaccessibility. This would provide more flexibility, but still indicate that there must be a justification for this approach based on good science related to patient safety.

Response: Comment not incorporated. Although there is some discussion about bioaccessibility vs. bioavailability, toxicologists generally refer to and set limits based on bioavailability. It is not within the purview of the USP to change the generally-accepted procedures of the toxicological profession. The inclusion of the leachate extraction (vs. digestion extraction, for example) already affords greater flexibility than the requirement for total solubility of a sample material.

Comment Summary #16: The commenter suggested revising the specification for *Indirect Solution* to include the statement, "before it is used it should be verified that the indirect solution is truly representative," because the current definition is not specific enough.

Response: Comment not incorporated. Indirect solution is intended to refer to samples that may need digestion. Good scientific practice dictates that the samples be representative under all conditions.

Comment Summary #17: The commenter suggested removing the reference to hydrofluoric acid, because hydrofluoric acid bears extreme safety hazards for the operator and as a result its use is prohibited or restricted in many organizations.

Response: Comment not incorporated**.** While the Expert Committee agrees that hydrofluoric acid should be handled with utmost care and only after proper training, its use may present the only way for a sample analysis to be performed. For this reason, it is included in <233>.

Comment Summary #18: The commenter suggested clarifying what is meant by 'dehydrate and pre-digest' in the section on "Sample preparation".

Response: Comment not incorporated. The terms used are commonly used in the arena of sample digestion--especially, microwave digestion. Dehydration refers to the removal of water, and sulfuric acid is known to be a good dehydrating agent. Pre-digestion normally refers to a digestion step before a sample is heated for digestion. A sample may be pre-digested at room temperature prior to being placed in a microwave digestion system, where heat is then applied.

Limit Procedures. Detectability

Comment Summary #15: The commenter indicated that the acceptance criteria for the limit procedure (Detectability) is far stricter than for the quantitative procedure (Recovery/Accuracy); therefore, the acceptance criteria for the limit procedure should be revised to the following, "The average value of the three replicate measurements of spiked sample solution 1 is within 70 and 150% of the average value obtained for the replicate measurements of the Standard solution."

Response: Comment not incorporated. Due to the less stringent analytical procedure for limit tests, the acceptance criteria are, therefore, stricter. Analysts are free to use the quantitative procedure.

Comment Summary #20: The commenter requested widening the accuracy in the matrix spikes, because a spike recovery of 85-115% for a limit test validation is overly restrictive, given

this level of acceptable instrumental measurement uncertainty, particularly when compared to the wider requirement of 70-150% for a quantitative test. The allowable drift for the calibration standards is 20% in the system suitability requirement stated in USP <233>.
Response: Comment not incorporated. The limit test, by its very nature, does not provide as much information as the fully quantitative test would. For this reason, the criteria for the limit test are tighter.

Quantitative Procedures
Comment Summary #21: The commenter indicated that the validation description for *Quantitative Procedures* is too prescriptive and must allow flexibility with respect to the range to be validated. Instruction to prepare standard solutions having concentrations ranging from 50 to 200% of the J value for the determination of Accuracy is too restrictive for several reasons.
Response: Comment not incorporated. Comments from others have indicated that the range should be from 0.5-1.5J, and the Expert Committee has agreed to keep that range, rather than changing it to 0.5-2J.
Comment Summary #22: The commenter indicated that the concept for "Ruggedness" under Quantitative procedures is not clear. What is meant by the definition 'three independent events' and how the data should be evaluated (N=12). An example should be added to clarify the requirements:
- Day 1, Instrument 1, Analyst 1
- Day 2, Instrument 1, Analyst 1
- Day 2, Instrument 1, Analyst 2

Response: Comment incorporated. The intent of this requirement is that the method be demonstrated to meet validation criteria on multiple instances. The Expert Committee is aware that many laboratories may have only one instrument and only one analyst experienced with ICP-OES or ICP-MS instrumentation, because of this, it is not prudent to require that three different analysts or three different instruments be used to demonstrate ruggedness. Therefore, it is possible to demonstrate ruggedness using three different events, and that those events take into account the availability of only one instrument and/or only one analyst.
Comment Summary #23: The commenter indicated that General Chapter <233> requires quantitation over a validation concentration range of 0.5J to 2J, where J is maximum limit permitted based upon PDE and dose. Scientifically, there is no basis for establishing such a limited validation range. Industry calibrates instruments over a much wider linear range (a few orders of magnitude concentration), typically from the method Limit of Quantitation to > 2J. This flexibility is absolutely required if this procedure is intended to influence clinical / analytical development in any manner, and may also be necessary in manufacturing if one intends to provide quantitative results without frequently having to remake standards in the necessary narrow (0.5 to 2J) concentration range.
Response: Comment not incorporated**.** Commonly accepted practice is to look at the range from 50-150%. Others have commented that changing to 50-200% is not in keeping with accepted practice; therefore, the range will revert to 50-150%.
Comment Summary #24: The commenter suggested adding (N=6) to the following statement under *Precision*, 'Relative standard deviation: NMT 20 % (N=6) for each target element'.
Response: Comment incorporated.

Appendix

Comment Summary #25: The commenter recommended modifying the definition of Target limit or Target concentration from " ... the linear dynamic range of the instrument, J would thus equal 5ng and 0.015~-tg/ml for lead and arsenic ... " to " 5 ng/ml and 15 ng/ml for lead and arsenic ... " in order to maintain unit consistency.

Response: Comment Incorporated.

Comment Summary #26: The commenter requested clarification on whether it is necessary to do quantitative validation for each API and excipient in order to generate individual elemental impurity data.

Response: Comment not incorporated**.** USP <232> permits a risk-based approach. It is incumbent on each company to determine how best to assess their products and what level of risk they wish to take. In some instances, companies will want to test each and every sample, whereas others may wish to use a less stringent approach. USP cannot advise as to which approach an individual company should take. Companies should consult with regulatory agencies.

Comment Summary #27: The commenter requested removing the following statement, "Include As, Cd, Pb, Hg in the target element evaluation when testing is done to demonstrate compliance," because routine testing should be focused on those impurities identified as a concern. As, Cd, Hg and Pb must be part of the risk assessment, but not necessarily routine test schedules.

Response: General Chapter <232> encourages the use of a risk-based approach to assess product compliance. It is not necessary to perform routine testing if a risk-based approach is used. The statement will remain in the General Chapter.

Comment Summary #28: The commenter indicated that there are still references to "verify" in the General Chapter and recommend changing these references to avoid confusion.

Response: Comment incorporated.

Comment Summary #29: The commenter indicated that the J value is applicable only to the Drug Product analysis option but this would not be appropriate for the USP summation approach which many companies may choose to use. There should be some reference to alternate procedures that can be used for the summation approach for testing components.

Response: Comment not incorporated. J values can be determined based on the individual components and then summed to determine compliance.

Monograph/Section: <755> Minimum Fill/Multiple Sections
Expert Committee: General Chapters—Dosage Forms
No. of Commenters: 6

Scope:

Comment Summary #1: The commenter requested retaining the upper limit of 150 mL or 150 g for containers subject to the General Chapter.

Response: Comment not incorporated. Minimum fill is an important attribute of a product at any labeled content. General Notices Section 6.30 *Alternative and Harmonized Methods* provide guidance on the use of alternative methods where they may provide advantages. Such methods should be submitted for consideration as potential replacement or addition to the standard.

Comment Summary #2: The commenter recommended that the term “jellies” be dropped from the list of dosage forms to which this General Chapter applies. The preferred dosage term is “gels” as discussed in <1151> Pharmaceutical Dosage Forms.
Response: Comment incorporated.
Comment Summary #3: The commenter indicated that sprays are not in pressurized containers and that the list of dosage forms to which this General Chapter applied should reflect that fact.
Response: Comment incorporated.
Comment Summary #4: The commenter recommended that the General Chapter cover liquid dosage forms such as topical solutions, topical suspensions, and ophthalmic solutions.
Response: Comment not incorporated. The Expert Committee will consider this recommendation for future revisions to the General Chapter.

Procedures for Dosage Forms other than Aerosols:
Comment Summary #5: The commenter indicated that the Stage 1 acceptance criteria are based on the average amount and do not limit the number of containers that are less than the limit for the average amount.
Response: Comment incorporated.
Comment Summary #6: The commenter suggested that alternatives such as the use of a hydrometer be mentioned as a means to measure density when working with containers labeled by volume.
Response: Comment not incorporated. The procedure for measuring density in this section is only one of several methods. Other methods are recognized and the General Chapter text indicates that they may also be employed.
Comment Summary #7: The commenter indicates that the procedure for measuring density is not consistent in initially characterizing the diluent as a miscible liquid and later as water.
Response: Comment incorporated.

Procedure for Aerosols:
Comment Summary #8: The commenter recommended that the title of this section include sprays.
Response: Comment incorporated.

General Chapters/Section(s): <1025> Pancreatin
Expert Committee: Monographs – Biologics and Biotechnology 1
No. of Commenters: 1
Comment Summary: The commenter inquired whether the hog, *Sus scrofa* L. var. *domesticus* Gray (Fam. Suidae) includes sub-species of mediterranea.
Response: Comment not incorporated. The hog, *Sus scrofa* L. var. *domesticus* Gray (Fam. Suidae) does include sub-species of mediterranea.

General Chapter/Section(s):	<1132> Residual Host Cell Protein Measurement in Biopharmaceuticals/Multiple Sections
Expert Committee:	General Chapters—Biological Analysis
No. of Commenters:	12

General Comments

Comment Summary #1: The commenter suggested adding a new section covering the need for antibody excess and appropriate stoichiometry to host cell protein (HCP) assays.
Response: Comment not incorporated. The topic is already sufficiently explained in section 4.3 as well as in other sections of the General Chapter.
Comment Summary #2: The commenter requested more information on risk assessment that will persuade regulators to accept higher than average HCP values in specific cases.
Response: Comment not incorporated. It is beyond the scope of this General Chapter to address regulatory filing strategies.
Comment Summary #3: The commenter stated that residual HCPs may impact product shelf life through product aggregation and fragmentation would be helpful to add their effect on product shelf life too.
Response: Comment not incorporated. The topic is sufficiently covered in the *Introduction and Scope* of the general chapter. For example the sentence, “For example, proteolytic HCPs, even in minute quantities, can cleave the desired protein product over time, reducing or eliminating biological potency or bioavailability” explains the impact of HCPs.
Comment Summary #4: The commenter suggested conducting an industry survey of HCP specifications for early and late stage products.
Response: Comment not incorporated. This i suggestion is beyond the scope of the general chapter.
Comment Summary #5: Two commenters suggested streamlining the General Chapter in various ways.
Response: Comment not incorporated. The Expert Committee determined that the style suggestions from the commenters would not add value to the General Chapter.
Comment Summary #6: The commenter recommended categorizing quantitative criteria throughout the General Chapter as “general practice” but may not be acceptable to all regulatory bodies.
Response: Comment not incorporated. The General Chapter is informational (numbered above 1000; see *USP–NF General Notices*) and does not contain any mandatory requirements.
Comment Summary #7: The commenter requested more definitions covering immunological-based tests.
Response: Comment not incorporated. The definitions described in this General Chapter are suitable for the General Chapter scope. Other General Chapters covering immunological-based tests explain some of the other basic terms suggested by the commenter.
Comment Summary #8: The commenter requested more references to support certain claims, e.g., size, number, and molecular distribution of HCPs in a given CCF.
Response: Comment not incorporated. Figure 3 already provides support for these issues and it is not common to include multiple peer reviewed references in a USP General Chapter.
Comment Summary #9: The commenter stated that UV detection is not suitable for HCP monitoring.

Response: Comment not incorporated. The Expert Committee agrees with the commenter, but no action is needed because UV detection was not recommended and Table 7 already states the disadvantages of using UV with HPLC.

1. Introduction and Scope

Comment Summary #10: The commenter requested the following edits: "During the manufacture of such products, some amount of nonproduct, host cell-derived material will inevitably be introduced into the process stream ~~due to either cell lysis or secretion by the cells~~. This process results in a mixture of the desired product and host cell-derived impurities, including host cell proteins (HCPs), and other process-related impurities that will ~~ultimately~~ be ~~cleared or minimized~~ targeted for clearance through bioprocessing.".

Response: Comment incorporated.

Comment Summary #11: The commenter requested the following edit: "For example, proteolytic HCPs, even in minute quantities, can cleave the desired protein product over time, reducing or eliminating biological potency or altering ~~bioavailability~~ stability."

Response: Comment incorporated.

Comment Summary #12: The commenter requested clarification regarding the statement "4) in some cases, a considerable effect from sample dilution effects."

Response: Comment not incorporated. The topic is sufficiently explained and clarified in various sections of the General Chapter.

Comment Summary #13: The commenter recommended changing "effects" to "from matrix interference," in the same phrase mentioned above.

Response: Comment not incorporated. Not all dilution effects are due to matrix interferences.

Comment Summary #14: The commenter asked to add "5) inherent limitations to measure single HCP components." after the same phrase above.

Response: Comment incorporated.

Comment Summary #15: The commenter requested that values be added to the Figure 3 axes.

Response: Comment incorporated.

1.1 Considerations for Manufacturing, Characterization, and Consistency

Comment Summary #16: Two commenters suggested adding "HT-1080" and one requested the addition of "HEK293" to the list of mammalian cell examples.

Response: Comment not incorporated. The list is intended to show a few examples and is not meant to be comprehensive.

Comment Summary #17: The commenter requested correction of the word "Spodoptera."

Response: Comment incorporated.

Comment Summary #18: The commenter requested clarification of the molecular weight range cited for HCPs.

Response: Comment incorporated by the revision "from ~5 kDa to at least 250 kDa)".

Comment Summary #19: The commenter requested the following edits: "Some harvest operations also lyse cells ~~via shear stress~~; therefore, the resulting harvested CCF typically contains both secreted and intracellular HCPs. While this mixture of proteins incubates in the fermenter, additional changes in the HCP population may occur, for example, as the result of enzymatic activity (e.g., proteinases or sialidases).

Response: Comment incorporated.

2. Terminology

Comment Summary #20: The commenter suggested more information on the pros and cons regarding the process specific versus generic HCP reagents be added.

Response: Comment not incorporated. The topic is sufficiently explained and clarified in various sections of the General Chapter.

Comment Summary #21: Two commenters stated concerns that downstream process specific methods were not recommended and that because some proteins undergo purposeful post-translational modification it would be more appropriate to use a downstream process specific method.

Response: Comments incorporated. Text was added to the following statement, "except for certain products with exceptional downstream processing."

Comment Summary #22: The commenter requested the following edits, "To help establish a common nomenclature in the literature and with regulatory agencies. ~~Terminology for HCP-related assays and reagents has not been consistent in the literature; therefore~~ Table 1 lists common terms with their definitions (indicating how they are used in this General Chapter) in addition to synonyms that have been used historically. ~~Note that~~ the term..."

Response: Comment partially incorporated. The edits were made except for deletion of "Note that," because it was a style change.

Comment Summary #23: The commenter requested that the definitions for validation and qualification be included in the *Terminology Table*.

Response: Comment not incorporated. The scope of the Table is HCP-specific. Definitions for validation and qualification are available in other USP General Chapters and guidance documents.

Comment Summary #24: Two commenters suggested that clarification and editorial changes could be added to the Table's "coverage" definition since HCPs in the process feed stream may not be identical to those in the immunogen.

