Commentary

USP–NF 2025 Issue 1

November 1, 2024; revised January 23, 2025

In accordance with USP's *Rules and Procedures of the Council of Experts ("Rules*"), and except as provided in Section 9.02 *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 (EC) deems appropriate, the proposal may advance to official status or be re-published in *PF* for further notice and comment, in accordance with the Rules. In cases when proposals advance to official status*,* a summary of comments received and the appropriate Expert Committee's responses, as well as Expert Committee initiated changes, are published in the Proposal Status/Commentary section of USPNF.com 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 or conflict between the contents of the *Commentary* and the official text, the official text prevails.

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Comments were received for the following when they were proposed in Pharmacopeial Forum (PF):

General Chapters
<86> Bacterial Endotoxins Test Using Recombinant Reagents
<129> Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies
<198> Nuclear Magnetic Resonance Spectroscopy Identity Testing of Bacterial Polysaccharides Used in Vaccine Manufacture
<208> Anti-factor Xa and Anti-factor IIa Assays for Unfractionated and Low Molecular Weight Heparins
<401> Fats and Fixed Oils
<1132.1> Residual Host Cell Protein Measurement in Biopharmaceuticals by Liquid Chromatography-Mass Spectrometry
<1243> Wetting Properties of Pharmaceutical Systems

Monographs

Adapalene and Benzoyl Peroxide Gel
Aspirin
Bendamustine Hydrochloride
Caffeine
Cefoxitin for Injection
Cefoxitin Sodium
Chlorhexidine Gluconate and Isopropyl Alcohol Topical Solution
Clioquinol
Daunorubicin Hydrochloride
DL-Lactide and Glycolide (50:50) Copolymer 12000 Acid
DL-Lactide and Glycolide (50:50) Copolymer 12000 Ethyl Ester
Hard Gelatin Capsule Shells
Hard Hypromellose Capsule Shells
Hard Pullulan Capsule Shells
Isradipine Compounded Oral Suspension
Ketamine Compounded Cream (updated 23-Jan-2025)
Mannose
Menthol
Nisoldipine
Permethrin
Sodium Nitroprusside
Sodium Nitroprusside Injection
Tolterodine Tartrate
Tolterodine Tartrate Extended-Release Capsules
Ulipristal Acetate
Vardenafil Tablets
Xanthan Gum

No comments were received for the following proposals:

Monographs
Acacia
Bendamustine Hydrochloride for Injection

Dextromethorphan Hydrobromide Oral Solution
Diethylene Glycol Stearates
Dimethyl Isosorbide
DL-Lactide and Glycolide (50:50) Copolymer 46000 Acid
Ethotoin
Ethotoin Tablets
Fish Oil Containing Omega-3 Acids
Fish Oil Containing Omega-3 Acids Capsules
Fish Oil Containing Omega-3 Acids Delayed-Release Capsules
Ibandronate Sodium
Krill Oil
Krill Oil Capsules
Krill Oil Delayed-Release Capsules
Methylene Blue Compounded Injection, Veterinary
Neomycin Sulfate and Hydrocortisone Cream
Neomycin Sulfate and Hydrocortisone Otic Suspension
Neomycin Sulfate and Hydrocortisone Acetate Ophthalmic Suspension
Neomycin Sulfate and Triamcinolone Acetonide Cream
Neomycin Sulfate and Methylprednisolone Acetate Cream
Omega-3 Acids Triglycerides
Omega-3 Free Fatty Acids
Oxymetholone Tablets
Polyoxyl 20 Cetostearyl Ether
Powdered Red Clover
Powdered Red Clover Extract
Red Clover
Streptococcus Salivarius
Trimipramine Capsules
Ulipristal Acetate Tablets

General Chapters

General Chapter/Section(s):	<86> Bacterial Endotoxins Test Using Recombinant Reagents
Expert Committee(s):	General Chapters – Microbiology
No. of Commenters:	40

GENERAL COMMENTS

Comment Summary #1: The commenter recommended modifying several monographs to include <86> as an option for *Bacterial Endotoxins Test* <85>, equating <85> to <86>. Specific monographs mentioned include *Water for Hemodialysis*, *Somatropin for Injection*, *Water for Injection*, and others.

Response: Comment not incorporated. The approach of including <86> as an alternative for these monographs is the scope of this current revision. The inclusion of this chapter in monographs will be considered as a future revision. USP accepts validated methods for inclusion in monographs.

Comment Summary #2: The commenter suggested modifying *Injections and Implanted Drug Products (Parenterals)* <1> – *Product Quality Tests*, *Bacterial Endotoxin* section to include <86> as an option, equating <85> to <86>.
Response: Comment not incorporated. This change is out of the scope of the current revision. This approach may be considered as a future revision to the *USP-NF*.
Comment Summary #3: The commenter encourages the adoption of a pyrogenicity test concept (e.g., Monocyte Activation Test (MAT) for the Rabbit Pyrogen Test (RPT)) in the USP *General Notices*, with language that specifically equivocates <85> to <86>.
Response: Comment not incorporated. This change is out of the scope of the current revision. The approach will be considered as a future revision.
Comment Summary #4: The commenter asked for clarification on showing comparability between recombinant and classic reagents, specifically whether inhibitory and enhancing products should be included and if this applies to medical devices.
Response: Comment not incorporated. While this change has not been incorporated in <86>, the topic has been incorporated into *USP*'s FAQs.
Comment Summary #5: The commenter requested clarity on whether companies can directly use <86> for new products and forego *Bacterial Endotoxins Test* <85>, noting contradictions between the FAQ provided by USP and the draft <86>.
Response: Comment not incorporated. The definition of alternative methods is outlined in *General Notices 6.30*, and no further clarification is needed at this time.
Comment Summary #6: The commenter emphasized that for <86> to offer a usable solution for industry, recombinant bacterial endotoxins test (rBET) methods must be placed on equal footing with current compendial methods. They highlighted the need for a statement that <86> is equivalent to <85>.
Response: Comment not incorporated. This chapter is considered to be an alternative method at this time. This change would require further data and consideration.
Comment Summary #7: The commenter recommended that Chapter <86> guidelines be applicable to both existing and new pharmaceutical products, emphasizing the need for global harmonization of rFC policies and the acceleration of the adoption of Chapter <86>.
Response: Comment not incorporated. Chapter <86> already applies to existing and new products. USP recognizes the need for global harmonization, but changes cannot be expedited without further data and review.
Comment Summary #8: The commenter advocated for the USP's consideration of synthetic alternatives for endotoxin testing to alleviate the dependence on horseshoe crab blood.
Response: Comment acknowledged.
Comment Summary #9: The commenter found it unusual for Chapter <86> to be published as an alternative microbiological method requiring validation and suggested incorporating <86> into <85>.
Response: Comment not incorporated. Modifying a globally harmonized chapter was previously proposed and comments were received that disagreed with the inclusion of recombinant reagents into <85>. Recombinant reagents will remain separate as chapter <86> for now.
Comment Summary #10: The commenter urged USP to finalize Chapter <86> as quickly as possible to address threats to ecosystems and supply chains.
Response: Comment not incorporated. USP followed its established review process and timeline for finalizing Chapter <86>.