Response: Comments incorporated.

Comment Summary #25: The commenter stated that the upstream process specific definition is difficult to classify this way and said that both upstream and downstream could be included in a "process-specific assay".

Response: Comment incorporated by adding "generally" to the text: "This is generally before any purification and may be applied..." The Expert Committee acknowledges that users could also combine the two types and just call them "process-specific" but the differences between them were important to state.

Comment Summary #26: The commenter asked that "from serum" be removed from both affinity purification definitions.

Response: Comments incorporated.

Comment Summary #27: The commenter asked for deletion of "and is incapable of product expression" from the null cell definition.

Response: Comment incorporated.

Comment Summary #28: The commenter stated multiple reasons why use of a kit was suitable and can also sometimes have less risk than using in-house reagents and method development.

Response: Comment not incorporated. The Expert Committee stated that the General Chapter's risk based assessment on these topics are practical considerations and issues for any publically available commercial reagent and are not specific to HCP methods.
Comment Summary #29: The commenter asked for additional text in this section to clarify appropriate use of commercially available kits.
Response: Comment not incorporated. Sufficient text already exists in section 3.1 regarding the appropriate use of these kits.
Comment Summary #30: The commenter recommended adding text to allow for future assay technologies that may become suitable.
Response: Comment incorporated. Text was revised as follows, "Other immunoassay formats (e.g., competitive immunoassays) may or may not be suitable, because…"

3. HCP Immunoassay Methods
Comment Summary #31: The commenter recommended adding text to allow for future assay technologies that may become suitable.
Response: Comment incorporated by revising the text: "Other immunoassay formats (e.g., competitive immunoassays) may or may not be suitable, because…"
Comment Summary #32: Two commenters recommended edits to the detection methods suitable for the immunoassay format.
Response: Comments incorporated. Text was revised as follows, "The format that is most commonly used for HCP testing is the sandwich ~~ELISA~~ immunoassay with ~~colorimetric detection, but other~~ detection systems such as colorimetric ~~(e.g.,~~ electrochemiluminescent (ECL), chemiluminescent, radioactive, or ~~and~~ others~~) have been used successfully~~."
Comment Summary #33: The commenter recommended adding the underlined text to the sentence, "Homogeneous immunoassays, including competitive assays, where all of the reagents are combined at once and the binding occurs in a single step without washing, are rarely used for HCP assays unless similar sensitivity and specificity has been demonstrated."
Response: Comment partially incorporated. An alternative revision was made to make a similar point, "Homogeneous immunoassays, including competitive assays, where all of the reagents are combined at once and the binding occurs in a single step without washing, may be problematic due to antigen excess leading to antibody insufficiency issues discussed later in the General Chapter; therefore these formats should be used with caution."
Comment Summary #34: The commenter recommended adding the underlined text to the sentence, "The heterogeneous sandwich immunoassay format described in General Chapter <1103> is preferred, because the dynamic range and sensitivity are reduced in the homogeneous format. ~~it separates the captured HCP from the high concentration of product before exposure to detecting antibodies~~..."
Response: Comment incorporated. The word "generally" was added before the word "preferred".
Comment Summary #35: The commenter recommended adding the underlined text to the sentence: "Data analysis is typically performed with a nonlinear fit or linear fit in the linear range of the assay of the sigmoidal curve generated by…"."
Response: Comment not incorporated. The General Chapter does not forbid the use of a linear fit but because the best practice is to use a nonlinear fit this is what the General Chapter states. Furthermore, the sentence already uses the word "typically" to show that it is not the only option.

3.1 *The Assay Development Cycle*

Comment Summary #36: The commenter requested consistency in the use of the terms bridging and crossover.

Response: Comment incorporated. The text was revised to use the term "bridging" throughout the General Chapter.

Comment Summary #37: The commenter requested additional guidance on the crossover study.

Response: Comment not incorporated. This topic is sufficiently explained and clarified in various sections of the General Chapter.

Comment Summary #38: Three commenters focused on aspects of Figure 1, including: it should show that commercial assays could be used in any phase if sufficiently qualified through a comprehensive validation and that the text should emphasize these aspects for any assay approach; and to add the word "Platform Assay" in the empty box of Figure 1B for greater clarity.

Response: Comments partially incorporated. Figure 1a was modified to add a dotted box for commercial assays to be used in later phases too; however, the Expert Committee has determined that the text already sufficiently covers the points made by the commenter that any assay type must be validated and suitable for its intended purpose. "Platform Assay" was added to the empty box in Figure 1B.

Comment Summary #39: Regarding Figure 1A, the commenter requested a clarification to the text to indicate that only one assay is needed, not both platform and upstream-process specific assays.

Response: Comment not incorporated. The text does not indicate that both are needed; therefore, no change is required. The Figure shows examples of when changes may occur.

Comment Summary #40: The commenter requested definition of "qualification" as used in the sentence, "The platform assay should be qualified for each new product."

Response: Comment not incorporated. The topic is sufficiently explained and clarified in various sections of the General Chapter.

Comment Summary #41: The commenter stated that the point of the sentence, "Thus, process development becomes very restricted and involves the risk of needing to develop multiple HCP assays…" is an industrial issue and regulators are not usually sensitive to this argument.

Response: Comment not incorporated. This is a real issue not an opinion and the General Chapter endeavors to inform readers about such issues independently.

Comment Summary #42: The commenter indicated that they were not sure about point #3 and questioned whether it supported the need to pool materials from several runs.

Response: Comment not incorporated. There is no strong evidence for pooling; therefore, the text was not modified.

Comment Summary #43: The commenter thought that the statement: "For these reasons, "downstream, process specific" HCP assays are not recommended but can still be an option," was strongly worded.

Response: Comment incorporated. The sentence was deleted and modified accordingly.

3.2 Development and Characterization of HCP Reagents

Comment Summary #44: The commenter requested the following edit, "Because the HCP assay is used to test DS samples that contain trace HCP impurities, any cross-reactivity of the anti-HCP antibodies with the product may compromise the test method and yield ~~false-positive~~ biased results…"

Response: Comment incorporated.
Comment Summary #45: The commenter requested adding that levels of endotoxin in antigen should be tested before immunization.
Response: Comment not incorporated. This is a general section and not possible for *E. coli*-derived HCP antigens.
Comment Summary #46: The commenter requested deletion of the non-process specific material in the suggested antigen preparation section because Figure 2 suggests combining cell lysate with CCF and that this practice is not suitable for secreted products.
Response: Comment not incorporated. Figure 2 reflects the vast experience of the Expert Committee knowing that shear stress and other issues can lead to the presence of non-secreted, cellular material in the bioreactors. However, the text was modified per one of the commenter's points on this topic, "To prepare immunogen from cell lysate, the cells are harvested by centrifugation, and the cell pellets are washed and lysed (e.g., by using repeated cycles of freeze/thaw, or high pressure homogenization~~, or by sonication~~)."
Comment Summary #47: Regarding Figure 2, four commenters requested greater clarity of the figure and more guidance comparing mock HCP to CCF or adding a table.
Response: Comments not incorporated. The figure is clear but may vary depending on individual computer monitors and the additional points regarding the Figure are already explained within the text. The selection of mock material will vary from process to process and an example is provided. The text already sufficiently explains options as well as the importance of comparing the antigen profile to the production cell line CCF profile.
Comment Summary #48: The commenter requested clarification on whether the mock transfected cells used to prepare the HCP antigen should be pools or clonal.
Response: Comment incorporated. A new sentence was added, "Using pools of mock transfected null lines which have not been cloned allows for the maximum potential genetic diversity and therefore gives the highest potential of generating a broad host cell protein population."
Comment Summary #49: The commenter requested adding of the underlined text. "Once the null cells have been established, the HCP antigen is prepared in a bioreactor, <u>cell culture flask, or cell culture bag</u>."
Response: Comment incorporated. The following was also added to the end of the sentence, "reflective of the product or process cell culture conditions."
Comment Summary #50: The commenter indicated that it was not clear which concentration of CCF is best to use for filtration or to achieve afterwards.
Response: Comment not incorporated. This level of detail is beyond the scope of this General Chapter.
Comment Summary #51: Two commenters asked if the filter cut-off should be 5 kDa instead of 10 kDa or otherwise modified.
Response: Comments not incorporated. Many lower molecular weight proteins will be retained on the 10 kDa filters and if a 5 kDa filter is used it will immediately clog so use of the 10 kDa is a best practice, but still shown as an example.
Comment Summary #52: Two commenters asked if there are specific proteins for certain host cell types (e.g., chaperones in *E. coli* or particular yeast proteins) that either required separate assay considerations or possibly specifications.
Response: Comments not incorporated. The current text already covers the most important points for these cell systems.

Comment Summary #53: Three commenters requested a recommendation to check for pre-existing anti-product antibodies before starting immunization.
Response: Comments incorporated. The underlined text was added, “The presence of significant levels of pre-existing anti-HCP antibodies may confound the analysis of the antibody responses; therefore, it is important to screen preimmune sera from animals for pre-existing antibodies prior to immunization.”
Comment Summary #54: Two commenters requested that testing for absence of product be recommended, not required.
Response: Comments incorporated. The text was edited as follows, “Several analyses are ~~required~~ recommended before immunization.”
Comment Summary #55: Two commenters requested alternatives to the list of total protein methods described and perhaps more advice on amino acid analysis.
Response: Comments partially incorporated. A sentence was added after the existing list of total protein examples, “Absorbance at 280 nm (A280) may also be used although it is less specific for protein (e.g., nucleic acids will also be measured).” General guidance on amino acid analysis is beyond the scope of this General Chapter and General Chapter <1052> *Biotechnology Derived Articles Amino Acid Analysis* already exists for further information on this topic.
Comment Summary #56: The commenter requested additional information on curve fit algorithms.
Response: Comment not incorporated. This level of detail is beyond the scope of this General Chapter. See also response to comment #34 for additional discussion.
Comment Summary #57: Two commenters requested more detail regarding the use of the product as a control.
Response: Comment incorporated. The following text was added, “Appropriate controls within the assay range may be established to monitor assay performance. Controls may be prepared from independent dilutions of the HCP standard, product samples or intermediate pools, or spiked product samples.” In addition, the word “samples” was added after control in the next sentence.
Comment Summary #58: The commenter requested addition of “C” after each degree in the General Chapter.
Response: Comment not incorporated. Per *General Notices* 8.180, all temperatures are expressed in centigrade (Celsius) degrees unless otherwise indicated. It is not USP style to show the “C.”
Comment Summary #59: The commenter requested the following text changes, "To mitigate the risk, data from several standard curve parameters are often assessed for each assay (e.g., slope, r2) and used to support HCP standard stability. Alternatively, material from a separate null cell production run, or an in-process upstream sample (for example, after column 1), can be used to prepare controls for the assay. Using material different from the reference to prepare controls will help ensure that the degradation rates will be independent ~~different~~, and HCP standard degradation can be detected easily.
Response: Comment partially incorporated. Additional edits were made to the first sentence to add more curve parameters, "To mitigate the risk, data from several standard curve parameters (e.g., signal, background, slope, coefficient of determination) are often assessed for each assay and used to support HCP standard stability.”

Comment Summary #60: The commenter requested addition of cascade immunization as a possible strategy.
Response: Comment incorporated. The sentence: "Other immunization strategies may also be helpful (e.g., cascade immunization or size fractionation of the HCP immunogen)" was added.
Comment Summary #61: The commenter requested the following text change: "In ~~rare~~ some cases, multiple animal species have been used."
Response: Comment incorporated.
Comment Summary #62: The commenter requested the following text change: "In contrast, if larger animals (goats or sheep) are used, investigators usually will immunize 3 ~~5~~–10 animals per protocol."
Response: Comment incorporated.
Comment Summary #63: The commenter requested the following text change, "A portion of the prepared HCP antigen is mixed with an appropriate adjuvant (most commonly, Freund's adjuvant or in combination with incomplete adjuvant) and used for the immunization of animals." The commenter also suggested text regarding animal treatment.
Response: Comment incorporated by the text change but the additional text on animal treatment is beyond the scope of this General Chapter.
Comment Summary #64: The commenter stated that it would be a lot of work to test individual bleeds by both titer and Western blot analyses; therefore, asked for an edit to this sentence.
Response: Comment not incorporated. The sentence already states, "perform titer or Western blot analyses." It does not say both. Consequently no change was made.
Comment Summary #65: The commenter asked for guidance on storage of neat sera.
Response: Comment incorporated. The underlined text was added: "…and stored frozen (typically -70^{o} or colder). Neat antisera should also be stored frozen" (see <1106>*Immunogenicity Assays-Design and Validation of Immunoassays to Detect Anti-Drug Antibodies* for additional information regarding storage of serum samples).
Comment Summary #66: The commenter requested additional guidance for resin storage and regeneration.
Response: Comment not incorporated. General chromatography guidance is beyond the scope of this General Chapter.
Comment Summary #67: The commenter suggested that the information in Table 2 is redundant and suggested editing the text.
Response: Comment not incorporated. There is some overlap but there are also unique points and the Expert Committee has determined it is suitably written.
Comment Summary #68: The commenter suggested the following edits, "The concentration of the unmodified antibody is determined most commonly by absorbance at 280 nm, Bradford~~AAA~~, or BCA. Each approach is acceptable, provided it is applied consistently and (if colorimetric) is standardized similarly. It is important to determine ~~match~~ the concentration of the capture and detection antibodies in each batch because they will be diluted to a certain concentration in the optimized immunoassay method."
Response: Comment partially incorporated. The Expert Committee does not agree that Bradford is a more common or preferable example to amino acid analysis but did agree to use the word "determine."
Comment Summary #69: One commenter suggested adding guidance on label interference with protein determination methods and another commenter did not understand how to quantitate the secondary antibody.

Response: Comments not incorporated. Suggestions for protein determination are already provided and users should select their particular method based on any expected interference. General guidance on total protein methods already exist in General Chapters <1057> *Biotechnology-Derived Articles-Total Protein Assay* and <1052> *Biotechnology-Derived Articles-Amino Acid Analysis.*

Comment Summary #70: The commenter asked to simplify the text, "Show that the antibody pairs are specific and sensitive in immunoassay format for the HCPs present…... because it is also possible that the purification process is not effective" by focusing on test evaluation.

Response: Comment not incorporated. Many suitable options are presented and evaluations of samples from the process train can also be informative.

Comment Summary #71: The commenter requested inclusion of difference gel electrophoresis (DIGE) as a possible approach.

Response: Comment incorporated. The underlined text was added, "For the purpose of sample comparison, difference gel electrophoresis (DIGE) technology could be helpful" to the section discussing affinity purification. The rest of the text is already written generally for any 2-D format.

Comment Summary #72: The commenter requested addition of the underlined text, "Coverage is evaluated using at least the capture antibodies using 2-D gel Western blots or immunoaffinity fractionation to the total HCP population by immobilizing the anti-HCP antibodies on a column. In addition it is recommended to test the coverage of the detection antibody, especially if different coating and detection antibodies are used."

Response: Comment incorporated. The word "especially" was deleted.

Comment Summary #73: Two commenters questioned the use of the SPR technique to characterize antibodies and whether it was measuring affinity or avidity.

Response: Comments incorporated. The SPR text in this section was deleted.

Comment Summary #74: The commenter requested clarification of the two methods cited in the sentence "Two methods are in fairly common usage."