INTRODUCTION
Comment Summary #11: Several commenters suggested clarification on the classification of tests as alternative tests unless specified in an individual monograph.
Response: Comment incorporated. The text was modified to clarify that tests are considered alternative methods unless specified in an individual monograph, with references to *Verification*

of Compendial Procedures <1226> and reference to *General Notices 3.10* added to the *Introduction*.
Comment Summary #12: The commenter suggested reconsidering the definition of a cascade reagent (rCR) as specifically containing recombinant Factor C (rFC), recombinant Factor B (rFB), and recombinant proclotting enzyme stating that this definition could limit future development of new recombinant reagents.
Response: Comment not incorporated. The Expert Committee would need to review data to include future rCR reagents.
Comment Summary #13: Several commenters requested the addition of *Tachypleus gigas* to the list of horseshoe crab species whose gene sequences are used for reagents in endotoxin tests.
Response: Comment not incorporated. This could be considered for future revisions if such a kit becomes commercially available, and data is provided for review.
Comment Summary #14: Commenters requested the removal of references to specific detection techniques—endpoint fluorescence technique and chromogenic technique—citing the possibility that other technologies may become available in the future.
Response: Comment not incorporated. The Expert Committee will need to review data to include new detection techniques.
Comment Summary #15: Four commenters requested more clarity regarding the user's responsibility in reviewing the supplier's primary validation package. They suggested adding specific text to ensure alignment with the criteria outlined in *Validation of Compendial Procedures* <1225>.
Response: Comment not incorporated. Requirements for alternative methods, including primary validation, are outlined in *General Notices*, *6.30 Alternative and Harmonized Methods and Procedures.*
Comment Summary #16: Several commenters suggested that the requirements for method verification should be more clearly defined. They recommended adding text to include using spiked samples and field samples of products found to be positive, as well as addressing variability in normal use and manufacturing processes.
Response: Comment not incorporated. The section *Verification – Tests for Interfering Factors* contains sufficient guidance on method verification.
Comment Summary #17: The commenter suggested changing the phrase "consult each regulatory authority" to "consult their relevant regulatory authority" for clarity.
Response: Comment incorporated.
Comment Summary #18: The commenter suggested that companies wishing to change from the method described in <85> to one in <86> may need to provide supplemental data to regulatory authorities. They recommended that users consult each regulatory authority and provided an example of supplemental data, such as a comparative study.
Response: Comment incorporated.
Comment Summary #19: Seven commenters requested examples or alignment of terminology.
Response: Comment partially incorporated. The Expert Committee is providing examples of verification testing.

APPARATUS
Comment Summary #20: The commenter suggested changing the title of the "Apparatus" section to "Material or Material Preparation" as the information refers to test tubes and their preparation rather than spectrophotometers or fluorimeters.
Response: Comment not incorporated. The title will remain as "Apparatus" to maintain alignment with <85>.
Comment Summary #21: The commenter proposed a change to specify that the minimum time and temperature for depyrogenating heat-stable materials is 30 minutes at 250°C. They also

suggested ensuring single-use labware is free of detectable endotoxin and does not interfere with the test.
Response: Comment incorporated.
Comment Summary #22: The commenter proposed adding a note at the end of the sentence to include a validity test for the procedure for inactivating endotoxins, referencing <85> and *Sterility Assurance* <1211>.
Response: Comment not incorporated. The requirement for a validated process ensures that an appropriate sensitivity is established, making the additional note unnecessary.

REAGENTS AND TEST SOLUTIONS
Comment Summary #23: Several commenters suggested specifying that all reagents, including the substrate and assay buffer, must be free of detectable endotoxin.
Response: Comment incorporated.

Water for Bacterial Endotoxins Test (BET)
Comment Summary #24: The commenter suggested that the term “LAL reagent water (LRW)” should be used instead of "Water for Injection" (WFI) to avoid errors, as WFI is not necessarily endotoxin-free. They recommended that any water source other than the manufacturer’s LALRW must be qualified before use.
Response: Comment not incorporated. The requirement aligns with <85>.

PREPARATION OF SOLUTIONS
Standard Endotoxin Stock Solutions
Comment Summary #25: The commenter questioned the use of "Endotoxin Units (EU)" and suggested using "International Units (IU)," stating that IU has been the international standard since the late 1990s.
Response: Comment not incorporated. The use of "Endotoxin Units (EU)" is in line with the Endotoxin Reference Standard (RS) nomenclature.
Comment Summary #26: The commenter suggested changing the word "specifications" to "instructions" in the phrase “follow the specifications in the package” for clarity.
Response: Comment incorporated.

Standard Endotoxin Solutions
Comment Summary #27: The commenter suggested specifying "vortexing" instead of "vigorous mixing" for the *Standard Endotoxin Stock solution* to ensure non-absorption of endotoxin and high homogeneity.
Response: Comment not incorporated. The term "vigorous mixing" will remain as there are multiple methods for mixing.
Comment Summary #28: The commenter suggested including a more specific time frame for using dilutions to avoid loss of activity by adsorption. They recommended specifying "immediately before use" instead of "as soon as possible."
Response: Comment not incorporated. The time frame for using dilutions should be verified through validation.

Sample Solutions
Comment Summary #29: The commenter suggested changing the phrase “may be more appropriately dissolved” to “may be better dissolved” for clarity.
Response: Comment not incorporated. The phrase is aligned with the language in <85>.
Comment Summary #30: The commenter recommended deleting the sentence about the validity of the positive product control indicating the potential necessity to adjust the sample pH.

Response: Comment incorporated.

DETERMINATION OF MAXIMUM VALID DILUTION

Endotoxin Limit

Comment Summary #31: The commenter suggested changing the formula to Endotoxin Limit instead of Result.

Response: Comment incorporated for clarity.

Comment Summary #32: Three commenters requested adding specific criteria similar to those found in *Guidelines on Endotoxins Test* <1085>.

Response: Comment not incorporated. The chapter is aligned with *European Pharmacopoeia* 2.6.32 and 5.1.10. Readers are referred to <1085> for additional details.

QUANTITATIVE TECHNIQUES

Fluorometric Technique

Comment Summary #33: The commenter suggested changing the terms to "Fluorometric Technique" and "Chromogenic Technique".

Response: Comment incorporated.

Comment Summary #34: The commenter suggested replacing the phrase with “This technique is an assay to measure the fluorescence [relative fluorescence units (RFU)] emitted by a fluorescent substrate as a result of cleavage by endotoxin and recombinant factor C” for clarity.

Response: Comment not incorporated. The current language is aligned with *European Pharmacopoeia* 2.6.32.

Comment Summary #35: The commenter suggested removing the word "typically" from the sentence "It is typically used as an endpoint fluorescence test."

Response: Comment incorporated.

Comment Summary #36: The commenter suggested removing the phrase about blank-corrected ΔRFU, stating that it is unnecessary if all wells in a microplate are read at the beginning of an assay and each well functions as its own blank-correction.

Response: Comment incorporated.

Chromogenic Technique

Comment Summary #37: The commenter requested to remove absorbance from the section title.

Response: Comment incorporated.

Comment Summary #38: The commenter suggested replacing the term "lysate" with "reagent" in the phrase "peptide by the reaction of endotoxins with reagent" to improve clarity.

Response: Comment incorporated

Comment Summary #39: The commenter suggested inserting "mAbs/min" to specify the rate of color development and avoid confusion.

Response: Comment not incorporated. These are vendor-specific instructions that are not appropriate for the general chapter.

Preparatory Testing

Comment Summary #40: The commenter suggested rephrasing the sentence to improve clarity, proposing: “These tests demonstrate that the criteria for the standard curve are valid and that the sample solution does not interfere with the test.”

Response: Comment not incorporated. The text is in alignment with <85> and *European Pharmacopoeia* 2.6.32.

Comment Summary #41: The commenter suggested changing the term "validation" to "verification" in the sentence: “Supplemental validation for the test method is required when any changes are made to the experimental conditions that are likely to influence the test result.”
Response: Comment not incorporated. The term "validation" aligns with the requirements described in *General Notices 6.30* for alternative methods.

Standard Curve Criteria
Comment Summary #42: The commenter suggested clarifying that instrument sensitivity adjustment refers to fluorometric techniques only. They recommended either specifying this or moving the statement to the section “Fluorometric Quantitative Technique.”
Response: Comment incorporated. The statement was moved to *Fluorometric Technique.*
Comment Summary #43: The commenter recommended changing the phrase “log change” to "log increase."
Response: Comment incorporated.
Comment Summary #44: Four commenters noted that <85> specifies using three replicates of each concentration of the endotoxin standard, suggesting alignment with this requirement.
Response: Comment not incorporated. The requirement for a minimum of two replicates aligns with commercial ready-to-use standard curve formats. Users may still use three replicates if desired.