Response: Comments incorporated. The underlined text was added, "Two methods (2-D gels followed by Western blot analysis and immunoaffinity purification followed by 2-D gel analysis) are in fairly…"

Comment Summary #75: The commenter asked if it was helpful to also run nonreduced 2-D gels for coverage evaluation.

Response: Comment not incorporated. Non-reduced gels for this purpose are not helpful or common.

Comment Summary #76: The commenter asked if it was helpful to add values for acceptable coverage levels.

Response: Comment not incorporated. An acceptable gel with blot are shown, but numerical values were purposely omitted, because they are not reproducible.

Comment Summary #77: Three commenters requested allowing use of a resin rather than only a column and also 1-D SDS-PAGE gels, with addition of the underlined text, "The second approach, immunoaffinity binding/SDS-PAGE, involves comparing the eluate or the flow-through to the load from the HCP calibration standard (or early process sample) passed over a column or mixed with a resin to which the capture antibodies have been covalently immobilized."

Response: Comment incorporated but with an edit to the suggestion "…involves comparing the flow through and eluate to the load…" and said "resin" rather than "mixed with a resin." Table 3 was also modified to include "resin" and "1-D."

Comment Summary #78: The commenter requested the following edit in Table 3, “Transfer efficiency of a broad range of HCPs difficult to optimize, leading to under- ~~and/or over-~~estimates due to…”
Response: Comment incorporated.
Comment Summary #79: The commenter indicated that Table 3 was biased and not useful.
Response: Comment not incorporated. The Expert Committee determined that the information is helpful.
Comment Summary #80: The commenter requested a higher quality Figure 3 containing pI and molecular weight labels.
Response: Comment incorporated. The figure legend was also edited accordingly.
Comment Summary #81: The commenter recommended that multiple animals and species be immunized in parallel to produce the best antibody reagents.
Response: Comment not incorporated. Multiple options for antibody are already presented and the Expert Committee determined that this addition is not justified.
Comment Summary #82: The commenter recommended avoiding generating HCP antigens and antibodies at the same time.
Response: Comment partially incorporated. The word “typically” was changed to “often” rather than dictating the timing of reagent generation.
Comment Summary #83: The commenter requested a revision of the replacement reagent text “…and a demonstration that they can detect similar or ~~more sensitively~~ HCP log reduction, from harvest to DS...”
Response: Comment partially incorporated. The text was revised as follows, “…and ~~a~~ demonstrating ~~on~~ that they can detect similar or ~~more sensitively~~ greater HCP log reduction, from harvest to DS...”
Comment Summary #84: The commenter requested the following revision, “In cases where better antibody response is desired, antibodies produced by different species may be ~~needed~~ evaluated. The selection of the species should take into account all the points discussed in this General Chapter. ~~In those cases,~~ A side-to-side comparison of results ~~from process samples using old and new antibodies in a sandwich immunoassay format~~ ~~is highly recommended~~ should be included ~~to demonstrate suitability of the replacement antibodies~~, in particular when changing antibodies from one species to another for an established test.”
Response: Comment partially incorporated. The text was revised as follows, “In cases in which better antibody response is desired, antibodies produced by different species may be evaluated. The selection of the species should take into account all the points discussed in this General Chapter. A side-by-side comparison of results from process samples using old and new antibodies in a sandwich immunoassay format should be included to demonstrate suitability of the replacement antibodies.”
Comment Summary #85: The commenter requested clarification of the statement, “Assay qualification or validation should also be performed,” because this may not always be necessary.
Response: Comment incorporated. The text was revised as follows, “Assay qualification or validation should also be considered after changing reagents ~~performed~~.”
Comment Summary #86: Two commenters requested clarification of the sentence, “The new assay should demonstrate good performance and similar or better log reduction of HCP, compared with the current process.”
Response: Comment incorporated. The sentence was deleted. Also, note the response to comment summary #83.

3.3 Immunoassay Method Development and Qualifying as Fit for Use

Comment Summary #87: The commenter requested addition of the underlined text, "Excess unconjugated reagents should be removed, either by dialysis, affinity purification, or by using a suitable desalting column."

Response: Comment incorporated.

Comment Summary #88: Two commenters requested revisions regarding the sentences that pertain to focusing on the low calibration standard.

Response: Comments partially incorporated by these minor edits, "Typically, HCP immunoassays do not always possess a full dose-response curve with both asymptotes; therefore, for the purposes of residual HCP detection, the assay for DS release should focus on the low end of the curve ~~near the quantitation limit (QL)~~." Readers should focus on the low end of the curve per ICH guidance for impurity methods.

Comment Summary #89: The commenter asked why a mock run was required and if a product-depleted production supernatant could be used instead for point 1 of qualifying a new product with a platform assay.

Response: Comment not incorporated. This approach is one of several suggestions, and is not a requirement.

Comment Summary #90: The commenter requested relevant criteria for the statement, "…the appearance of a few new spots…is not a basis for invalidating the application of the platform assay."

Response: Comment not incorporated. It is impossible to give specific criteria that would fit all cases.

Comment Summary #91: Three commenters requested clarification of the text "In general, results within a factor of 2 are considered similar," because it is not clear if that is acceptable to all regulators and to specify that this is in reference to the ELISA/immunoassay.

Response: Comments incorporated. The following edits were made, "In general, immunoassay results within a factor of 2 are often considered similar."

Comment Summary #92: Two commenters stated concerns with approach 4.

Response: Comments not incorporated. Approach 4 is only one of several options available to users.

4. HCP Immunoassay Method Validation

Comment Summary #93: Three commenters recommended general structural changes to the section by adding more about what is unique to HCP assays, and that more information on precision and robustness be added to this section.

Response: Comments not incorporated. This section focuses on validation aspects that are more challenging for HCP procedures and the Expert Committee has determined the overall content and structure are suitable.

Comment Summary #94: One commenter recommended the following text revision, "All validation parameters for a quantitative impurity test are needed when used for the final DS, whereas validation for in-process samples usually focuses on dilution linearity, ~~interference~~ selectivity, and precision. Whether the assay is an in-process assay or a ~~C of A~~ release testing assay (DS), both alert ~~(trend)~~ and reject limits…." Another commenter asked for more guidance on alert/action/trend limits.

Response: Comments partially incorporated. The following change was made, “All validation parameters for a quantitative impurity test are needed when used for the final DS, whereas validation for in-process samples usually focuses on dilution linearity, interference, and precision. Regardless of whether the assay is an in-process assay or a ~~C of A~~ release testing assay (DS), action ~~both alert (trend) and reject~~ limits…” (interference was preferred over selectivity and the existing text on limits is suitable for the scope of the General Chapter).
Comment Summary #95: The commenter requested the total number of sample dilutions recommended for the ELISA for release testing.
Response: Comment not incorporated. This is too specific and will vary depending on the samples and process.
Comment Summary #96: Three commenters requested clarification of the acceptable spike recovery in the accuracy section.
Response: Comments not incorporated. Typical ranges are given and where they differ are due to specific issues (e.g., near the QL the range is widened to 50-200% often) and again these are not intended as requirements but just common practice and suggestions. The text was also clarified as follows: “Spikes near the quantitation limit (QL) help to evaluate the accuracy and repeatability of the assay near the QL, which is where the measurement is often the most variable. A spike recovery of 50-200% may be acceptable for a spike at or near the QL.”
Comment Summary #97: The commenter recommended deleting the sentence, “To accomplish that, if the HCP is present in antigen excess…”
Response: Comment partially incorporated by the following clarification, “Although these are minimum requirements for assay validation, they should not be interpreted as demonstrating accuracy for ~~the specific~~ any one specific HCP that may co-purify with the product. To accomplish that, ~~if the HCP is present in antigen excess in the assay,~~ comparison to a standard of that particular HCP species is needed; however, because this HCP is rarely known, this may not be ~~it is usually not~~ possible.”
Comment Summary #98: The commenter requested adding a high dose hook effect test within the validation section.
Response: Comment not incorporated. This is usually a sample specific issue and the suggested spike experiment will not necessarily reflect that seen in a given sample; therefore, the General Chapter recommends multiple sample dilutions to uncover any unique antigen excess issues.
Comment Summary #99: The commenter asked if reporting could continue to be in ppm.
Response: Comment not incorporated. The terminology section already covered this topic and the General Chapter use of ng/mg is a recommendation not a requirement.
Comment Summary #100: Eight commenters asked for clarification of the second part of the QL determination.
Response: Comments incorporated. The following text edits were suggested by one of the commenters, “~~If the DS sample does not have detectable HCP, it can be used for the second part of the QL determination; otherwise, a formulation buffer may be used. The product MRD (or the formulation by itself, if necessary) is used as the first dilution in a series to which increasingly lower concentrations of HCP are spiked (e.g., six replicates at each of these levels: 1, 2, 5, and 10 ng/mL). This study is typically repeated at least three times, preferably by different analysts or on different days. The HCP spike level that can be recovered in all experiments (e.g., within 70%–130% or 50%–150%) in the majority of tests (e.g., four of six), divided by the protein concentration, is confirmed as the QL.~~ For the second part of the QL determination, the DS is

tested at the MRD (if the DS sample has high levels of detectable HCP, a formulation buffer may be used). The QL is generally determined by the analysis of spiked concentrations of HCP and by establishing the minimum level at which the HCP can be determined with acceptable accuracy and precision. This study is typically performed at least 3 times, preferably by different analysts or on different days. For example, a spike of 3 ng/mL of HCP in a product protein concentration of 5 mg/mL has a QL of 0.6 ng/mg or 3 ng/mL if spike recovery is achieved in, e.g., at least four of six tests or if mean spike recovery criteria are met. Typically, assays are set up to ~~cover~~ measure the range from a few ng/mL to >100,000 ng/mL." Additional information for QL (the ICH term, so will not be changed to LOQ) can be found in ICH guidances and General Chapter <1225>. In addition, General Chapter <1132> contains best practices, examples, and guidance, not requirements.

Comment Summary #101: Four commenters requested changes to the paragraph beginning with "For routine DS testing…"

Response: Comment incorporated. The text was edited as follows: "For routine commercial DS ~~testing~~ manufacture, where the protein product concentration is known and the process impurities are well understood, testing at a single dilution ~~(>MRD), which produces results not lower than the QL (in ng/mL), is typically~~ may be used for release testing. ~~By fixing the protein product concentration,~~ The results are reported ~~in consistent units (ng/mg)~~ as the ratio of measured HCP (ng/mL) to the product concentration (mg/mL) resulting in units of (ng/mg). ~~This is helpful~~ When the DS has undetectable levels, ~~therefore, results "less than" are consistent~~ the results are reported as "less than" the assay QL (ng/mL) divided by the product concentration (mg/mL) (e.g., <0.6 ng/mg in the example above). Before setting this target concentration for testing, however, the dilution linearity of the samples should be well understood ~~in a development study~~, and a robust manufacturing process established. In the event that the level or species of HCPs vary run-to-run it may be necessary to test each sample at multiple dilutions (see below)."

Comment Summary #102: The commenter stated that the *Linearity* section was sample linearity not linearity of the assay.

Response: Comments incorporated. The section title to "4.3 *Sample Linearity*" was changed. Linearity of the assay is already covered in the first paragraph of Section 4.

Comment Summary #103: The commenter suggested that non-specific adsorption of HCPs to the tubes could also lead to lack of dilution linearity and this concept should be added to the General Chapter.

Response: Comment not incorporated. HCP samples generally have a high overall protein concentration. The most common causes are mentioned in the general chapter.

Comment Summary #104: The commenter referred to the text "…multiple batches of a given sample type (e.g., from several clinical DS lots or process validation batches)…" and stated that they focus on matrix and spike recovery, not sample dilutions.

Response: Comment not incorporated. Sample linearity is important to demonstrate in most cases, particularly for the early phase type of samples mentioned in this part of the text.

Comment Summary #105: The commenter referred to the text, "In this example, all were diluted initially to 10 mg/mL (the MRD, where…" and stated that in this case the MRD for each sample has to be redetermined and not all the dilutions in the example meet the MRD criteria.

Response: Comment not incorporated. Matrix interference is not the only source of nonlinearity which is why these recommendations are suitable as written.

Comment Summary #106: Two commenters requested revision of the NOTE regarding sample dilutions.
Response: Comments incorporated. The following revision was made, "NOTE- If large sample dilutions are required to get into the range of the HCP assay, consider making intermediate dilutions to limit dilution-related errors."
Comment Summary #107: The commenter requested disclosure of the product and samples types found in Table 4.
Response: Comment not incorporated. The Expert Committee determined that Information is not needed for this purpose.
Comment Summary #108: Four commenters requested greater clarity of the Table 4 examples and also a third potential worst-case guide.
Response: Comments incorporated. Table 4 was modified and a third guide to the Table 4 footnote was added. In addition, the following was added to the text: "In contrast, the third potential guide, which reports the highest value measured above the QL, would yield results that are at least 10% higher. In addition, the validity of the third guide would depend highly on the quality of the method development."
Comment Summary #109: The commenter requested the following revision, "Another specificity issue to evaluate is the potential cross-reactivity of the anti-HCP antibodies with the product itself. ~~because the product will be present at 10,000-fold to 1-million-fold more than the HCPs to be quantified, increasing the likelihood that a homologous epitope might be recognized by the antibodies."~~
Response: Comment incorporated.
Comment Summary #110: The commenter requested the following revision: "If bands are detected that are unrelated to the product ~~(e.g., light and heavy IgG chains of monoclonal antibodies seen by reduced SDS-PAGE)~~, this suggests that cross-reactivity is not occurring, and further process development may be required if lower HCP levels are sought."
Response: Comment incorporated.
Comment Summary #111: The commenter indicated that Western blot results can also be dependent on Western blot conditions.
Response: Comment not incorporated. This information is sufficiently covered in the text of the general chapter.

5. Supporting Technologies for Residual HCP Detection, Identification, and Measurement
Comment Summary #112: The commenter suggested adding that LC-MS/MS detects individual HCPs whereas an immunoassay detects total HCP and these approaches are complementary.
Response: Comment not incorporated. The General Chapter already contains sufficient text on this concept but, per the comment, did revise the following to add clarity: "Mass spectrometric techniques for the detection, identification, and quantitation of individual HCPs are rapidly…"
Comment Summary #113: The commenter stated that electrophoretic separation methods are not acceptable or suitable orthogonal methods for detecting HCP in DS samples and lack sufficient sensitivity.
Response: Comment not incorporated. The General Chapter does not recommend them as an alternative to immunoassays. Separation methods using sensitive detection systems can provide additional information.
Comment Summary #114: The commenter requested a correction to the word "proteolytic".