Verification—Test for Interfering Factors
Comment Summary #45: The commenter suggested removing the term “verification” from the phrase because verification is used in <1226> for different requirements.
Response: Comment not incorporated. The term “verification” involves method verification, ensuring the procedure can be used for its intended purpose under actual conditions.
Comment Summary #46: The commenter suggested changing the phrase to "potential difference in interference."
Response: Comment not incorporated. The addition does not add clarity.
Comment Summary #47: The commenter recommended mentioning suitable treatments for interference, such as filtration, neutralization, dialysis, heat treatment, or endotoxin-specific binding steps, to harmonize with *European Pharmacopoeia* 2.6.32.
Response: Comment not incorporated. Suitable treatments for interference should refer to the manufacturer's instructions.
Comment Summary #48: The commenter asked whether "the result with Solution D does not exceed the limit of the blank value required in the description of the reagent mixture employed or is less than the endotoxin detection limit of the recombinant reagent employed" refers to a negative control.
Response: Comment not incorporated. The current text aligns with <85> and *European Pharmacopoeia* 2.6.32, which states *Solution A*. No changes will be made as Solution D cannot be on the curve.
Comment Summary #49: The commenter asked for clarification on the term "blank-corrected mean endotoxin concentration" They also noted that *European Pharmacopoeia* 2.6.32 does not refer to "blank-corrected mean endotoxin concentration."
Response: Comment incorporated. “Blank-corrected” was removed.
Comment Summary #50: The commenter suggested editing the sentence for clarity regarding whether the subtraction of endotoxin is from the product positive control or from the standard.
Response: Comment not incorporated. The text will remain unchanged to maintain alignment with <85>.

TEST PROCEDURE

Comment Summary #51: The commenter requested changes for clarity in the "Test procedure, Calculation" section.
Response: Comment not incorporated. The current text and calculation method will remain unchanged to maintain alignment with <85>.
Comment Summary #52: The commenter recommended a revised calculation method for endotoxin recovery, and another suggested rephrasing the sentence to specify using conditions that show no interference.
Response: Comment not incorporated. The current text and calculation method will remain unchanged to maintain alignment with <85>.

Calculation
Comment Summary #53: The commenter suggested replacing the criteria for the negative control Solution D with a simpler criterion to make it easier to determine.
Response: Comment not incorporated. The current criterion aligns with <85> and *European Pharmacopoeia*. 2.6.32. Simplifying the language and specifying the use of the average would make the chapter too prescriptive.

General Chapter:	<129> Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies
Expert Committee(s):	Biologics Monographs 1 – Peptides and Oligonucleotides
No. of Commenters:	5

Introduction
Comment Summary #1: The commenter requested clarification on the statement “Such alternative procedures and methods shall be validated as described in *Validation of Compendial Procedures* <1225> and must be shown to give equivalent or better results.”
Response: Comment incorporated. The paragraph was revised to “Alternative methods and/or procedures may be used if such alternative procedures and methods are appropriately validated as described in *Validation of Compendial Procedures* <1225>.”

Size-Exclusion Chromatography, Method 1
Comment Summary #2: The commenter suggested removing System suitability blank because there is no specified use for it.
Response: Comment incorporated.

Size-Exclusion Chromatography, Method 2
Comment Summary #3: The commenter indicated that they have a different validated method and would like to submit it for consideration.
Response: Comment not incorporated. The Expert Committee will consider further revisions upon receipt of supporting data.
Comment Summary #4: The commenter suggested using 0.2 µm filter or less to filter the mobile phase for the UHPLC application.
Response: Comment not incorporated. 0.45 µm filter was used in the validation and NMT 0.45 µm includes 0.2 µm and less.
Comment Summary #5: The commenter suggested including the stability of *System suitability solution*.
Response: Comment partially incorporated. The stability of *System suitability solution* in Method 1 was deleted with additional data support. A note of “store at 2°–8° if not used immediately” was added for *System suitability solution* in both Method 1 and Method 2.
Comment Summary #6: The commenter suggested adding a note to describe formulation buffer.

Response: Comment not incorporated. Formulation buffer is based on product formulation and could vary among manufacturers.
Comment Summary #7: The commenter indicated that there is no representative chromatogram in the certificate of USP Monoclonal IgG System Suitability RS for Method 2.
Response: Comment not incorporated. Representative chromatogram is included in the certificate of USP Monoclonal IgG System Suitability RS for Method 2.
Comment Summary #8: The commenter suggested adding an injection of a *Blank* as the final injection before the final *System suitability solution injection* in the *Analysis*.
Response: Comment partially incorporated. The statement was revised to "An injection of a *Blank* should be included as the final injection after the final *System suitability solution injection*." in both Method 1 and Method 2.
Comment Summary #9: The commenter suggested adding equations to calculate HMWS & LMWS peaks.
Response: Comment incorporated. Equations of HMWS, main peak and LMWS calculations were added in both Method 1 and Method 2.

Capillary SDS Electrophoresis (Reduced and Nonreduced)
Comment Summary #10: The commenter suggested including "Capillary Gel Electrophoresis" as a parenthetical within the section.
Response: Comment incorporated.
Comment Summary #11: The commenter recommended including the composition of the SDS gel buffer or including a statement to only use the manufactured kit.
Response: Comment partially incorporated. The composition of the gel buffer is proprietary. The SDS gel buffer preparation was revised to "Buffer at a pH of 8.0 containing 0.2% (w/w) SDS and separation using entangled polymer network for the SDS-protein complexes."
Comment Summary #12: The commenter recommended replacing neat β-mercaptoethanol with undiluted β-mercaptoethanol and adding the grade of β-mercaptoethanol in the Reduced system suitability solution preparation.
Response: Comment partially incorporated. Neat was replaced with undiluted. The users can choose any suitable grade of β-mercaptoethanol.
Comment Summary #13: The commenter suggested specifying the appropriate instrument to use.
Response: Comment not incorporated. USP does not endorse any brand of instruments. Users can choose any suitable instrument from the market.
Comment Summary #14: The commenter suggested keeping consistency in the use of *Blank* solution in the reducing and nonreducing conditions.
Response: Comment not incorporated. The *Blank* solution is only used in the *Suitability requirements* for nonreducing conditions.
Comment Summary #15: The commenter recommending listing an injection sequence and number of replicates to determine RSD for the system suitability of nonreducing conditions.
Response: Comment not incorporated. Injection sequence is not included in monographs or general chapters. Users can determine the number of replicates for RSD calculation.

Oligosaccharide Analysis—Analysis of N-Linked Oligosaccharides of Monoclonal Antibodies
Comment Summary #16: The commenter suggested replacing structure with glycans in the statement of "If applicable, one or more of the following analytical approaches can be employed for oligosaccharide profiling or quantitation of individual structure." For clarity.
Response: Comment incorporated.
Comment Summary #17: The commenter suggested revising the incubation conditions in the sample solution preparation.

Response: Comment incorporated. The incubation was changed to “Incubate at appropriate temperature for suitable time for the PNGase F used.”
Comment Summary #18: The commenter suggested either replacing the sample solution preparation procedure with solid-phase extraction (SPE) method or including text to recommend SPE as an alternative method.
Response: Comment incorporated. Suitable solid-phase extraction (SPE) was added as an alternative method.

Monosaccharide Analysis—Sialic Acid Analysis
Comment Summary #19: The commenter suggested clarifying oxygen level for water used in the Solution A preparation.
Response: Comment partially incorporated. The note about water used was deleted since *General Notices 8.230* and <1231> contain detailed information.

Expert Committee initiated change #1: The molecular weight of USP N-Glycolylneuraminic Acid RS was changed to 325.27 from 325.3 to be consistent with USP style of molecular weights.