Response: Comment incorporated.
Comment Summary #115: Three commenters suggested changes to Table 5 regarding the amount of protein loaded and the type of stain used (one preferred Coomassie).
Response: Comments partially incorporated. The values for loading were deleted. The stain was not changed, because the Expert Committee does not agree that Coomassie is currently the best choice when more sensitive stains exist.
Comment Summary #116: The commenter requested changing "coulombic heating" to "joule heating".
Response: Comment not incorporated. The text was simplified to "over-heating" to accomplish the same purpose.
Comment Summary #117: The commenter requested the text edit, "A related issue is standardization of the densitometer or imager, which must be…"
Response: Comment incorporated. The text "and calibration" was added after the word "standardization."
Comment Summary #118: The commenter requested a clarification to Table 6 that Western blots used for ID release testing are not likely appropriate for purity determination
Response: Comment not incorporated. The General Chapter sufficiently states that Western blot is not recommended as a release test and provides supportive information.
Comment Summary #119: The commenter stated that there are other immunoassay formats beyond those mentioned in the General Chapter, as well as LC-MS and asked if regulators have accepted such submissions.
Response: Comment not incorporated. This topic is not within the scope of the General Chapter.
Comment Summary #120: Three commenters suggested revisions to the mass spectrometric text.
Response: Comments incorporated. The suggestions were adapted with the following revision, "These methods (see also proposed General Chapter <1736> Applications of Mass Spectrometry for additional information) often combine sample preparation such as reduction, alkylation, and proteolytic digestion, followed by separation (e.g. reversed-phase chromatography [RP-HPLC ~~or RP-UHPLC...~~]) before introduction into a mass spectrometer that fragments all proteins thus providing an amino acid sequence for each peptide. The resulting sequence information is compared to the product sequence to identify product-related fragments and to a database related to the host (e.g., E. coli, CHO) sequences to identify HCPs. A challenge for MS analysis stems from the overwhelming number of product-derived peptides relative to impurity peptides. One approach to address this issue of competitive ionization of the peptides (also called ion suppression of the HCP peptides by the product peptides), is to apply LC-MS/MS analysis on partially resolved HCP preparations (e.g. HPLC fractions). This purification reduces the product contribution to total ions in the mass spectrometer. ~~For example, bands cut from 1-D gels can be selected to avoid gel regions that are overloaded in product.~~ Other approaches are in development. Recent technological improvements, such as (1) chromatography resins able to resolve effectively using MS-friendly mobile phases, (2) improved interfaces to front end LC and/or IEF separation systems, and (3) mass spectrometers with higher mass resolution, accuracy and faster scan rates, now make it possible to identify and quantify specific HCPs in DS with a high degree of confidence. Major challenges in terms of sensitivity and quantitation of sufficiently large sets of heterogeneous HCPs, cost, and QC-related issues remain to be met before this technology can replace immunoassays for the

control of HCPs in DS. As a characterization method orthogonal to the HCP immunoassay, LC-MS/MS data may be used in two ways: First, if the MS-based method does not find HCPs in samples that were also below QL in the immunoassay, then these orthogonal techniques can increase confidence that the HCP ELISA did not miss an HCP that has co-purified. The detection limit for many LC-MS/MS methods is currently in the range of 10-100 ng of HCP per mg of product. It is therefore sufficiently sensitive to rule out a single HCP being present at a high level and is more sensitive than gels ~~stained with sensitive fluorescent dyes~~." In addition and in support of this revision, the phrase "(or better may be possible)" was added to the MS-sensitivity cell of Table 7.

5.4 *Concluding Remarks on Supporting Technologies for HCPs*

Comment Summary #122: The commenter stated that the General Chapter should emphasize coverage assessment of polyclonal antibodies not DS characterization.

Response: Comment not incorporated. The topic is sufficiently covered throughout the General Chapter.

Comment Summary #123: The commenter requested the following revision, "One example is fractionation of HCP standards by ion exchange, followed by RP chromatography, and then an immunoassay or MS-analysis (2-D MS) on the proteins in the final fractions. Alternatively, "product subtraction" has been evaluated to remove the bulk of the total protein from final product samples before gel electrophoresis or ~~of~~ LC-MS/MS analysis."

Response: Comment incorporated.

6. Use of HCP Immunoassays for Process Development, Characterization, and Validation

Comment Summary #124: The commenter requested a correction to the space and comma in the first sentence of the section.

Response: Comment incorporated.

Comment Summary #125: The commenter stated that interference due to matrix or DS can also be a reason regarding the text, "The product concentration in the assay may be increased with each purification step, and thus there is a similar increase in the concentration of the co-purifying HCP impurity. By column 3, the amount of the impurity likely exceeds the capacity of the available antibodies."

Response: Comment not incorporated. This concept is already sufficiently covered elsewhere in the General Chapter and the text also says "likely" implying other sources.

6.1 Assays for Individual HCPs

Comment Summary #126: The commenter requested additional guidance on assays for individual HCPs.

Response: Comment not incorporated. These are standard immunoassays and are beyond the scope of this General Chapter. General immunoassay guidance can also be found in General Chapters <1102> *Immunological Test Methods—General Considerations* and <1103> *Immunological Test Methods—Enzyme-Linked Immunosorbent Assay (*ELISA).

6.3 Control Strategy

Comment Summary #127: The commenter asked if a target of 100 ppm is well accepted by regulators.

Response: Comment not incorporated. Although this value is common for starting purification goals the value can ultimately vary a lot and must be as low as achievable; therefore, it is not appropriate to place the value in this General Chapter.
Comment Summary #128: The commenter requested deletion of the sentence, "During product development, knowledge about the process and the product increases; therefore, it is expected that reject limits can be tightened as one moves from toxicology materials to phase I/II material, to phase II, and finally to commercial material."
Response: Comment not incorporated. This is best practice and expected as product and process knowledge increases therefore the sentence is not deleted.
Comment Summary #129: The commenter requested the following revision, "….acceptable levels ~~are based on experience gathered during the clinical trials~~ for early stage products should be determined through a risk assessment (including non-clinical data, available literature, previous experience with products manufactured using the same or a similar cell line, etc). For commercial products, the acceptable levels are also based on the experience gathered during the clinical trials."
Response: Comment incorporated.
Comment Summary #130: The commenter requested the following revision, "With the emergence of orthogonal measures of purity, ~~any significant signal observed that is not product related is to be identified~~ any non-product related atypical signals should be evaluated. Efforts should be made to revise the purification process to remove ~~the~~ any unwanted HCPs present at higher than desirable levels (based on clinical and non-clinical data, available literature, previous experience with products manufactured using the same or a similar cell line, etc)…."
Response: Comment incorporated. The text "for example" was added before "clinical" and the examples shortened, because the list was almost identical to the text above.
Comment Summary #131: The commenter suggested adding a list of USP references.
Response: Comment not incorporated. The other USP General Chapters cited are within the same compendium, sufficiently cited and linked (online) within the text, and it is not USP style to list them at the end again.
Expert Committee-initiated Change #1: The following edit was made, "Because of the complexity of HCP immunoassays, careful development and characterization of critical reagents are required, particularly for the immunogen used ~~required~~ to elicit the anti-HCP antibodies…" within section 1.
Expert Committee-initiated Change #2: The following edit was made, "In the case of platform HCP assays, the antibodies to HCP are obtained from animals immunized with HCP antigens generated from a common upstream process that is applicable to many products, even if the downstream purifications are ~~is~~ different." within section 2.
Expert Committee-initiated Change #3: The following edit was made, "Commercially available" assays produced by vendors are often derived from a combination of strains and harvest/purification procedures, and these assays are intended to have a broad application; but these commercially available assays are not specifically designed for ~~as well matched to~~ a given manufacturer's proprietary cell line,…" within section 2.
Expert Committee-initiated Change #4: The following edit was made, "This assay format offers a combination of high sensitivity, specificity, throughput, automation potential, rapid turnaround, ~~a~~ quantitative results ~~readout,~~ and low cost per assay that is unmatched by any other currently available assay technology." within section 3.

Expert Committee-initiated Change #5: The following edit was made, “For these reasons, orthogonal measures of product purity are often ~~also~~ needed.” within section 3.
Expert Committee-initiated Change #6: The following edit was made, “An additional consideration ~~risk~~ is that the reagents are…” within section 3.1.
Expert Committee-initiated Change #7: The following edit was made, “…in practice the bacterial HCP antigen is usually generated from the lysates of washed cells ~~washed previously~~, using null cell fermentations…” within section 3.2.1.2.
Expert Committee-initiated Change #8: The following edit was made, “Lastly, the 1-D and 2-D gels help characterize the pattern of HCPs, show that a broad spectrum of proteins is ~~are~~ present…” within section 3.2.1.3.
Expert Committee-initiated Change #9: The following edit was made, “The following approaches and concepts may be useful in ~~qualifying a new product for a platform HCP assay or a new HCP standard with pre-existing antibodies~~ determining if a platform assay is suitable for a product made with a new process:” within section 3.3.1.
Expert Committee-initiated Change #10: The following edit was made, “This is the biggest problem with these very specialized assays and why platform-based assays are usually preferred, because they often do not…” in section 3.3.2.
Expert Committee-initiated Change #11: The following edit was made, “As with the electrophoretic methods discussed in this General Chapter, reversed-phase separation of proteolytically cleaved product samples with UV detection may already be included in a GMP control system as ~~a control for~~ part of product ~~integrity~~ testing.” within section 5.3.
Expert Committee-initiated Change #12: The following edit was made, “In cases where the process is shown to clear HCPs robustly, process validation may also be used to justify not having ~~the~~ a routine HCP test ~~ELISA~~ as part of the cGMP control system.” within section 6.
Expert Committee-initiated Change #13: The following sentence was made to Figure legends 5 and 6 for greater clarity, “Within a sample set, the first value is the most dilute in its dilution series.”
Expert Committee-initiated Change #14: The following edit was made, “Ideally, changes in HCP level and thus clinical exposure...” within section 6.2.1.
Expert Committee-initiated Change #15: The following edit was made, “Some proteins may not induce an immune response in the animal species used to generate antibodies, and the HCP immunoassay will be blind to those proteins.” within section 7.

General Chapter/Section(s):	<1223> Validation of Alternative Microbiological Methods
Expert Committee:	General Chapters—Microbiology
No. of Commenters:	6

Comment Summary #1: The commenter suggested adding a paragraph in the *Introduction* section that highlights the important contents of the General Chapter.
Response: Comment incorporated.
Comment Summary #2: The commenter suggested adding text to indicate a) that microbial identification is not covered by this General Chapter and b) a reference to General Chapter <1113> *Microbial Identification, Characterization and Strain Typing*.
Response: Comment incorporated.

Comment Summary #3: The commenter suggested adding a reference to support the statement that traditional plate count methods can only recover or detect 0.1 to 1% of Microbial Cells present in comparison to Flow Cytometry.
Response: Comment incorporated.
Comment Summary #4: The commenter suggested replacing the terms “Modern Molecular Methods and Molecular Biochemical Methods” with the generic term “Alternative Microbiological Methods.”
Response: Comment incorporated.
Comment Summary #5: The commenter suggested clarifying the statement that indicates a higher cell count obtained by an alternate method that utilizes a signal other than cfu does not translate to a higher risk to the end user.
Response: Comment incorporated.
Comment Summary #6: The commenter suggested clarifying the statement that classical growth based microbiology methods constitutes logarithmic science.
Response: Comment incorporated.
Comment Summary #7: The commenter asked about the relevance of the sub-section on *Submission of Alternative Procedures to* USP and whether it should be deleted since it is covered in other parts of *USP–NF*.
Response: Comment not incorporated. The Expert Committee has determined that this information should be reinforced to encourage submission of validated alternate procedures.
Comment Summary #8: The commenter suggested replacing the reference to <111> *Design and Analysis of Biological Assays* with <1034> *Analysis of Biological Assays*.
Response: Comment incorporated.
Comment Summary #9: The commenter suggested adding text that clarifies the purpose of the User Requirements Specification (URS).
Response: Comment incorporated.
Comment Summary #10: The commenter suggested replacing the section with a reference to *PDA Technical Report* #33.
Response: Comment not incorporated. The Expert Committee has determined that *PDA Technical* Report #33 is not a public standard and a standalone subsection on URS within General Chapter <1223> is warranted.
Comment Summary #11: The commenter recommended adding text that clarifies the statement which suggests that if a prior submission from a company on an alternate method to the regulatory agency exists then mere confirmation of the performance in the new use is needed.
Response: Comment incorporated
Comment Summary #12: The commenter recommended clarifying the statement that use of alternate sample size needs justification.
Response: Comment incorporated
Comment Summary #13: The commenter suggested adding text that supports the statement that the cfu signal is completely dependent on the growth or recovery of microorganisms.
Response: Comment incorporated.
Comment Summary #14: The commenter suggested replacing the term “refereed scientific publication” with the term “peer reviewed scientific publication” in the *Signals from Alternate Microbiological Methods* section.
Response: Comment incorporated.

Comment Summary #15: The commenter suggested replacing the term “genetic analytical methods” with the term “nucleic acid-based methods.”
Response: Comment incorporated.
Comment Summary #16: The commenter suggested revising of text in the sub-section *Success Criteria* that clarifies the statement that it is generally possible to correlate cell counts obtained by the traditional and alternative methods.
Response: Comment incorporated.
Comment Summary #17: The commenter suggested clarifying situations in the sub-section on Success Criteria in which alternative methods use a combination of traditional methods (compendial) with new instrumental detection (alternative) methods, like digital imaging, only the detection (alternative) method needs to be validated.
Response: Comment incorporated.
Comment Summary #18: The commenter suggested revising the text in the sub-section on *Sample Size* that clarifies that one may use any sample size and number of tests sufficient to produce an equivalent decision.
Response: Comment incorporated.
Comment Summary #19: The commenter suggested that in the sub-section on *Statistics* and *Alternate Methods*, revision of text that indicates use of statistics to compare signals from classical and alternate methods is probably of limited value and should be deleted.
Response: Comment incorporated.
Comment Summary #20: The commenter suggested that in the sub-section on *Validation Criteria* all parameters listed in the table should be defined and detailed in the same order
Response: Comment incorporated
Comment Summary #21: The commenter suggested clarifying the text in the sub-section on *Validation Criteria* that indicates only the accuracy and precision validation parameters are required for quantitative methods and the accuracy parameter for qualitative methods.
Response: Comment incorporated.
Comment Summary #22: The commenter suggested that in the sub-section on *Validation Criteria,* addition of text that clarifies after an alternative method has been shown to be equivalent to the compendial test with one product, it is not necessary to repeat the equivalency parameters for every new product.
Response: Comment incorporated.
Comment Summary #23: The commenter suggested that in the sub-section *Validation Criteria*, the sentence “all microorganisms should be recovered and identified” should be modified. Not all new methods allow for the identification of the microorganism detected, mainly when the methods are presence/absence methods.
Response: Comment incorporated.
Comment Summary #24: The commenter suggested that in the sub-section *Validation Criteria*, clarity is needed on what to do with MPN results.
Response: Comment incorporated.
Comment Summary #25: The commenter suggested that that equivalency should not be grouped under method suitability but under method validation in the sub-section *Validation Criteria.*
Response: Comment not incorporated. Equivalency is a sub-section under *Validation Criteria* and not grouped under *Method Suitability*.