General Chapter:	<198> Nuclear Magnetic Resonance Spectroscopy Identity Testing of Bacterial Polysaccharides Used in Vaccine Manufacture
Expert Committee(s):	Biologics Monographs 3 – Complex Biologics and Vaccines
No. of Commenters:	2

1.2 *O*-Acetylated Polysaccharides
Comment Summary #1: The commenter questioned the degree of *O*-acetylation is considered as part of the Identity test.
Response: Comment partially incorporated. The statement was revised to “For those polysaccharides that are *O*-acetylated, the presence of *O*-acetylation of the polysaccharides is considered part of the identity test and the degree of *O*-acetylation is important in consistency of manufacture.” for clarity.

2.1 Equipment Requirements
Comment Summary #2: The commenter requested clarification on temperature calibration within ±3° in the NMR Spectrometer section.
Response: Comment incorporated. The statement was revised to “A temperature accurate to within ±3° of the desired temperature” for clarity.
Comment Summary #3: The commenter requested clarification on the relationship between system suitability requirements and NMR tube quality in the NMR Tubes section.
Response: Comment partially incorporated. The statement was revised to “The line width criterion of the system suitability test using the USP procedural reference material (3.3 Acceptance Criteria) is an appropriate means to establish the equipment, including the NMR tubes, is fit for purpose.”

2.2 Reagents for Vaccine Polysaccharide Sample Solutions
Comment Summary #4: The commenter suggested removing DSS preferred in the Chemical Shift Reference Compounds section.
Response: Comment not incorporated. DSS is an accepted standard for NMR and has been widely used.

2.4 Experimental Procedures
Comment Summary #5: The commenter suggested providing information about this target concentration instead of the volume to be used in the *Sample Preparation* section.
Response: Comment not incorporated. The volume is important for complete filling of the coil. *Section 2.3* contains the sample amount between 0.5 and 20 mg for analysis. The volume is included in *Section 2.4*. It is sufficient for the target concentration estimate.

2.7 Procedure 2
Comment Summary #6: The commenter suggested removing the base-catalyzed de-*O*-acetylation inside the NMR tube in the Scope.
Response: Comment partially incorporated. The statement was revised to "Base-catalyzed de-O-acetylation of the polysaccharide can be conducted in a separate vial, however, the reaction in the NMR tube provides advantages and has shown to prove useful."

3.1 System Suitability Solution
Comment Summary #7: The commenter suggested adding stability information of the solution prepared with USP PS NMR System Suitability RS.
Response: Comment not incorporated. Manufacturers could assess the stability under their storage conditions.

4.2 *Haemophilus Influenzae* Type b Polysaccharide
Comment Summary #8: The commenter suggested removing the signal-to-noise ratio information as the Section *5 Assay Criteria* already contains the information.
Response: Comment partially incorporated. All signal-to-noise ratio information was moved to Section *5.3*.
Comment Summary #9: The commenter suggested adding identity can be confirmed using Procedure 1a or 1b in Section *4.2*.
Response: Comment incorporated. The statement "Alternatively, identity can be confirmed using *2.6 Procedure 1b* with numerical comparison of test and reference spectra" was added to the end of Assessing Identity section.

General Chapter:	<208> Anti-factor Xa and Anti-factor IIa Assays for Unfractionated and Low Molecular Weight Heparins / Multiple sections
Expert Committee(s):	Biologics Monographs 3-Complex Biologics & Vaccines
No. of Commenters:	5

General Comments
Comment Summary #1: The commenter requested to clarify if either an endpoint measurement or kinetic measurement is used, and to provide the necessary details for data collection and analysis.
Response: Comment incorporated. The endpoint measurement or kinetic measurement can be used for unfractionated Heparin and low molecular weight heparin. Endpoint measurement and kinetic point measurement instructions were added to all the tests.
Comment Summary #2: The commenter suggested changing the reagent albumin bovine serum to the more commonly used bovine serum albumin.
Response: Comment incorporated.

Introduction
Comment Summary #3: The commenter requested to add **amidolytic test** to the sentence, "The residual factor IIa or factor Xa not inhibited by the heparin–AT complex is quantified

by a chromogenic substrate that is specific for either factor IIa or factor Xa **amidolytic test** and is added in the final step."
Response: Comment incorporated. Amidolyitic test was added to the sentence.

General Considerations
Comment Summary #4: The commenter requested to remove the phrase, "but it is basically the same requirement" from the sentence "However, the need to prewarm the reagents, and the mode of incubation are described slightly differently for all four assays~~, but it is basically the same requirement~~."
Response: Comment partially incorporated. The entire sentence was deleted instead of just the phrase, "but it is basically the same requirement" because the sentence is repetitive with the text for each procedure that is later in the chapter.
Comment Summary #5: The commenter suggested clarifying whether the protease used in each test is factor Xa or IIa**.**
Response: Comment incorporated. To clarify the protease that is used, Factor Xa or Factor IIa was added after the word protease in the *General Considerations* section.
Comment Summary #6: The commenter requested clarification on how the suitability of new batches is verified.
Response: Comment not incorporated. A description of how different batches of reagents are verified is outside this document's scope.
Expert Committee initiated change #1: To clarify that endpoint and kinetic assays can be used, the phrase, "kinetic assays" was added to this statement, "The procedures described as endpoint assays" The sentence was changed to, "The procedures are described as endpoint **and kinetic** assays."

Anti-Factor Xa and Anti-Factor IIa for Unfractionated Heparin
Expert Committee initiated change #2: The statement "All incubations should be performed at 37°" was removed because this is already stated in the *General Considerations* section.
Comment Summary #7: The commenter suggested editing the statement to, "All reagents, Standard solutions, and Sample solutions should be prewarmed when required ~~to 37° just before use~~.
Response: Comment partially incorporated. The sentence, "All reagents, Standard solutions, and Sample solutions should be prewarmed to 37° just before use." was removed from the *Note* in all assays because the prewarming of solutions is covered in the *General Considerations* section.
Expert Committee initiated change #3: The wording in the *Analysis* section, under Kinetic measurement was changed follows, "Follow the change in absorbance for each solution for **at least** ~~over~~ 1 min at 405 nm…"
Expert Committee initiated change #4: The following sentence was deleted from the Slope ratio assay and the *Parallel-line assay* calculation, "Express the potency of heparin sodium per milligram, calculated on the dried basis." This sentence was deleted because it only applies to the drug substance, and the chapter can be used for drug products or drug substances.
Comment Summary #8: The commenter recommended to add consistency and choose to describe every solution preparation by weight or molar concentration.
Response: Comment incorporated. The description of the preparation of the *pH 8.4 buffer* solution was aligned so that all solutions are prepared by weight.
Comment Summary #9: The commenter requested clarification of the reason for a range of weight (0-10.0 g) of polyethylene glycol 6000 is provided in the *pH 8.4 buffer* solution preparation and suggested that 1.0 grams of polyethylene glycol would be more appropriate for this preparation.

Response: Comment not incorporated. A range of weight is provided for the polyethylene glycol 6000 because flexibility is needed due to instrumental differences.
Comment Summary #10: The commenter requested that additional information be provided to clarify if it is necessary to use a spectrophotometer blank if a multi-well microtiter plate is used to take the endpoint measurement.
Response: Comment not incorporated. The procedures in the chapter are described for performing the analysis using tubes. If multi-well plates are used, the user should follow their own procedures for performance in multi-well plate because blanking of plates may be specific for the instrument type. There is no need to add details about using a microtiter plate or a blank for the microtiter plate because this information can be specific for the instrument type and manufacturer.
Comment Summary #11: The commenter recommended moving the incubation step to the "Endpoint measurement" description later in the section.
Response: Comment incorporated. The phrase "incubate for exactly 2 min" was moved to the *Endpoint Measurement* section of the *Analysis*.
Comment Summary #12: The commenter recommended revising the procedure for Endpoint Measurement: Endpoint measurement: **Incubate the mixture for exactly 2 min**. Add 150 µL of Stopping solution to each tube, and mix. To zero the spectrophotometer.
Response: Comment incorporated. The phrase "incubate for exactly 2 min" was moved to the *Endpoint Measurement* section of the *Analysis*.

Anti-Factor Xa Assay for Unfractionated Heparin
Comment Summary #13: The commenter requested details of how to perform a kinetic measurement using multi-well microtiter plates and a microplate reader, including how long to read the absorbance for the Anti-Factor Xa assay.
Response: Comment not incorporated. Kinetic measurement can be done using microtiter plate, but the procedure is dependent on the instrument and how the chromogenic substrate is added to the 96 wells. Describing this detail for the number of ways the microtiter plates can be used is out of scope of the chapter.

Anti-Factor IIa Assay for Unfractionated Heparin
Comment Summary #14: The commenter requested to include 20% v/v acetic acid (or less concentrated) to avoid impact on existing applications.
Response: Comment not incorporated. The method has been developed and validated using 2% acid and not using 20% acid. As specified in *General Notices 6.30*. *Alternative and Harmonized Methods and Procedures of General Notices*, an alternative method using a concentration of 20% v/v acetic acid can be used if it is shown to be equivalent or better than the method in <208>, and the method is validated.
Comment Summary #15: The commenter requested to add an option to perform 4 or 5 blanks to avoid impact on existing applications.
Response: Comment incorporated. The wording was changed to "include at least four reagent blanks" in the test.
Comment Summary #16: The commenter requested to adjust the volumes to use multi-well microtiter plates.
Response: Comment not incorporated. Changing the procedure to use a microtiter plate deviates from the validated compendial procedure which would need to be validated and shown to be equivalent or better than the compendial method.
Comment Summary #17: The commenter recommended removing the duplication of the statement "Solutions should be prewarmed to 37°C just before use."
Response: Comment incorporated. The duplicate statement, "Solutions should be prewarmed to 37° just before use" was removed.

Comment Summary #18: The commenter suggested updating the 150 µL buffer amount to align with the amount or volume used in the *Assay*.
Response: Comment not incorporated. The volumes used in the assay should be consistent with other assays in the general chapter. The 150ul was not changed because it was the correct volume for the other volumes of the assay.
Comment Summary #19: The commenter requested clarification on how the change in absorbance per minute is measured in the anti-factor IIa assay for UFH and if the data should be collected after a certain period of time after the chromogenic substrate is added or measure immediately after adding the chromogenic substrate.
Response: Comment not incorporated. The reading from the spectrophotometer is not immediate. Measurement can be read for a certain amount of time, but this is dependent on the user to determine what time is appropriate as different instruments may have different lag times before reading. The user will also want to establish reading in the linear part which may vary due to equipment or assay conditions.
Comment Summary #20: The commenter recommended changing the *System suitability* criteria to 10% RSD if 5 blanks are used.
Response: Comment not incorporated. There is data to support an RSD of NMT 5% for 4 reagent blanks.
Comment Summary #21: The commenter requested that USP consider changing the system suitability criteria to “RSD of four reagent blanks is NMT 10%”
Response: Comment not incorporated. There is data to support an RSD of NMT 5% for 4 reagent blanks.

Anti-Factor Xa and Anti-Factor IIa for Low Molecular Weight Heparin
Comment Summary #22: The commenter requests to clarify when the Chromogenic substrate solution is pre-heated because there is a note to preheat reagents for 15 minutes before use in the Anti-Factor Xa Activity for Low Molecular Weight Heparin, but this note is not included in other assays in the chapter.
Response: Comment partially incorporated. The *Note* was deleted, as it does not appear in the other assays in the General Chapter, and instructions to prewarm the reagents are included in the *General Considerations* section.
Comment Summary #23: The commenter suggested to delete “log change in absorbance per minute” in the Anti Factor Xa and IIa activity for Low Molecular Weight Heparin the analysis is for endpoint measurement only, but the calculation specifies “log change in absorbance per minute.”
Response: Comment partially incorporated. The Anti Factor Xa and IIa activity for Low Molecular Weight Heparin can be determined using endpoint measurement or kinetic measurement. To clarify this, instructions on how to perform and Endpoint measurement or a kinetic measurement were added to both the Anti-factor Xa and IIa activity assays for Low Molecular Weight Heparin.

General Chapter/Section(s):	<401> Fats and Fixed Oils/Water and Sediment in Fixed Oils
Expert Committee(s):	Excipients Test Methods
No. of Commenters:	1

Comment Summary #1: The commenter requested to delete the test which uses benzene for *Water and Sediment in Fixed Oils* because it is not used in any monograph and the change will support the USP initiative for reduction of the environmental footprint.
Response: Comment not incorporated. This test is not part of the proposed revision. The Expert Committee will consider a future revision based on available data and information.

General Chapter/Section(s): <1132.1> Residual Host Cell Protein Measurement in Biopharmaceuticals by Liquid Chromatography-Mass Spectrometry/Multiple Sections
Expert Committee(s): Biologics Monographs 2 – Proteins
No. of Commenters: 15

GENERAL COMMENTS
Comment Summary #1: The commenter suggested adding column priming into the general chapter.
Response: Comment not incorporated. Column priming is a normal HPLC practice. The Expert Committee determined that this level of detail is beyond the scope of this general chapter.
Comment Summary #2: The commenter requested to provide additional information about how to identify signals when non-proteins may be visualized on LCMS systems with the increased amount of data generation.
Response: Comment not incorporated. The comment has been addressed in section 4. *Sample Preparation, Chromatographic Separation, and Mass Spectrometry Analysis* (e.g., detergents).
Comment Summary #3: The commenter indicated that correct quantification may be more challenging with MS than in current HCP detecting techniques such as ELISA. However, it is recognized that MS may provide satisfactory detection of HCP.
Response: Comment acknowledged.
Comment Summary #4: The commenter suggested using MS after ELISA demonstrates increase in HCP to identify the HCP.
Response: Comment not incorporated. This topic has been addressed in other parts of this general chapter. Comment may relate to process comparability.
Expert Committee initiated change #1: Different terms such as “drug product”, “protein product”, “product protein”, “active product”, and “product” were used throughout the chapter. Each term was reviewed, and the most accurate one was selected for the specific context.

TITLE
Comment Summary #5: The commenter suggested adding LC to the title because LC is discussed in the chapter.
Response: Comment incorporated.

INTRODUCTION AND SCOPE
Comment Summary #6: The commenter suggested revising the second sentence of the first paragraph as follows: “During the manufacture of such products, HCPs are molecularly heterogenous class of process-related impurities that are coproduced during upstream expression of the desired product.”
Response: Comment not incorporated. The description of HCPs as a "molecularly heterogenous" class of process-related impurities" is correct and has already been described this way. The term "significant" reflects that HCPs are a main class of impurities and always required for drug substance testing, thus worthy of attention.
Comment Summary #7: The commenter suggested adding “other activities” along with protease, glycosides, and lipase because all of these can impact product quality and stability.
Response: Comment incorporated.
Comment Summary #8: The commenter suggested indicating that other types of enzymatic activity, besides protease, glycosides, and lipase, can also impact product quality and stability.