Comment Summary #26: The commenter suggested clarification on why *Acceptable Procedures* was listed under equivalence options rather than in the sub-section on *Equivalency.*
Response: Comment partially incorporated. While the Expert Committee acknowledged that *Acceptable Procedure* is not strictly an equivalence option, it was determined that grouping it with the equivalence options is logical from a user perspective when comparing options to implement alternative procedures.
Comment Summary #27: The commenter suggested that in the sub-section on *Equivalency,* the paragraph on Performance Equivalence needs clarification on the meaning of the term "test functions."
Response: Comment incorporated.
Comment Summary #28: The commenter suggested that in the sub-section on *Equivalence Demonstration for Alternative Qualitative Microbiological Procedures* clarification is needed on the term "close enough".
Response: Comment incorporated.
Comment Summary #29: The commenter suggested demonstrating non-inferior recovery with a delta of 0.2 is severe with the variability in microbiology. General Chapter <1227> for instance mentions a difference in recovery of 30 % (delta of 0.3) in comparison to the control (i.e. reference) as appropriate.
Response: Comment not incorporated. The commenter is referring to method suitability (delta of 0.3) while the delta of 0.2 is in reference to comparison between two methods.
Comment Summary #30: The commenter suggested that the term R be defined in the equation for calculation involving Independent Samples.
Response: Comment incorporated.
Comment Summary #31: The commenter suggested that in the sub-section on *Equivalence Demonstration for Alternative Qualitative Microbiological Procedures* clarification is needed on details on how the first two experiments are to be evaluated in Approach 1.
Response: Comment incorporated.
Comment Summary #33: The commenter suggested that in the sub-section *Correlation* clarification is needed on what is meant by the specification limit.
Response: Comment incorporated.
Comment Summary #34: The commenter suggested that in the sub-section *Correlation* clarification is needed on the relationship of the compendial cut point of 200 cfu and the microbiological quality count of NMT 10^2
Response: Comment incorporated.
Comment Summary #35: The commenter indicated that the sub-section on *Statistical Tools* makes no connection with the General Chapter and should be deleted.
Response: Comment incorporated.
Comment Summary #36: The commenter indicated that the *Glossary Section* definitions for Multivariate Analysis, Multiple Partial Least Square Analysis and Principal Component Analysis have no relation to the General Chapter and should be deleted. Also definitions for Non-inferiority and Users Responsibility should be added and the definition for Specificity be modified to include guidance on the type of microorganisms to be used for determining specificity.
Response: Comment incorporated.
Comment Summary #37: The commenter recommended that a general reference to the revised *PDA Technical Report* #33 should be included as was the case in the previous revision of General Chapter <1223>.

Response: Comment not incorporated. Unlike the original version of <1223> which resembled PDA *Technical Report* #33 in a number of sections, this version of <1223> is significantly different from PDA *Technical Report* #33; therefore, a reference was not relevant.

General Chapter/Section(s):	<1223.1> Validation of Alternative Methods to Antibiotic Microbial Assays
Expert Committee:	General Chapters—Microbiology
No. of Commenters:	3

Comment Summary #1: The commenter suggested adding text that clarifies the control strategy for those organic impurities which do not have antimicrobial activity, if found using the HPLC method.
Response: Comment incorporated.
Comment Summary #2: The commenter recommended reformatting or revising the formula in Equation 6 for clarity since the multiple division symbols may not be correctly applied as it is written.
Response: Comment incorporated.
Comment Summary #3: The commenter suggested correcting the calculations for bias in relation to the acceptance criteria listed for the Bland-Altman Plots.
Response: Comment incorporated.
Comment Summary #4: The commenter indicated that reference is made to a specific publication which discusses how predefined acceptance criteria are set using Two One-Sided Tests (TOST).
Response: Comment incorporated.
Comment Summary #5: The commenter suggested that the minimum number of samples mentioned in both the Simple and Complex Antibiotics examples may not be enough to apply TOST and the number of replicates should be chosen depending on the acceptance criteria (referred to theta or delta) and the method standard deviation.
Response: Comment incorporated.
Comment Summary #6: The commenter suggested, in the section *Data Evaluation—Step 2*, changing the word “trend”…to “important trend” as one could conceive a situation where the variability is very small and thus a trend could be detected but it would not be practically important.
Response: Comment incorporated.
Comment Summary #7: The commenter indicated that in *Appendix 2: TOST Formulas for Paired Samples* Equation 8 contained an error.
Response: Comment incorporated.
Comment Summary #8: The commenter pointed that the axis labels in Figure. 1 are the reverse of what they should be.
Response: Comment incorporated.

Monographs

Monograph/Section: American Ginseng/Specific Tests—Botanical Characteristics
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.

Monograph/Section: Amlodipine and Valsartan Tablets/Multiple sections
Expert Committee: Monographs—Small Molecules 2
No. of Commenters: 6
Comment Summary #1: The commenter requested revising the test for *Organic Impurities* to specify which *Sample solution* is used to evaluate the disregard limit.
Response: Comment not incorporated**.** The Expert Committee determined that the monograph adequately describes the disregard limit as 0.1%, which applies to all three *Sample solutions*.
Comment Summary #2: The commenter requested including a note in the test for *Dissolution* to indicate that the paddles should be covered with Teflon or be made of any inert material other than steel because amlodipine degrades when exposed to stainless steel.
Response: Comment not incorporated**.** The changes do not reflect approved procedures. The Expert Committee will consider revising the monographs based on FDA approved specifications.
Comment Summary #3: The commenter requested widening the acceptance criteria in the *Assay* from 95.0% – 105.0% to 90%– 110%.
Response: Comment not incorporated**.** The acceptance criteria in the monograph reflect FDA approved requirements. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.
Comment Summary#4: The commenter requested widening the acceptance criteria for amlodipine related compound A in the test for *Organic Impurities* from NMT 0.5% to NMT 1.0% for consistency with other amlodipine drug product monographs and widening the acceptance criteria for total degradation products to align with the wider limit for related compound A.
Response: Comment not incorporated. The acceptance criteria in the monograph reflect FDA approved requirements. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.
Comment Summary #5: The commenter requested including a microbial limit test.
Response: Comment not incorporated**.** The Expert Committee considers revising monographs based on supporting data and FDA approved specifications.
Comment Summary#6: The commenter requested revising the acceptance criteria for *Dissolution* tolerances from NLT 80% for both analytes to NLT 75% for amlodipine and NLT 80% for valsartan.
Response: Comment not incorporated**.** The tolerances in the monograph reflect FDA approved specifications. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.
Comment Summary #7: The commenter requested including *D*-valsartan as a degradation product with an acceptance criterion of NMT 1.0% in the test for *Organic Impurities.*
Response: Comment not incorporated. The acceptance criteria in the monograph reflect FDA approved requirements. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.

Comment Summary #8: The commenter requested widening the acceptance criteria for total degradation products in the test for *Organic Impurities* from NMT 0.8% to NMT 2.0% and to exclude amlodipine related compound A and *D*-valsartan from this limit.
Response: Comment not incorporated. The acceptance criteria in the monograph reflect FDA approved requirements. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.
Comment Summary #9: The commenter requested widening the suitability requirements for signal-to-noise ratio and the relative standard deviation in the test for *Organic Impurities* as the commenter is unable to meet the requirements.
Response: Comment not incorporated. The Expert Committee determined that the system suitability criteria in the PF proposal are suitable for the procedure. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.
Comment Summary #10: The commenter requested revising the *Organic Impurities* procedure, because of the co-elution of devaleryl valsartan with a process-specific impurity in their product.
Response: Comment not incorporated. The Expert Committee determined that the procedure is adequately selective. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.

Monograph/Section: Asian Ginseng/Specific Tests-Botanical Characteristics
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.

Monograph/Section: Aspartame/Multiple sections
Expert Committee: Monographs—Excipients
No. of Commenters: 1
Comment Summary #1: The commenter recommended adding information stating that 5-benzyl-3, 6-dioxo-2-piperazineacetic acid and USP Aspartame Related Compound A RS are equivalent.
Response: Comment not incorporated. The Expert Committee determined that the USP Aspartame Related Compound A RS is linked to 5-benzyl-3, 6-dioxo-2-piperazineacetic acid in the *USP Reference Standard <11>* section of the monograph.
Comment Summary #2: In the *Chromatographic Purity* test, the commenter requested to specify a limited time span for the samples analysis after the samples were prepared to prevent biased high results.
Response: Comment not incorporated. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.

Monograph/Section: Aspartic Acid
Expert Committee: Monographs—Dietary Supplements
No. of Commenters: 1
Comment Summary #1: The commenter requested that the packing L58 in the *Briefing* section and *Chromatographic system* subsection be changed to L17 to reflect the description of the column packing material.
Response: Comment incorporated.

Comment Summary #2: The commenter indicated that they could not detect aspartic acid at the concentration of 0.1 mg/mL in the *Related Compounds* test.
Response: Comment not incorporated. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.
Comment Summary #3: The commenter requested the amount of 6 N hydrochloric acid added to the Sample solution be increased from a few drops to 20 mL to help dissolution of aspartic acid in the test for *Related Compounds*.
Response: Comment not incorporated. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.

Monograph/Section: Bacopa/Specific Tests—Botanical Characteristics
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.

Monograph/Section: Beclomethasone Dipropionate Compounded Oral Solution
Expert Committee: Compounding
No. of Commenters: 1
Comment Summary #1: The commenter suggested the time required for the active ingredient to dissolve into solution may indicate that the solution precipitates out easily. The commenter also suggested the labeling statement for the compounded preparation to be well-shaken is not needed if it is a solution.
Response: Comment not incorporated. The Expert Committee confirmed that the compounded preparation formed a solution and remained in solution throughout the duration of the stability study. The labeling statement was maintained because the Expert Committee determined that it was best practice to shake the solution well prior to administration.

Monograph/Section: Benzocaine Topical Solution/Multiple sections
Expert Committee: Monographs—Small Molecules 4
No. of Commenters: 1
Comment Summary #1: The commenter requested reordering the identification tests in the monograph for consistency across several monographs of the Benzocaine family.
Response: Comment incorporated.
Comment Summary #2: The commenter requested revising the concentration of the *System suitability solution* in the *Assay* for consistency across the Benzocaine family of monographs.
Response: Comment incorporated.
Comment Summary #3: The commenter requested revising the concentration of the *Standard solution* in the test for *Organic Impurities* for consistency across the Benzocaine family of monographs.
Response: Comment incorporated.

Monograph/Section: Black Cohosh/Identification
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: A reference to the new General Chapter <203> *High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin* was added to the Identification B.

Monograph/Section: Black Pepper/Specific Tests—Botanical Characteristics/Identification
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.
Expert Committee-initiated Change #2: A reference to the new General Chapter <203> *High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin* was added to the Identification B.

Monograph/Section: *Boswellia serrata*/Specific Tests—Botanical Characteristics
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.

Monograph/Section: Bupropion Hydrochloride/Multiple sections
Expert Committee: Monographs—Small Molecules 4
No. of Commenters: 4
Comment Summary #1: The commenter requested tightening the limit for bromochloropropiophenone (also known as 2-bromo-3'-chloropropiophenone) from NMT 0.1% to NMT 4 ppm for consistency with the NMT 1.5 µg/day exposure and to consider using a new analytical procedure to control this impurity at ppm levels.
Response: Comment not incorporated. Tightening the limit and the addition of a new analytical procedure may be considered by the Expert Committee in a future revision.
Comment Summary #2: The commenter requested the use of a different analytical procedure to control 3-chlorobenzoic acid, because the proposed test may not be sufficiently specific for 3-chlorobenzoic acid.
Response: Comment not incorporated. The Expert Committee received data demonstrating that the test is suitable when the analytical solutions are protected from light and used within 1 day.
Expert Committee-initiated Change #1: The test for *Limit of 3-Chlorobenzoic Acid* was revised to include storage conditions for analytical solutions.

Monograph/Section: Butylated Hydroxytoluene/Organic Impurities
Expert Committee: Monographs—Excipients
No. of Commenters: 2
Comment Summary #1: In the *System suitability* section, the commenter recommended including information about a critical pair and setting requirements for resolution between the components of the critical pair.
Response: Comment not incorporated. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.
Comment Summary #2: The commenter requested including a detailed explanation for calculation of total impurities.
Response: Comment incorporated.
Comment Summary #3: The commenter recommended including a detailed explanation for calculation of individual impurities.
Response: Comment incorporated.

Monograph/Section: Carbachol/Multiple sections
Expert Committee: Monographs—Small Molecules 4
No. of Commenters: 1
Comment Summary #1: The commenter requested modernizing the *Assay* and *Organic Impurities* procedures by replacing the existing procedures with ion chromatographic procedures.
Response: Comment not incorporated. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.

Monograph/Section: Carbamazepine/Multiple sections
Expert Committee: Monographs—Small Molecules 4
Expert Committee-initiated Change #1: All references to USP Carbamazepine Related Compound F RS and USP Iminodibenzyl RS were deleted from the monograph. These reference standards were originally intended as markers for system suitability, but it was not possible to develop a reference standard for carbamazepine related compound F due to stability issues.

Monograph/Section: Carbamazepine Oral Suspension/Organic Impurities
Expert Committee: Monographs—Small Molecules 4
Expert Committee-initiated Change #1: All references to USP Carbamazepine Related Compound F RS and USP Iminodibenzyl RS were deleted from the monograph. These reference standards were originally intended as markers for system suitability but it was not possible to develop a reference standard for carbamazepine related compound F due to stability issues.

Monograph/Section: Carboprost Tromethamine/Multiple sections
Expert Committee: Monographs—Small Molecules 4
Expert Committee-initiated Change #1: The molecular formula for carboprost tromethamine in the *Definition* and *Assay* was corrected to be consistent with the molecular formula in the *Chemical information* section.

Monograph/Section: Cat's Claw/Specific Tests-Botanical Characteristics
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.

Monograph/Section: *Centella asiatica* /Specific Tests—Botanical Characteristics
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.

Monograph/Section: Cisapride Compounded Injection, Veterinary
Expert Committee: Compounding
Expert Committee-initiated Change #1: The Expert Committee clarified the *Beyond-Use Date* section to indicate that in the absence of performing and completing a sterility and endotoxins test, the storage conditions defined in <797> *Pharmaceutical Compounding—Sterile Preparations* apply.

Monograph/Section: Crypthecodinium Cohnii Oil Capsules
Expert Committee: Monographs—Dietary Supplements
No. of Commenters: 1
Comment Summary: The commenter highlighted that text in the *Identification* test is not consistent with referred General Chapter <401> *Fats and Fixed Oils*, which was recently revised and currently official.
Response: Comment incorporated.

Monograph/Section: Cyclosporine Compounded Ophthalmic Solution, Veterinary
Expert Committee: Compounding
Expert Committee-initiated Change #1: The Expert Committee clarified the *Beyond-Use Date* section to indicate that in the absence of performing and completing a sterility and endotoxins test, the storage conditions defined in <797> *Pharmaceutical Compounding—Sterile Preparations* apply.

Monograph/Section: Desmopressin Acetate/Multiple Sections
Expert Committee: Monographs—Biologics & Biotechnology 1
No. of Commenters: 2

Specific Tests
Comment Summary #1: The commenter requested retaining Specific Rotation test as chiral purity is essential for the activity. Also, chiral purity of the compound amino acid residues is highly susceptible to racemization due to change in pH, temperature and water content. Because specific optical rotation is the only test for chiral purity of the peptide in the monograph, it is recommended to retain the test.
Response: Comment not incorporated. Based on experience with other peptides, under normal storage conditions, this is not expected to occur. Specific Rotation is non-specific and not sensitive enough to detect low level of racemization. Furthermore, an HPLC-based impurities method would be able to control racemization impurities.

Identification
Comment Summary #2: The commenter requested revising the mass of Desmopressin to include the M+H peak that is present in positive ion mode.
Response: Comment incorporated. The correct monoisotopic mass for Desmopressin has been added.

Additional Requirements/Labeling

Comment Summary #3: The commenter requested revising the label to state the strength in micrograms per mL to reflect the FDA approved drug product labels.
Response: Comment incorporated.