Response: Comment incorporated. The sentence was revised as follows: “it is increasingly of interest to understand whether trace levels of HCPs with protease, glycosidase, lipase, or other enzymatic activities impact product quality or stability.”
Comment Summary #9: Regarding ELISA limitations, the commenter suggested adding text around the fact that each set of HCP antibodies is unique making critical reagent management difficult through the assay lifecycle and making comparisons across products impossible when different assays are used.
Response: Comment incorporated. A new sentence, “this methodology is reagent-specific and has a number of other limitations,” was added.
Comment Summary #10: Regarding ELISA limits, the commenter suggested revising the sentence, “…such as its high sensitivity, specificity, throughput, automation capability, and quantitative nature”, to indicate that despite having limitations (unlisted), the methodology has many advantages (listed).
Response: Comment incorporated. The sentence was changed to: “…despite the many advantages of using immunoassay for HCP measurement, such as its high sensitivity, specificity, throughput, automation capability, and quantitative nature, this methodology is reagent-specific and has a number of other limitations.”
Comment Summary #11: The commenter suggested defining the acronym “MS” the first time it appears.
Response: Comment incorporated.
Comment Summary #12: The commenter suggested highlighting that lab equipment and lab personnel for both analysis of sample and of data are more complex using LC-MS, which could result in a significant increase in cost and personnel.
Response: Comment not incorporated. The comment has been addressed in section 1. *Introduction and Scope*. The Expert Committee received conflicting comments and struck a balance on detail.
Comment Summary #13: The commenter suggested adding the following MS challenge to the bullet list: Method relies on quality of database available for expression cell line.
Response: Comment incorporated. The text “availability of quality database” was added to the 3rd bullet point and further elaborated in the MS data analysis section.
Comment Summary #14: The commenter indicated the challenge for GMP compliance is broader than a lack of clear validation requirements and suggested including these challenges.
Response: Comment incorporated. The second to last bullet point was revised as follows: “Instrument and software validation requirements for current good manufacturing practice (cGMP) can require significant effort and collaboration with instrument vendors; appropriate vendor software solutions need to be considered.”
Comment Summary #15: The commenter suggested rewording the last sentence before the bullet list to improve clarity.
Response: Comment incorporated. The sentence was revised as follows: “However, the following challenges should be considered in order to apply the MS-based techniques for HCP quantitation during drug substance release testing and for in-process testing.”
Comment Summary #16: The commenter indicated some listed MS challenges are a fact associated with the technique, but not a limitation of the method.
Response: Comment not incorporated. The bullet points are a list of the challenges faced by implementing MS-based methods for DS release testing and in process control (IPC), not the limitations of MS technique itself.
Comment Summary #17: The commenter recommended revising the text in the first paragraph to distinguish analytical control (specifications) vs analytical characterization and comparability.
Response: Comment incorporated. The content was revised as follows:
“Consequently, residual HCP levels are commonly tested during drug substance release and for process characterization to monitor clearance. HCP analysis is also a critical component of

biopharmaceutical product comparability studies where the impact on the upstream expression and/or downstream clearance of HCPs is evaluated before and after process changes."
Comment Summary #18: For the statement of "The analytical challenges for quantifying all of the thousands of potential HCPs are well known" in the first paragraph, the commenter suggested using a new paragraph for readability and revising it to describe HCP identification and quantitation separately because each element has different constraints in ELISA vs MS methods.
Response: Comment not incorporated. The limitations of ELISA are thoroughly described in <1132>. This new general chapter <1132.1> articulates the limitations in quantitation of HCPs by LC-MS/MS. The Expert Committee found that the topic is adequately addressed in both general chapters and further elaboration is not needed.
Comment Summary #19: For the first sentence in the second paragraph, "The major risks of the presence of HCPs in biological products are their potential to ….", the commenter asked if USP general chapters routinely include literature references for the statements like this.
Response: Comment not incorporated. It is not routine for USP general chapters to include a full bibliography, but USP does provide the critical references.
Comment Summary #20: The commenter suggested revising the second paragraph to separate clinical risks (product batch to batch consistency) from reliable shelf life (variation in stability data)
Response: Comment not incorporated. The risks are not only clinical, but commercial for approved products. The separation of risks to human health and product quality is already articulated.
Comment Summary #21: The commenter suggested adding "original and" to the first sentence of the third paragraph as follows: "HCP immunoassay, often in the form of a sandwich enzyme-linked immunosorbent assay (ELISA), remains the original and most common methodology for HCP measurement…"
Response: Comment not incorporated. The Expert Committee does not believe ELISA is the "original" methodology. This is SDS-PAGE stained with silver then westerns.
Comment Summary #22: The commenter suggested revising the sentence, "This assay relies on the quality of…", to separate the parameters of detection and quantitation in HCP ELISA to set up a clear and logical comparison with MS for detection versus quantitation.
Response: Comment not incorporated. This is well covered in USP GC<1132> already. The relationship between <1132> and <1132.1> is well established.
Comment Summary #23: The commenter suggested revising the second to last sentence in the third paragraph as follows: "While this may be acceptable for a drug substance specification limit as part of a total HCP control strategy, a multiplex HCP ELISA cannot detect slight variations of individual HCPs in the process or product. Lastly, the inability to identify individual HCPs from multiplex HCP ELISA data requires the use of other single-protein identity techniques to assess clearance and consistency of particular HCPs."
Response: Comment not incorporated. The original text is clearer and more consistent with the position of the Expert Committee.
Comment Summary #24: The commenter suggested deleting the sentence, "Therefore, ⟨1132⟩ and many other recent publications have emphasized the importance of including orthogonal assays to provide additional assurance of product quality".
Response: Comment not incorporated. This sentence establishes the connection between the chapters of <1132> and <1132.1> where the need for another general chapter was articulated.
Comment Summary #25: The commenter suggested deleting "are rapidly evolving" from the sentence: "MS capabilities are rapidly evolving and have demonstrated value..." because it is not objective.
Response: Comment incorporated.

Comment Summary #26: The commenter suggested deleting the sentence, “In addition, liquid chromatography coupled with tandem MS (LC-MS/MS) methods usually need less development time for a new product derived from either the same or a different expression system”, because it is not always true.
Response: Comment partially incorporated. The original statement is true. However, the point is taken, and a statement, “due to the fact that antibody generation and qualification for ELISA takes months; and each cell line type requires its own assay, whereas LC-MS/MS is more amenable to platforming” was added to explain why this is true.
Comment Summary #27: The commenter suggested revising the sentence to highlight this MS advantage as follows: “A major advantage of MS methods is that with optimized procedural conditions and a sufficiently accurate HCP sequence database, MS can be used to identify a wide array of HCPs without the use of staining or immunological reagents.”
Response: Comment incorporated. The sentence was revised as follows: “A major advantage of MS methods is that with optimized procedural conditions and a sufficiently accurate HCP sequence database, MS can be used to identify and quantify a wide array of HCPs without the use of staining or immunological reagents.”
Comment Summary #28: The commenter suggested adding more information about HCP quantification as follows: “And an individual HCP can be quantified by comparison to a purified reference standard of that same protein. When used appropriately, MS-based techniques for HCP analysis may be orthogonal to ELISA and other analytical methods and enable the user to build an understanding of HCP profiles and clearance patterns throughout the process, identify potential problematic HCPs and monitor their adequate removal, and facilitate process and product risk assessment.”
Response: Comment not incorporated. The Expert Committee found the current description to be sufficient.
Comment Summary #29: For the sentence, “However, the use of MS-based techniques for HCP detection and quantitation….”, the commenter suggested using a new paragraph because it is a new topic and recommended revising it as follows: “However, the use of MS-based techniques for HCP detection and quantitation in upstream/downstream drug substance samples and bulk drug substance still face the following challenges:”
Response: Comment not incorporated. The Expert Committee has reviewed the section and determined the text is sufficiently clear as written.
Comment Summary #30: The commenter suggested deleting the second to last bullet point, “Instrument and software validation requirements not well established for cGMP; and”. The commenter indicated that this has been done by several lab groups and their vendors.
Response: Comment partially incorporated. The Expert Committee agreed it has been done successfully by a few companies, but it is still challenging to do. The statement has been modified as follows: “Instrument and software validation requirements for current good manufacturing practice (cGMP) can require significant effort and collaboration with instrument vendors;”.
Comment Summary #31: The commenter suggested revising the fifth paragraph as follows: “Despite these technical and operational challenges, the MS technology is routinely used for successful characterization, comparability, and troubleshooting to detect and measure low levels of HCPs in samples even when orders-of-magnitude more protein product is present. The information provided by emerging advances in MS technology on the identity of individual HCPs represents another advance in facilitating risk of HCPs in biopharmaceuticals.”
Response: Comment not incorporated. The Expert Committee found that the original text is more appropriate. In addition, this is the first place that nanogram per milligram is introduced, so keeping it front and center will help readers understand what is meant by sensitivity in this context.

Comment Summary #32: The commenter suggested revising the first sentence of the last paragraph as follows: “This chapter provides an overview of the capability of current MS methods for HCP identification and quantitation. Technical considerations for instrument selection, sample preparation, LC separation, …”
Response: Comment not incorporated. The Expert Committee found that the proposed changes did not improve the general chapter.
Comment Summary #33: The commenter suggested deleting “Residual” from this sentence: ““Residual HCP ELISA is a multi-analyte immunoassay that uses polyclonal antibodies raised against a broad HCP population from a host organism”.
Response: Comment incorporated.
Comment Summary #34: The commenter suggested replacing “particular” with “individual” as follows: “First, polyclonal antibodies used in the immunoassay often have no or limited coverage to **individual** HCPs that are nonimmunogenic or weakly immunogenic in the animals used to raise antibodies.”
Response: Comment partially incorporated. The Expert Committee agreed in concept and changed it to a simpler and factual statement as follows: “First, polyclonal antibodies used in the immunoassay may have no or limited coverage to HCPs that are nonimmunogenic…” The Expert Committee also changed “often” to “may” since most HCPs do elicit responses in animals.
Comment Summary #35: The commenter suggested revising the last sentence of this section as follows: “While this chapter focuses on the use of MS-based techniques for residual HCP analysis in recombinant therapeutic proteins, the general principles discussed are applicable to all types of biologically-derived products including vaccines, gene therapies, cellular- and tissue-based products, and biocatalysis products. However, for any given biological product, specific regulatory requirements for HCP characterization, comparability, and control, as well as approaches to HCP risk assessment, should be discussed with the relevant regional health authorities.”
Response: Comment not incorporated. Regulatory involvement is not within scope and the suggestion is obvious.

TERMINOLOGY
Comment Summary #36: The commenter suggested modifying the definition of “top-down proteomics” in Table 1 to indicate it is applicable to HCP analysis.
Response: Comment not incorporated. Top-down proteomics is not commonly applied to HCP analysis and specifically refers to the mass spectrometry-based fragmentation technology towards intact protein. Visible peaks in RP-HPLC will typically be collected as fractions and bottom-up proteomics will be applied to identify the HCPs. Hence, top-down proteomics is considered not applied to HCP analysis currently. But “top-down” definition was rephrased as follows to soften the language: “…and is generally not currently applicable for HCP analysis.”
Comment Summary #37: The commenter recommended softening the language used to describe the applicability of top-down proteomics in HCP analysis across the general chapter.
Response: Comment partially incorporated. “Top-down” definition was rephrased in different sections across the general chapter to soften the language. Please see the justification described in comment summary #36.
Comment Summary #38: The commenter suggested adding more descriptive wording to MS/MS definition in *Table 1*.
Response: Comment incorporated. The definition of MS-MS was revised as follows: “Tandem mass spectrometry (MS/MS) uses two mass analyzers in tandem for the identification of peptides, and this process is typically achieved in two separate scan events. The initial full scan event separates the precursor ions (i.e., peptides) in one of the mass analyzers by their mass-to-charge ratio (m/z) to establish a list of precursor ion m/z values and intensities at each point

in elution time. Subsequently, the second scan event (i.e., MS/MS scan) allows selection of certain or all precursor ions for fragmentation in the collision cell. The product ions from this fragmentation process are then scanned by a mass analyzer and detected. The combination of each precursor ion m/z value and its product ions' m/z values is analyzed via a proteomics search engine algorithm to identify the peptide and its source protein."
Comment Summary #39: The commenter suggested removing "cell line" from the DDA definition in *Table 1* because proteomic database suffices.
Response: Comment incorporated.
Comment Summary #40: The commenter suggested adding more description to DIA definition in *Table 1* for the layperson.
Response: Comment not incorporated. The Expert Committee found the current description of DIA definition to be sufficient.
Comment Summary #41: For targeted mass spectrometry definition in *Table 1*, the commenter suggested keeping only the text required for explaining the abbreviation and the concept. Comments about "significant method development" can be deleted.
Response: Comment incorporated.
Comment Summary #42: The commenter suggested starting the definition of FDR with a more general description, e.g., something like "The false discovery rate is a statistical approach typically used by database searching software tools to measure the expected amount of false positives. It is calculated...."
Response: Comment partially incorporated. A general description of FDR is added with minor changes to explain how FDR is used to control numbers of false positives as follows: "The false discovery rate (FDR) is adapted from large-scale proteomics analysis, and it is a statistical approach for controlling the expected proportion of false positives among all significant hypotheses within a dataset. FDR is applied at both the MS/MS spectrum matching and protein identification levels to assist with correct assignment of the peptide and protein sequences, respectively. FDR is calculated by the ratio between the false positives and the total number of hypotheses. Hence, lower FDR indicates search results with higher confidence. Selection of acceptable percentages based on application are included in 4.4 *Data Analysis*."
Comment Summary #43: The commenter indicated that it could be misleading to define FDR only at PSM level since FDR is calculated at both peptide and protein level. Therefore, the commenter recommended adding a definition for FDR at the protein level, especially because protein FDR is typically used to set the threshold for confident identification data reporting.
Response: Comment incorporated. The FDR definition was revised as described in comment summary #42.
Comment Summary #44: The commenter indicated that FDR can be calculated at either the protein or peptide level. However, when using the PSMs (Peptide-Spectrum Matches), the FDR is specifically reflective of the peptide-level FDR only, so the commenter suggested modifying the definition.
Response: Comment incorporated. The FDR definition was revised as described in comment summary #42.
Comment Summary #45: The commenter suggested defining PSM acronym in the FDR definition.
Response: Comment incorporated. The FDR definition was revised as described in comment summary #42.
Comment Summary #46: Regarding the DIA definition, the commenter suggested indicating the spectral library-free DIA methods also exist.
Response: Comment incorporated. A sentence was added at the end of the definition as follows: "Identification of the fragment ions without the need for a spectral library is also possible."

Comment Summary #47: The commenter suggested modifying the DIA definition to indicate DIA can be done without a spectral ion library.
Response: Comment incorporated. The definition was revised as described in comment summary #46.
Comment Summary #48: The commenter asked why "native approach" is not mentioned in the definition of "proteomics" in *Table 1*.
Response: Comment not incorporated. Comment has been addressed in the text. Native approach is a choice of digestion and will be considered as part of bottom-up proteomics.
Comment Summary #49: The commenter recommended expanding the definition of "proteomics" in *Table 1* as follows: "Studies of the expressed protein in a system to identify what is present (such as HCPs) and to characterize changes in expression pattern, including spatial and temporal protein interactions with other biomolecules. MS-based proteomics includes both top-down and bottom-up proteomics approaches."
Response: Comment not incorporated. Proteomics used in the context of HCP analysis do not involve analyzing protein interactions. Data obtained from these experiments will not be sufficient to elucidate the interactions, either spatial or temporal, between biomolecules.
Comment Summary #50: The commenter recommended revising the definition of "Bottom-up proteomics" in *Table 1* as follows: "Refers to the analysis of peptides resulting from proteolytic cleavage of their parent proteins and the characterization of their amino acid sequences using LC-MS/MS. At present, HCP analysis by LC-MS/MS can only be done on the peptide level via bottom-up proteomics."
Response: Comment partially incorporated. Revised to "enzymatically produced peptides from their parent proteins" because it is sufficient to describe the source of the peptides.
Comment Summary #51: The commenter recommended defining "MRM", "PRM", and "SRM" in the definition of "Targeted Mass Spectrometry" in *Table 1* because this is the first time that these acronyms appear in this general chapter.
Response: Comment incorporated. Three acronyms were defined.