Monograph/Section: Doxorubicin Hydrochloride/Multiple Sections
Expert Committee: Monographs—Small Molecules 1
No. of Commenters: 8

Comment Summary #1: The commenter requested adding a statement that precipitate in *Identification C* may turn black when ammonium hydroxide is added.
Response: Comment not incorporated. The test was evaluated in the laboratory and no problems were reported. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.
Comment Summary #2: The commenter requested revising the resolution requirement in the test for *Organic impurities*
Response: Comment incorporated. The resolution requirement was revised from NLT 3.0 to NLT 1.5, which is adequate to demonstrate baseline resolution.
Comment Summary #3: The commenter indicated that peaks resulting from the diluent may interfere with the analyte peak in the *Assay* procedure.
Response: Comment not incorporated. No interference was observed during method validation. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.
Comment Summary #4: The commenter requested revising the relative retention times to two decimal points in the test for *Organic impurities* to align with the drug product monograph.
Response: Comment incorporated.
Comment Summary #5: The commenter requested replacing the impurity reference standards with relative response factors to minimize testing costs.
Response: Comment not incorporated. The Expert Committee determined that the use of impurity reference standards is consistent with the method validation; manufacturers can use appropriately validated alternative procedures that replace the reference standards with relative retention times.
Comment Summary #6: The commenter requested replacing the UHPLC procedure with an HPLC procedure to minimize costs.
Response: Comment not incorporated. The Expert Committee determined that the higher selectivity and shorter run time of a UHPLC procedure offers advantages over an HPLC procedure for this drug substance.
Comment Summary #7: The commenter indicated that the test for *Organic impurities* procedure did not resolve doxorubicinone from epirubicin and expressed concerns about method selectivity.
Response: Comment not incorporated. The Expert Committee determined that epirubicin is not a known impurity so resolution between doxorubicinone and epirubicin is not a concern. A footnote was added to *Table 2* to indicate that epirubicin is added to the *System suitability solution* as a marker to evaluate the resolution between epirubicin and doxorubicin.
Comment Summary #8: The commenter requested revising the resolution requirement from NLT 3.0 to NLT 1.5 which is adequate to demonstrate baseline resolution.
Response: Comment incorporated.

Expert Committee-initiated Change #1: The *Assay* and the test for *Organic impurities* were revised to include a note about light protection for solutions containing doxorubicin.
Expert Committee-initiated Change #2: The *Assay* and the test for *Organic impurities* were revised to change the injection volume from 2.0 µL to 2 µL for consistency with current USP format.
Expert Committee-initiated Change #3: The calculation section in the *Assay* was updated to include the reference standard potency and a conversion factor.
Expert Committee-initiated Change #4: The relative standard deviation requirement in the test for *Organic Impurities* was changed from NMT 1.5% to NMT 5.0%, which is a more suitable limit for an impurities procedure.
Expert Committee-initiated Change #5: The calculations for each known impurity and unspecified impurities in the test for *Organic impurities* were separated for clarity. Reference standard potencies and conversion factors are included as needed.
Expert Committee-initiated Change #6: The name of *"Procedure 2: Limit of solvent residues (as acetone and alcohol)*" was changed to "*Limit of Acetone and Alcohol*," the numbering format for impurities procedures is generally used only for flexible monographs*.*
Expert Committee-initiated Change #7: The *Limit of Acetone and Alcohol* test was revised to update the name of Solution A to "Internal standard solution," which is more informative about the function of the solution.
Expert Committee-initiated Change #8: The flow rate in the *Limit of Acetone and Alcohol* test was updated to current USP format.
Expert Committee-initiated Change #9: The *Limit of Acetone and Alcohol* test was revised to indicate that peak response ratios are used in the relative standard deviation requirement*.*
Expert Committee-initiated Change #10: The note about using the results of the *Limit of Acetone and Alcohol* test to calculate *Assay* results was removed from the acceptance criteria because the Assay acceptance criteria indicate that the calculation should be on the solvent-free basis.
Expert Committee-initiated Change #11: The statement, "protect from light" was deleted from the *Packaging and Storage* section. This statement can be added to the monograph in the future upon receipt of supporting data.

Monograph/Section: Doxorubicin Hydrochloride Injection/Multiple sections
Expert Committee: Monographs—Small Molecules 1
No. of Commenters: 3
Comment Summary #1: The commenter requested the rationale for the use of the mass spectrometry-compatible mobile phase is used in the *Assay*, which uses a UV detector.
Response: Comment not incorporated. The mass spectrometry-compatible mobile phase allows analysts to use Mass Spectrometry to identify impurities if needed.
Comment Summary #2: The commenter requested replacing the UHPLC procedure with an HPLC procedure to minimize costs.
Response: Comment not incorporated. The Expert Committee determined that the higher selectivity and shorter run time of a UHPLC procedure offers advantages over an HPLC procedure.
Comment Summary #3: The commenter requested revising the resolution requirement in the test for *Organic impurities*

Response: Comment incorporated. The resolution requirement was revised from NLT 3.0 to NLT 1.5, which is adequate to demonstrate baseline resolution.
Expert Committee-initiated Change #1: The *Assay* and the test for *Organic impurities* were revised to include a note about light protection for solutions containing doxorubicin.
Expert Committee-initiated Change #2: The *Sample solution* in the *Assay* and the test for Organic Impurities is updated based on current USP format.
Expert Committee-initiated Change #3: The Assay and the test for *Organic impurities* were revised to change the injection volume from 2.0 µL to 2 µL for consistency with current USP format.
Expert Committee-initiated Change #4: The calculation section in the *Assay* was updated to include the reference standard potency and a conversion factor.
Expert Committee-initiated Change #5: The relative standard deviation requirement in the test for *Organic Impurities* was changed from NMT 1.5% to NMT 5.0%, which is a more suitable limit for an impurities procedure.
Expert Committee-initiated Change #6: The calculations for each known impurity and unspecified impurities in the test for *Organic impurities* are separated for clarity. Reference standard potencies and conversion factors are included as needed.
Expert Committee-initiated Change #7: *Table 2* in the test for *Organic Impurities* was updated to add a footnote to indicate that epirubicin is a resolution marker and is not to be reported.

Monograph/Section: Doxorubicin Hydrochloride for Injection/Multiple sections
Expert Committee: Monographs—Small Molecules 1
Expert Committee-initiated Change #1: The *Assay* and the test for *Organic impurities* were revised to include a note about light protection for solutions containing doxorubicin.
Expert Committee-initiated Change #2: The Assay and the test for *Organic impurities* were revised to change the injection volume from 2.0 µL to 2 µL for consistency with current USP format.
Expert Committee-initiated Change #3: The resolution requirement in the *Assay* and the test for *Organic impurities* was revised from NLT 3.0 to NLT 1.5, which is adequate to demonstrate baseline resolution.
Expert Committee-initiated Change #4: The calculation section in the *assay* was updated to include the reference standard potency and a conversion factor
Expert Committee-initiated Change #5: The relative standard deviation requirement in the test for *Organic Impurities* was changed from NMT 1.5% to NMT 5.0%, which is a more suitable limit for an impurities procedure.
Expert Committee-initiated Change #6: The calculations for each known impurity and unspecified impurities in the test for *Organic impurities* were separated for clarity. Reference standard potencies and conversion factors are included as needed.
Expert Committee-initiated Change #7: *Table 2* in the test for *Organic Impurities* was updated to add a footnote to indicate that epirubicin is a resolution marker and is not to be reported.
Expert Committee-initiated Change #8: The pH test was updated to current USP format.

Monograph/Section: Eleuthero/Identification
Expert Committee: Monographs—Dietary Supplements
Expert Committee-Initiated Change #1: The Reference to General Chapter <201> *Thin-Layer Chromatographic Identification Test* in the Identification A was replaced with the reference to a

new General Chapter <203> *High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin*.

Monograph/Section: Enrofloxacin Compounded Oral Suspension, Veterinary
Expert Committee: Compounding
Expert Committee-initiated Change #1: The Expert Committee corrected a typographical error in the detector wavelength from 227 nm to 277 nm based on the developed and validated stability-indicating method for the compounded preparation.

Monograph/Section: Ezetimibe/Multiple sections
Expert Committee: Monographs—Small Molecules 2
No. of Commenters: 1
Comment Summary #1: The commenter requested clarification that the requirements in the *Definition* and *Assay* are on the solvent free basis, consistent with FDA approved acceptance criteria.
Response: Comment incorporated.
Comment Summary #2: The commenter requested including the option of *Method 1c* for *Water Determination* along with *Method 1a* to be consistent with approved FDA specifications.
Response: Comment incorporated.
Comment Summary #3: The commenter requested widening the acceptance criteria for *Water Determination* from NMT 0.6% to NMT 1.0% for the anhydrous form and including an acceptance criterion of NMT 5.0% for monohydrate.
Response: Comment not incorporated. The acceptance criteria in the monograph reflect FDA approved requirements. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.
Comment Summary #4: The commenter suggested revising *Organic Impurities Procedure 1*, because the desfluoroaniline analog coelutes with process impurities in their product.
Response: Comment not incorporated. The Expert Committee determined that the system suitability criteria in the *PF* proposal are suitable for the procedure. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.
Comment Summary #5: The commenter suggested revising *Organic Impurities Procedure 1* because of lengthy run time coupled with limited solution stability.
Response: Comment not incorporated. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.
Comment Summary #6: The commenter requested widening the acceptance criteria for ezetimibe cyclic ether and ezetimibe ketone from NMT 0.1% to NMT 0.15% based on ICH guidelines.
Response: Comment not incorporated. The acceptance criteria in the monograph reflect FDA approved requirements. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.
Comment Summary #7: The commenter suggested revising *Organic Impurities Procedure 2* because of the SSR-ezetimibe coelutes with chiral process impurities in their product and because the procedure controls only 6 of 8 possible isomers.
Response: Comment not incorporated. The Expert Committee determined that the system suitability criteria in the *PF* proposal are suitable for the procedure. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.

Comment Summary #8: The commenter indicated that they were unable to meet the resolution requirements in the test for *Organic Impurities Procedure 2*.
Response: Comment not incorporated. The Expert Committee determined that the system suitability criteria in the *PF* proposal are suitable for the procedure. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.
Comment Summary #9: The commenter indicated that the concentration of the *Sample solution* in the test for *Organic Impurities Procedure 2* is very low and expressed concern about the sensitivity and precision of the method.
Response: Comment not incorporated. The Expert Committee determined that the sensitivity and precision of this test is adequate. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.

Monograph/Section: Ezetimibe Tablets/Multiple sections
Expert Committee: Monographs—Small Molecules 2
No. of Commenters: 2
Comment Summary #1: The commenter requested deleting *Identification test B* as the commenter does not have diode array capabilities at the manufacturing sites.
Response: Comment not incorporated. The Expert Committee determined that there is a need for two specific orthogonal *Identification* tests in the public standard.
Comment Summary #2: The commenter requested deleting *p*-chloroaniline analog and p-methylbenzene analog from *Table 1* in the test for *Organic Impurities* to be consistent with the FDA approved specifications.
Response: Comment incorporated.
Comment Summary #3: The commenter requested deleting the note about *m*-fluoroaniline in the *System suitability solution* and revising the resolution requirement in the test for *Organic Impurities* procedure as the *System suitability solution* does not produce *m*-fluoroaniline upon basic degradation at 55°C.
Response: Comment not incorporated. The Expert Committee determined that available data supports the fact that m-fluoroaniline is generated in the System suitability solution. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.

Monograph/Section: Fenugreek Seed/Multiple Sections
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The solvent in all three HPTLC procedures: Thin Layer Chromatography—Amino Acid Profile, Thin Layer Chromatography—Steroidal Saponins Profile, and Specific Tests—Presence of Trigonelline was changed from methanol to 70% ethanol, which exhibits better extractive properties and facilitates chromatographic similarity.
Expert Committee-initiated Change #2: The chromatographic plate heating conditions in all three HPTLC procedures: Thin Layer Chromatography—Amino Acid Profile, Thin Layer Chromatography—Steroidal Saponins Profile, and Specific Tests—Presence of Trigonelline was uniformly changed to 3 min at 105°. This change effects more uniform conditions beneficial to the assessment of the chromatographic separation outcome.
Expert Committee-initiated Change #3: The requirement for the R_F reproducibility was deleted from the *System Suitability* in all three HPTLC procedures: Thin Layer Chromatography—Amino

Acid Profile, Thin Layer Chromatography—Steroidal Saponins Profile, and Specific Tests—Presence of Trigonelline. This requirement was found to be unnecessarily constraining.
Expert Committee-initiated Change #4: The tabular format used for *System Suitability* in all three HPTLC procedures: Thin Layer Chromatography—Amino Acid Profile, Thin Layer Chromatography—Steroidal Saponins Profile, and Specific Tests—Presence of Trigonelline was replaced with a verbal description.
Expert Committee-initiated Change #5: The visualization of the chromatogram in Specific Tests—Presence of Trigonelline was modified to eliminate Dragendorff reagent in favor of inspection at 254nm against the fluorescent background. Trigonelline is a sole prominent band, and the use of the derivatization reagent was largely superfluous.
Expert Committee-initiated Change #6: Throughout the monograph, the USP Trigonella Foenum-graecum Seed Powdered Extract RS was renamed USP Trigonella Foenum-graecum Seed Dry Extract RS to reflect the current nomenclature, and in agreement with the official title of the USP Reference Standard.
Expert Committee-initiated Change #7: Throughout the monograph, the USP Trigonelline Hydrochloride RS was replaced with USP Trigonelline RS.

Monograph/Section: Fenugreek Seed Powder /Multiple Sections
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The solvent in all three HPTLC procedures: Thin Layer Chromatography—Amino Acid Profile, Thin Layer Chromatography—Steroidal Saponins Profile, and Specific Tests—Presence of Trigonelline was changed from methanol to 70% ethanol, which exhibits better extractive properties and facilitates chromatographic similarity.
Expert Committee-initiated Change #2: The chromatographic plate heating conditions in all three HPTLC procedures: Thin Layer Chromatography—Amino Acid Profile, Thin Layer Chromatography—Steroidal Saponins Profile, and Specific Tests—Presence of Trigonelline was uniformly changed to 3 min at 105°. This change effects more uniform conditions beneficial to the assessment of the chromatographic separation outcome.
Expert Committee-initiated Change #3: The requirement for the R_F reproducibility was deleted from the *System Suitability* in all three HPTLC procedures: Thin Layer Chromatography—Amino Acid Profile, Thin Layer Chromatography—Steroidal Saponins Profile, and Specific Tests—Presence of Trigonelline. This requirement was found to be unnecessarily constraining.
Expert Committee-initiated Change #4: The tabular format used for *System Suitability* in all three HPTLC procedures: Thin Layer Chromatography—Amino Acid Profile, Thin Layer Chromatography—Steroidal Saponins Profile, and Specific Tests—Presence of Trigonelline was replaced with a verbal description.
Expert Committee-initiated Change #5: The visualization of the chromatogram in Specific Tests—Presence of Trigonelline was modified to eliminate Dragendorff reagent in favor of inspection at 254nm against the fluorescent background. Trigonelline is a sole prominent band, and the use of the derivatization reagent was largely superfluous.
Expert Committee-initiated Change #6: Throughout the monograph, the USP Trigonella Foenum-graecum Seed Powdered Extract RS was renamed USP Trigonella Foenum-graecum Seed Dry Extract RS to reflect the current nomenclature, and in agreement with the official title of the USP Reference Standard.
Expert Committee-initiated Change #7: Throughout the monograph, the USP Trigonelline Hydrochloride RS was replaced with USP Trigonelline RS.

Monograph/Section: Fenugreek Seed Powdered Extract/Multiple Sections
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The solvent in all three HPTLC procedures: Thin Layer Chromatography—Amino Acid Profile, Thin Layer Chromatography—Steroidal Saponins Profile, and Specific Tests—Presence of Trigonelline was changed from methanol to 70% ethanol, which exhibits better extractive properties and facilitates chromatographic similarity.
Expert Committee-initiated Change #2: The chromatographic plate heating conditions in all three HPTLC procedures: Thin Layer Chromatography—Amino Acid Profile, Thin Layer Chromatography—Steroidal Saponins Profile, and Specific Tests—Presence of Trigonelline was uniformly changed to 3 min at 105°. This change effects more uniform conditions beneficial to the assessment of the chromatographic separation outcome.
Expert Committee-initiated Change #3: The *System Suitability* in all three HPTLC procedures: Thin Layer Chromatography—Amino Acid Profile, Thin Layer Chromatography—Steroidal Saponins Profile, and Specific Tests—Presence of Trigonelline no longer includes the requirement for the R_F reproducibility. This requirement was found to be unduly constraining.
Expert Committee-initiated Change #4: The tabular format used for *System Suitability* in all three HPTLC procedures: Thin Layer Chromatography—Amino Acid Profile, Thin Layer Chromatography—Steroidal Saponins Profile, and Specific Tests—Presence of Trigonelline was replaced with a verbal description.
Expert Committee-initiated Change #5: The visualization of the chromatogram in Specific Tests—Presence of Trigonelline was modified to eliminate Dragendorff reagent in favor of inspection at 254nm against the fluorescent background. Trigonelline is a sole prominent band, and the use of the derivatization reagent was largely superfluous.
Expert Committee-initiated Change #6: The content of water in the Specific Tests—Water Determination was changed from NMT 6.0% to NMT 9.0% to accommodate a wider range of industry-supplied specifications.
Expert Committee-initiated Change #7: Throughout the monograph, the USP Trigonella Foenum-graecum Seed Powdered Extract RS was renamed USP Trigonella Foenum-graecum Seed Dry Extract RS to reflect the current nomenclature, and in agreement with the official title of the USP Reference Standard.
Expert Committee-initiated Change #8: Throughout the monograph, the USP Trigonelline Hydrochloride RS was replaced with USP Trigonelline RS.

Monograph/Section: Forskohlii/Specific Tests—Botanical Characteristics
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.

Monograph/Section: *Garcinia cambogia* /Specific Test—-Botanical Characteristics
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.

Monograph/Section: *Garcinia indica* /Specific Tests—Botanical Characteristics/Identification
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.
Expert Committee-initiated Change #2: A reference to the new General Chapter <203> *High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin* was added to the Identification B.

Monograph/Section: Ginkgo/Identification
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: A reference to the new General Chapter <203> *High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin* was added to the Identification A.

Monograph/Section: Guggul/Specific Tests-Botanical Characteristics
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.

Monograph/Section: Gymnema/Specific Tests-Botanical Characteristics/Identification
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.
Expert Committee-initiated Change #2: A reference to the new General Chapter <203> *High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin* was added to the Identification B.

Monograph/Section: Isoflurane
Expert Committee: Monographs—Small Molecules 4
No. of Commenters: 1
Comment Summary #1: The commenter requested adding a test for *Acidity or Alkalinity* for consistency with other anesthetics of this type.
Response: Comment not incorporated. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.

Monograph/Section: Lactulose Concentrate
Expert Committee: Monographs—Small Molecules 3
No. of Commenters: 1
Comment Summary #1: The commenter recommended adding a test for sulfites.
Response: Comment not incorporated. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.

Monograph/Section: Lamivudine Tablets/Multiple sections
Expert Committee: Monographs—Small Molecules 1

No. of Commenters: 1
Comment summary #1: The commenter requested reducing the concentration of the *Sample solution* in the *Assay* from 0.2 mg/mL to 0.05 mg/mL.
Response: Comment not incorporated. The concentration in the monograph reflects the validated procedure. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.
Comment Summary #2: The commenter requested increasing the concentration of the *Sample solution* in the test for *Organic impurities* from 0.2 mg/mL to 0.5 mg/mL.
Response: Comment not incorporated. The concentration in the monograph reflects the validated procedure.
Comment Summary #3: The commenter requested revising the quantitation of impurities in the test for *Organic impurities* to replace the area normalization with comparison to the drug substance reference standard and relative response factors.
Response: Comment not incorporated. The calculations in the monograph are consistent with the validated procedure and FDA approved acceptance criteria. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.

Monograph/Section: Lamotrigine Extended-Release Tablets/Multiple Sections
Expert Committee: Monographs—Small Molecules 4
No. of Commenters: 3
Comment Summary #1: The commenter requested including an orthogonal procedure for identification.
Response: Comment not incorporated. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.
Comment Summary #2: The commenter requested including a test and acceptance criteria for *Water determination*.
Response: Comment not incorporated because the moisture content of solid oral dosage forms is dependent on the formulation. The Expert Committee will consider revising this monograph based on supporting data showing the necessity to control moisture content in the product.
Comment Summary #3: The commenter indicated that the dissolution procedure time points and medium are inconsistent with the FDA approved parameters.
Response: Comment not incorporated. The dissolution parameters were confirmed by the FDA before the proposal was published in the *Pharmacopeial Forum.*

Monograph/Section: Levocetirizine Dihydrochloride Tablets/Multiple Sections
Expert Committee: Monographs—Small Molecules 4
No. of Commenters: 2
Comment Summary #1: The commenter requested increasing the disregard level in the test for *Organic Impurities* from 0.02% to 0.1% to be consistent with the ICH reporting threshold.
Response: Comment incorporated.
Comment Summary #2: The commenter requested identifying eight specified unidentified process impurities listed in *Table 1* in the test for *Organic Impurities* for clarity.
Response: Comment not incorporated. The Expert Committee determined that the information about the specified unidentified impurities is not needed; *Table 1* was revised to remove reference to the specified unidentified impurities.

Comment Summary #3: The commenter requested additional details regarding the acceptance criteria for *Identification test A, Ultraviolet Absorption <197U>.*
Response: Comment not incorporated. The Expert Committee determined that the acceptance criteria as stated are clear and additional details are provided in the General Chapter.

Monograph/Section: Loperamide Hydrochloride Tablets/Multiple Sections
Expert Committee: Monographs—Small Molecules 3
No. of Commenters: 2
Comment Summary #1: The commenter requested revising the *Ion-pairing solution* in the *Assay* to indicate that the solvent is water.
Response: Comment incorporated.
Comment Summary #2: The commenter requested tightening the limits in the *Organic Impurities* procedure to be consistent with the FDA-approved limits.
Response: Comment not incorporated. The acceptance criteria in the proposal are consistent with FDA approved limits; the public standard is intended to address all approved drug products.

Monograph/Section: Malabar-Nut-Tree, Leaf/Specific Tests—Botanical Characteristics
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.

Monograph/Section: Mercaptopurine/Organic Impurities
Expert Committee: Monographs—Small Molecules 3
No. of Commenters: 1
Comment Summary #1: The commenter requested adding two process impurities as specified impurities with relative response factors in *Table 2* in the test for *Organic impurities*.
Response: Comment not incorporated. These process impurities are controlled as unspecified impurities in accordance with FDA approved specification. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.

Monograph/Section: Methimazole/Multiple sections
Expert Committee: Monographs—Small Molecules 3
No. of Commenters: 2
Comment Summary #1: The commenter requested retaining the test for *Melting Range*.
Response: Comment not incorporated. The test does not add value to the monograph and is not included in the corresponding *European Pharmacopoeia* monograph.
Comment Summary #2: The commenter requested revising the chemical name for methimazole related compound C from ‘1-Methyl-2-(methylsulfanyl)-1*H*-imidazole’ to ‘1-Methyl-2-(methylthio)-1*H*-imidazole’.
Response: Comment incorporated.

Monograph/Section: Mycophenolate Mofetil Capsules/Assay
Expert Committee: Monographs—Small Molecules 3
No. of Commenters: 1

Comment Summary #1: The commenter requested widening the acceptance criteria in the *Assay* from 95.0%-105% to 94.0%-105.0% based on the FDA approved stability limits.
Response: Comment incorporated.

Monograph/Section: Mycophenolate Mofetil Tablets/Assay
Expert Committee: Monographs—Small Molecules 3
No. of Commenters: 1
Comment Summary #1: The commenter requested widening the acceptance criteria in the *Assay* from 95.0%-105% to 94.0%-105.0% based on the FDA approved stability limits.
Response: Comment incorporated.

Monograph/Section: Nicotine/Multiple Sections
Expert Committee: Monographs—Small Molecules 4
No. of Commenters: 1
Comment Summary #1: The commenter requested revising the calculations in the test for *Organic Impurities* which account for the water of crystallization twice.
Response: Comment incorporated. The molecular weight of nicotine bitartrate dihydrate was replaced with the molecular weight of anhydrous nicotine bitartrate in the calculations, because the reference standard label states that *Water Determination* must be performed on the standard at the time of use.
Expert Committee-initiated Change #1: In the test for *Organic Impurities,* the system suitability solution was replaced with the *Standard solution* for determination of relative standard deviation. The limit for relative standard deviation was increased from 2.0% to NMT 5.0% because of the low concentration of the *Standard solution*. These changes are made to maintain consistency across the monograph family; the *Nicotine Polacrilex* monograph was revised similarly to address comments received.
Expert Committee-initiated Change #2: The chemical information in *the Reference Standards* section of the monograph was revised to align with the salt forms of some of the *Reference Standards* that were developed in support of the monograph.

Monograph/Section: Nicotine Polacrilex/Multiple Sections
Expert Committee: Monographs—Small Molecules 4
No. of Commenters: 3
Comment Summary #1: The commenter requested revising the calculations in the test for *Organic Impurities* which account for the water of crystallization twice.
Response: Comment incorporated. The molecular weight of nicotine bitartrate dihydrate was replaced with the molecular weight of anhydrous nicotine bitartrate in the calculations, because the reference standard label states that *Water Determination* must be performed on the standard at the time of use.
Comment Summary #2: The commenter recommended removing the reference to the System suitability solution for the relative standard deviation requirement in the *Assay* and using only the Standard solution.
Response: Comment incorporated.
Comment Summary #3: The commenter recommended replacing the *System suitability solution* in the test for *Organic Impurities,* with the *Standard solution* for determination of relative

standard deviation and increasing the % RSD to NMT 5.0% because of the low concentration of the Standard solution.
Response: Comment incorporated.
Expert Committee-initiated Change #1: The chemical information in the *USP Reference Standards* section of the monograph was revised to align with the salt forms of some of the reference standards that were developed in support of the monograph.

Monograph/Section: Northern Schisandra Fruit/Labeling
Expert Committee: Monographs—Dietary Supplements
No. of Commenters: 1
Comment Summary #1: The commenter indicated that the labelling as written repeats the information provided in the monograph title.
Response: Comment incorporated. The *Labeling* section was revised to read, “The label states the Latin binomial following the official name.”

Monograph/Section: Northern Schisandra Fruit Powder/Labeling
Expert Committee: Monographs—Dietary Supplements
No. of Commenters: 1
Comment Summary #1: The commenter indicated that the *Labelling* as written repeats the information provided in the monograph title.
Response: Comment incorporated**.** The *Labeling* section was revised to read, “The label states the Latin binomial following the official name.”

Monograph/Section: Oleyl Alcohol/Multiple Sections
Expert Committee: Monographs—Excipients
No. of Commenters: 3
Comment Summary #1: By supplying the rationale and supporting data, the commenter recommended revising the lower assay limit from “80%” to “75%”
Response: Comment incorporated.
Comment Summary #2: In the test for *Limit of Related Fatty Alcohols*, the commenter suggested changing the acceptance criteria for linoleyl alcohol, linolenyl alcohol, and arachidyl alcohol from “NMT 3.0%”, “NMT 0.5%”, and “NMT 0.3%” to “NMT 7.0%”, “NMT 1.0%” and “NMT 1.0%”, respectively based on the data provided.
Response: Comment incorporated.
Comment Summary #3: In the test for *Peroxide Value*, the commenter recommended revising the acceptance criteria from “NMT 5.0” to “NMT 10.0” based on the supporting data provided.
Response: Comment incorporated.

Monograph/Section: Phenylephrine Bitartrate/Multiple Sections
Expert Committee: Monographs—Small Molecules 2
Expert Committee-initiated change #1: The chemical name for USP Norphenylephrine Hydrochloride RS in the *USP Reference Standards* section is revised to remove the chiral designation ‘*R*’ to be consistent with available characterization data for the reference standard.
Expert Committee-initiated change #2: The chemical formulas for USP Norphenylephrine Hydrochloride RS and USP Phenylephrine Related Compound C RS in the *USP Reference Standards* section are revised to indicate that they are hydrochlorides.

Expert Committee-initiated change #3: The chemical information (chemical name, formula and molecular weight) for USP Phenylephrine Related Compound E RS in *USP Reference Standards* section is updated to indicate that this is the hydrochloride. The analysis of phenylephrine related compound E in the test for Organic Impurities is revised to reflect the correct salt form.

Monograph/Section: Phenylephrine Hydrochloride/Multiple Sections
Expert Committee: Monographs—Small Molecules 2
No. of Commenters: 1
Comment Summary #1: The commenter recommended retaining the test of *Content of Chloride* to ensure a 1:1 ratio of phenylephrine to hydrochloride.
Response: Comment not incorporated. The Expert Committee determined that the chloride identification test together with other tests in the monograph is sufficient to ensure the quality and strength of this drug substance.
Comment Summary #2: The commenter requested revising the test for *Chloride and Sulfate, Sulfate <221>* to clarify how the test is performed.
Response: Comment not incorporated. The language of the test is consistent with current USP format. Only the sulfate test is required for this monograph as indicated by the title of this test. This test refers to the analytical procedure for sulfate in the General Chapter, using the *Sample solution* and *Standard solution* as described in the monograph.
Comment Summary #3: The commenter recommended retaining the test for *Melting Range or Temperature <741>*.
Response: Comment not incorporated. The Expert Committee determined that the newly proposed HPLC procedure for *Organic Impurities* together with other tests in the monograph is sufficient to ensure the quality of this drug substance.
Expert Committee-initiated change #1: The chemical name for USP Norphenylephrine Hydrochloride RS in the *USP Reference Standards* section is revised to remove the chiral designation '*R*' to be consistent with available characterization data for the reference standard.
Expert Committee-initiated change #2: The chemical formulas for USP Norphenylephrine Hydrochloride RS and USP Phenylephrine Related Compound C RS in the *USP Reference Standards* section are revised to indicate that they are hydrochloride salts.
Expert Committee-initiated change #3: The chemical information (chemical name, formula and molecular weight) for USP Phenylephrine Related Compound E RS in *USP Reference Standards* section is updated to indicate that this is the hydrochloride salt. The analysis of phenylephrine related compound E in the test for Organic Impurities is revised to reflect the correct salt form.

Monograph/Section: Phenytoin/Multiple Sections
Expert Committee: Monographs—Small Molecules 4
No. of Commenters: 2
Comment Summary #1: The commenter indicated that the 55-minute run time in the *Assay* procedure is excessively lengthy, given that phenytoin elutes in 9 minutes.
Response: Comment not incorporated. The Expert Committee determined that the procedure long run time is necessary in the public standard to prevent interference from late-eluting impurities.

Comment Summary #2: The commenter indicated that the signal-to-noise ratio of the *Standard solution* is significantly higher than the system suitability requirement for signal-to-noise ratio. The concern is that the signal-to-noise ratio is not a good indicator of the sensitivity of this procedure and should be deleted.
Response: Comment not incorporated. The Expert Committee determined that the signal-to-noise ratio is appropriate for this procedure and accounts for differences between labs, instrumentation, and detection capabilities

Monograph/Section: *Phyllanthus amarus* /Specific Tests—Botanical Characteristics
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.

Monograph/Section: Powdered American Ginseng/Specific Tests—Botanical Characteristics
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.

Monograph/Section: Powdered Ashwagandha Root/Specific Tests—Botanical Characteristics
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.

Monograph/Section: Powdered Asian Ginseng/Specific Tests—Botanical Characteristics
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.

Monograph/Section: Powdered Bacopa/Specific Tests—Botanical Characteristics
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.

Monograph/Section: Powdered Black Cohosh/Specific Tests—Botanical Characteristics/Identification
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.
Expert Committee-initiated Change #2: A reference to the new General Chapter <203> *High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin* was added to the Identification B.

Monograph/Section: Powdered Black Pepper/Specific Tests—Botanical Characteristics/Identification
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.
Expert Committee-initiated Change #2: A reference to the new General Chapter <203> *High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin* was added to the Identification B.

Monograph/Section: Powdered *Centella asiatica* /Specific Tests—Botanical Characteristics
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.

Monograph/Section: Powdered Chaste Tree/Specific Tests—Botanical Characteristics
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.

Monograph/Section: Powdered Eleuthero/Specific Tests—Botanical Characteristics
Identification
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.
Expert Committee-initiated Change #2: The reference to the General Chapter <201> *Thin-*Layer Chromatographic Identification Test in the Identification A was replaced with the reference to a new General Chapter <203> High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin.

Monograph/Section: Powdered Forskohlii/Specific Tests—Botanical Characteristics
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.

Monograph/Section: Powdered *Garcinia cambogia* /Specific Tests—Botanical Characteristics
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.

Monograph/Section: Powdered *Garcinia indica* /Specific Tests—Botanical Characteristics/Identification
Expert Committee: Monographs—Dietary Supplements

Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.
Expert Committee-initiated Change #2: A reference to the new General Chapter <203> *High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin* was added to the Identification B.

Monograph/Section: Powdered Goldenseal/Specific Tests—Botanical Characteristics
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.

Monograph/Section: Powdered Gymnema/Specific Tests—Botanical Characteristics/Identification
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: A reference to the new General Chapter <203> *High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin* was added to the Identification C.

Monograph/Section: Powdered Horse Chestnut /Specific Tests—Botanical Characteristics
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.

Monograph/Section: Powdered Licorice/Specific Tests—Botanical Characteristics
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.

Monograph/Section: Powdered Malabar-Nut-Tree, Leaf /Specific Tests—Botanical Characteristics
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.

Monograph/Section: Powdered *Phyllanthus amarus* /Specific Tests—Botanical Characteristics
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.

Monograph/Section: Powdered St. John’s Wort/Specific Tests—Botanical Characteristics/Identification
Expert Committee: Monographs—Dietary Supplement

Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.
Expert Committee-initiated Change #2: A reference to the new General Chapter <203> *High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin* was added to the Identification B.

Monograph/Section: Powdered Turmeric /Specific Tests—Botanical Characteristics
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.

Monograph/Section: Propafenone Hydrochloride Extended-Release Capsules/Multiple Sections
Expert Committee: Monographs—Small Molecules 2
No. of Commenters: 2
Comment Summary #1: The commenter requested including microbial limit tests in the monograph.
Response: Comment not incorporated**.** The proposed monograph is consistent with the sponsor's FDA approved acceptance criteria. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data. **Comment Summary #2:** The commenter requested deleting the relative response factors for the process impurities in the test for *Organic Impurities* as these impurities are not reported for the drug product.
Response: Comment incorporated.
Expert Committee-initiated change #1: The term 'total unspecified impurities' is revised to 'total degradation products' to be consistent with the ICH naming convention.

Monograph/Section: Rizatriptan Benzoate Tablets/Multiple Sections
Expert Committee: Monographs—Small Molecules 4
No. of Commenters: 3
Comment Summary #1: The commenter requested revising the *Sample solution* concentration in the test for *Identification A* from 1 mg/mL to 5 mg/mL to allow the same procedure to be used for all dosage strengths.
Response: Comment incorporated.
Comment Summary #2: The commenter requested tightening of the *Assay* acceptance criteria from 90.0%–110.0% to the commenter's approved criteria of 94.0–105.0%.
Response: Comment not incorporated. The acceptance criteria reflect FDA approved acceptance criteria; the public standard is intended to address all approved drug products.
Comment Summary #3: The commenter requested revising the limit of total impurities in the test for *Organic impurities* from NMT 0.75% to the commenter's approved limit of NMT 0.7%.
Response: Comment not incorporated. The acceptance criteria reflect FDA approved acceptance criteria; the public standard is intended to address all approved drug products.
Comment Summary #4: The commenter requested revising the *System suitability solution* in the test for *Organic impurities* to indicate that the volumetric flask used in the test should be pre-rinsed with hydrogen peroxide.

Response: Comment not incorporated. USP laboratory evaluation indicated that it was not necessary to rinse the volumetric flask with peroxide.
Comment Summary #5: The commenter requested deleting the resolution requirement between benzoic acid and rizatriptan-N-oxide in the test for *Organic impurities* as it is not part of the commenter's approved procedure.
Response: Comment not incorporated. The Expert Committee determined that a resolution requirement is needed in the public standard. The USP laboratory has successfully evaluated the procedure and demonstrated that the resolution requirement can be met.
Comment Summary #6: The commenter requested including two *Dissolution* procedures with the UV spectroscopy based test as Test 1 and HPLC based test as Test 2.
Response: Comment not incorporated. Submissions from two sponsors that use the identical dissolution parameters and have the identical approved tolerance values were used to develop the *Dissolution* test. There is no need for two separate *Dissolution* tests because the only difference is the analytical procedure used to determine the amount dissolved.

Monograph/Section: Rizatriptan Benzoate Orally Disintegrating Tablets/Multiple Sections
Expert Committee: Monographs—Small Molecules 4
No. of Commenters: 3
Comment Summary #1: The commenter requested including a second orthogonal test for Identification.
Response: Comment not incorporated. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.
Comment Summary #2: The commenter requested revising the heating time required in the preparation of the *System suitability solution* in the test for Organic impurities from NLT 30 minutes to 30 ± 5 minutes to provide flexibility.
Response: Comment incorporated. The Expert committee revised NLT 30 min to "about 30 minutes."
Comment Summary #3: The commenter requested replacing 6M sodium hydroxide in the *System suitability solution* in the test for *Organic impurities* with 25% sodium hydroxide.
Response: Comment incorporated.
Comment Summary #4: The commenter requested widening of the *Assay* acceptance criteria from 93.0–107% to the commenter's approved criteria of 92.5–107.5%.
Response: Comment incorporated. The *Assay* acceptance criteria were widened to 90.0–110.0% based on FDA approved limits.
Comment Summary #5: The commenter requested the inclusion of their approved *Dissolution* tolerances as *Dissolution Test 2*.
Response: Comment incorporated.
Comment Summary #6: The commenter requested deleting the limit for rizatriptan desmethyl, which is a process impurity.
Response: Comment not incorporated. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.

Monograph/Section: Timolol Maleate/Multiple Sections
Expert Committee: Monographs—Small Molecules 2

No. of Commenters: 1

Comment Summary #1: The commenter requested replacing the UHPLC procedure in the *Assay* and the test for *Organic impurities* with an HPLC procedure.

Response: Comment not incorporated. The Expert Committee determined that the proposed procedure is suitable for its intended use.

Comment Summary #2: The commenter requested widening of the relative standard deviation requirement in test for *Organic impurities* from 2.0% to 4.0%.

Response: Comment not incorporated. The Expert Committee determined that the procedure is suitable as proposed. The USP laboratory has successfully evaluated the procedure and demonstrated that the resolution requirement can be met.

Monograph/Section: Tolterodine Tartrate/Multiple Sections
Expert Committee: Monographs—Small Molecules 3
No. of Commenters: 3

Comment Summary #1: The commenter recommended adding a test and associated acceptance criteria for *Tartrate Content.*

Response: Comment not incorporated. The acceptance criteria in the proposal are consistent with FDA approved limits.

Comment Summary #2: The commenter recommended tightening the acceptance criteria for the *Assay*.

Response: Comment not incorporated. The acceptance criteria in the proposal are consistent with FDA approved limits; the public standard is intended to address all approved drug products.

Comment Summary #3: The commenter recommended tightening the acceptance criteria in the test for *Enantiomeric Purity*.

Response: Comment not incorporated. The acceptance criteria in the proposal are consistent with FDA approved limits; the public standard is intended to address all approved drug products.

Comment Summary #4: The commenter indicated that the test for *Organic Impurities* was not adequately selective for their product.

Response: Comment not incorporated. The Expert Committee determined that the procedure is adequately selective. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.

Comment Summary #5: The commenter indicated that the acceptance criteria for the *Loss on Drying* test are not suitable for their product.

Response: Comment not incorporated. The acceptance criteria in the proposal are consistent with FDA approved limits. The Expert Committee will consider future revisions to this monograph upon the receipt of the necessary supporting data.

Comment Summary #6: The commenter requested revising the relative retention time for 6-methyl-4-phenylchroman-2-one from 1.59 to 1.82 based on supporting data.

Response: Comment incorporated.

Comment Summary #7: The commenter requested correcting the chemical name for tolterodine dimer from *N*,*N*-Bis[3-(2-hydroxy-5-methylphenyl)-2-phenylpropyl]-N-isopropylamine to N,N-Bis[3-(2-hydroxy-5-methylphenyl)-3-phenylpropyl]-N-isopropylamine.

Response: Comment incorporated.

Monograph/Section: Scaffold Silk Fibroin/Multiple Sections
Expert Committee: Monographs—Biologics and Biotechnology 2

Number of Commenters: 1
Comment Summary #1: The commenter requested removing the words, “Before testing” in the *Analysis* section of *Dimensional analysis.*
Response: Comment incorporated.
Comment Summary #2: The commenter requested deleting the “s” in the phrase, “the sutures pulls through the *Sample*” in the *Analysis* section of *Suture Retention Force*.
Response: Comment incorporated.
Comment Summary #3: The commenter requested removing the words, “is determined” in the statement, “Calculate the average suture retention is determined using the equation” in the *Analysis* section of *Suture Retention Force*.
Response: Comment incorporated.
Comment Summary #4: The commenter requested removing the word, “from” in the phrase, “tear resistance load is calculated by the software from of the mechanical instrument” in the *Analysis* section of *Tear Testing*.
Response: Comment incorporated.

Monograph/Section: Schizochytrium Oil Capsules/Identification
Expert Committee: Monographs—Dietary Supplements
No. of Commenters: 1
Comment Summary: The commenter highlighted that text In the *Identification* test is not consistent with referred General Chapter <401> *Fats and Fixed Oils*, which was recently revised and is currently official.
Response: Comment incorporated.

Monograph/Section: Spirulina/Multiple Sections
Expert Committee: Monographs—Dietary Supplements
No. of Commenters: 2

Definition:
Expert Committee-initiated Change #1: The nomenclature of the spirulina species was revised to reflect the correct naming practices.

***Fatty Acid Profile*:**
Comment Summary #1: The commenter requested changing the column initial temperature from 70° to 170° in order for the fatty acids to elute within a 30-min run time.
Response: Comment incorporated.
Comment Summary #2: The commenter requested reversing the elution order of methyl alpha linolenate and methyl gamma linolenate shown in *Table 1*. The methyl gamma linolenate should elute before the methyl alpha linolenate.
Response: Comment incorporated.
Comment Summary #3: The commenter suggested the concentration of individual methyl esters in the *Standard solution* be the same for ease of preparation purposes.
Response: Comment incorporated.
Comment Summary #4: The commenter requested the percentage range of individual fatty acids in *Table 3* be broadened to reflect new data recently received.
Response: Comment incorporated.

***Content of Beta Carotene and Total Carotenoids*:**
Comment Summary #5: The commenter requested the cis-isomers be included in the determination of beta carotene content. Thus, beta carotene is the sum of all-*trans*- and *cis*-isomers.
Response: Comment incorporated.

Content of C-Phycocyanin:
Comment Summary #6: The commenter requested increasing the test sample size from 15 mg to 100 mg and the extraction time from 12 h–14 h to 16 h–24 h to improve the accuracy of the result.
Response: Comment incorporated.

Microbial Enumeration Tests <2021>:
Comment Summary #1: The commenter requested that the use of antibiotics in the culture media be allowed for suppressing bacterial growth if the total combined yeasts and molds count is expected to exceed the acceptance criteria due to bacterial growth.
Response: Comment incorporated.

Monograph/Section: Spirulina Tablets/Multiple Sections
Expert Committee: Monographs—Dietary Supplements
No. of Commenters: 2

Fatty Acid Profile
Comment Summary #1: The commenter requested changing the column initial temperature from 70° to 170° in order for the fatty acids to elute within a 30-min run time.
Response: Comment incorporated.
Comment Summary #2: The commenter requested reversing the elution order of methyl alpha linolenate and methyl gamma linolenate shown in *Table 1*. The methyl gamma linolenate should elute before the methyl alpha linolenate.
Response: Comment incorporated.
Comment Summary #3: The commenter suggested the concentration of individual methyl esters in the *Standard solution* be the same for ease of preparation purposes.
Response: Comment incorporated.
Comment Summary #4: The commenter requested the percentage range of individual fatty acids in *Table 3* be broadened to reflect new data recently obtained.
Response: Comment incorporated.

Content of Beta Carotene and Total Carotenoids
Comment Summary #5: The commenter requested the cis-isomers be included in the determination of beta carotene content. Thus, beta carotene is the sum of all-*trans*- and *cis*-isomers.
Response: Comment incorporated.

Content of C-Phycocyanin
Comment Summary #6: The commenter requested increasing the test sample size from 15 mg to 100 mg and the extraction time from 12 h─14 h to 16 h─24 h to improve the accuracy of the result.
Response: Comment incorporated.

Microbial Enumeration Tests <2021>:
Comment Summary #7: The commenter requested the total aerobic bacterial count be changed from NMT 10^4 cfu/g to NMT 5 x 10^4 cfu/g and the total combined molds and yeasts count from NMT 10^3 cfu/g to NMT 10^2 cfu/g to reflect new data recently obtained.
Response: Comment incorporated
Comment Summary #8: The commenter requested that the use of antibiotics in the culture media be allowed for suppressing bacterial growth if the total combined yeasts and molds count is expected to exceed the acceptance criteria due to bacterial growth.
Response: Comment incorporated.

Monograph/Section: St. John's Wort/Identification
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: A reference to the new General Chapter <203> *High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin* was added to the Identification B.

Monograph/Section: Turmeric /Specific Tests—Botanical Characteristics
Expert Committee: Monographs—Dietary Supplements
Expert Committee-initiated Change #1: The decision was made to retain organoleptic characteristics of the article within the body of the monograph.