INTRODUCTION TO INDENTIFICATION AND QUANTITATION OF HCPS USING LC-MS/MS ANALYSIS
Comment Summary #52: The commenter suggested revising step 3 as follows: "Tandem mass spectrometry (MS/MS) to fragment the separated peptides for sequence information."
Response: Comment incorporated. Step 3 was revised as follows: "MS/MS analysis to fragment the separated peptides for sequence information."
Comment Summary #53: The commenter suggested revising the note about "top-down" proteomics. The commenter stated that if the HCP is prominent, it can be identified by its intact mass with top-down fragmentation techniques and high-resolution spectrometry.
Response: Comment not incorporated. Top-down proteomics is not commonly applied to HCP analysis and specifically refers to the mass spectrometry-based fragmentation technology towards intact protein. Visible peaks in RP-HPLC will typically be collected as fractions and bottom-up proteomics will be applied to identify the HCPs. Hence, top-down proteomics is considered not applied to HCP analysis currently. But the language was softened as follows: "…or "top-down" proteomics, is generally not feasible due to the following challenges."
Comment Summary #54: The commenter indicated there are several other important reasons why top-down proteomics is not feasible for HCP identification and/or quantitation. Therefore, the commenter suggested adding these reasons to the note about "top-down" proteomics.
Response: Comment incorporated. The note was revised as follows: "[Note-The approaches described in this chapter require digestion using a protease prior to analysis. This is referred to as "bottom-up" proteomics. MS analysis of intact proteins for HCP identification, or "top-down" proteomics, is generally not feasible due to the following challenges: limited method sensitivity, diversity of individual intact protein masses due to post-translational processing and

modification, complex data analysis, deconvoluted masses measured in top-down experiments that cannot be searched against a background database, requirement for a homologous reference standard for quantitation, and less ionization efficiency compared to peptides.]"

SAMPLE PREPARATION, CHROMATOGRAPHIC SEPARATION, AND MASS SPECTROMETRY ANALYSIS

Comment Summary #55: The commenter stated that non-mAbs usually have challenging formulation components that interfere with LC-MS/MS.

Response: Comment not incorporated. Different formulation buffer applies to different products at different organizations. There are descriptions at the beginning of Section 4.1 for buffer/matrix impact.

4.1 SAMPLE PREPARATION

Comment Summary #56: The commenter suggested adding in-depth discussion about reproducibility of the sample preparation because it is crucial to prepare samples side-by-side, which should be compared/analyzed together.

Response: Comment incorporated. The following sentences were added before Table 2: "Given that the use of different sample preparation strategies can lead to very different HCP identification results and there is inherent variability in the sample preparation step even if the same strategy is being used, it is highly recommended to standardize the sample preparation and digestion procedure. Also, including replicates will help to understand the variability introduced by this step and gain more reproducible results from run-to-run. For trending or comparability purposes, side-by-side preparation of samples using the same procedure can help reduce the variability introduced in this step."

Comment Summary #57: The commenter recommended modifying the language regarding trypsin digestion to indicate that it is often feasible to improve digestion efficiency without having to use an enzyme with a different specificity than trypsin.

Response: Comment incorporated. The last sentence of the second paragraph was revised as follows: "However, in some cases it may be useful to use a different enzyme for proteolysis if specific proteins of interest cannot be effectively digested by trypsin."

Comment Summary #58: The commenter recommended adding the reversed-phase liquid chromatography into the methods which can be used to fractionate samples prior to LC-MS/MS analysis.

Response: Comment incorporated. The reversed-phase liquid chromatography (RP-LC) was added to the chapter as follows: "Size exclusion chromatography (SEC), reversed phase liquid chromatography (RP-LC), hydroxyapatite (HA) chromatography, and hydrophilic interaction liquid chromatography (HILIC) have also been used to separate HCPs from product protein before LC-MS/MS analysis."

Comment Summary #59: The commenter recommended adding hydroxyapatite into the methods which can be used to fractionate samples prior to LC-MS/MS analysis.

Response: Comment incorporated. Hydroxyapatite was added to the chapter as described in comment summary #58.

Comment Summary #60: The commenter recommended adding "low MWCO filter" into "Separation of product proteins by a molecular weight cutoff (MWCO) filter" in Table 2. The commenter stated that the use of a low MWCO filter can remove detergents and other undesirable buffer components, which additionally achieves efficient concentration of dilute samples.

Response: Comment not incorporated. Detergents usually form micelles, so it is hard to assess what MW cut off can remove them. In addition, dialysis and buffer exchange mentioned at beginning of Section 4.1 also help to clear the concern. Therefore, it is not necessary to add low MWCO description.

Comment Summary #61: The commenter suggested editing the sentence “These methods will be less quantitative than the standard bottom-up approach” to “These methods may sacrifice some level of quantitative accuracy compared to the standard bottom-up approach.”
Response: Comment incorporated. The sentence was revised as follows: “These methods may sacrifice some level of quantitative accuracy for total HCP detection, compared to the standard bottom-up approach…”
Comment Summary #62: For ProA affinity example in *Table 2*, the commenter suggested adding text indicating that once bound to ProA, the product can be washed with buffers that tend to favor disruption of any interaction of HCPs with the protein product. However, it may not be possible to recover all HCPs with this method, and those with the highest affinity to product may go undetected.
Response: Comment not incorporated. This level of detail is not the intention of this general chapter.
Comment Summary #63: Regarding the paragraph describing another option for HCP enrichment using immobilized polyclonal-anti-HCP antibodies, the commenter suggested editing the sentence as follows: “The primary drawback of this method is that HCPs for which there are no or insufficient antibodies (i.e.: non-covered HCPs), would flow through...”
Response: Comment incorporated. The sentence was revised as follows: “The primary drawback of this method is that HCPs for which there are no antibodies or low affinity would not be captured or detected.”
Comment Summary #64: Regarding the paragraph describing another option for HCP enrichment using immobilized polyclonal-anti-HCP antibodies, the commenter suggested not limiting this method to coverage determination. The commenter indicated that HCPs can be enriched in downstream pools to understand which HCPs are being detected in the ELISA. This can be an important part of risk assessment.
Response: Comment incorporated. The paragraph was revised as follows: “This differs from the traditional 2D SDS-PAGE and 2D Western blot methods for coverage assessment. HCP capture from process samples, prior to LC-MS/MS analysis, may also be used to enrich HCPs detected by ELISA for identification and quantitation by LC-MS/MS.”
Comment Summary #65: The commenter suggested adding more references, for example, references about HCP enrichment methods using combinatorial library of peptides or aptamers**.**
Response: Comment not incorporated. The Expert Committee found that sufficient references have been provided in this general chapter. A general chapter is not intended as a comprehensive review article.
Comment Summary #66: The commenter pointed out that two sentences under product protein depletion in Table 2 conflict with each other. “HCPs are fully digested, and product protein is not (lower susceptibility);” and “Some HCPs may also be incompletely digested and be underrepresented.”
Response: Comment incorporated. The first sentence was revised as follows: “HCPs are more likely to be fully digested, whereas product antibody proteins (e.g., mAb) are not. This is due to lower susceptibility vs. HCPs.”
Comment Summary #67: In the paragraph of “To increase sensitivity…”, the commenter suggested changing “lipases and proteases” to “enzymatically active HCPs.”
Response: Comment incorporated. The sentence was revised as follows: “…for example, when enzymatic activity on the product protein (or polysorbate) is observed but HCPs are not detected in the standard bottom-up approach.”
Comment Summary #68: The commenter indicated that the total digestion method is the most straightforward method, however, also the least sensitive method. The commenter suggested adding more information about the total digestion sample prep application**.**

Response: Comment not incorporated. The current description clearly states this is the method to digest product and HCPs, and then the readers can refer to methods described in this section for more sample prep information (1st item in *Table 2*).
Comment Summary #69: The commenter suggested providing clarity on "specificity of ligands" under the caveats of enrichment of HCPs using affinity reagents in *Table 2* as it is an incomplete sentence. The commenter also suggested starting the second sentence as "saturated ligands," not "saturation ligands."
Response: Both points were incorporated. Changed to "Ligand specificity to HCPs is hard to assess."
Comment Summary #70: The commenter suggested including Antibody Affinity Extraction (AAE) in the content and *Table 2*.
Response: Comment not incorporated. Although AAE is a method for assessing HCP antibody coverage and HCP enrichment, it is closely associated with a vendor, and USP general chapter prefers describing the method and its application not under specific potential trademark of a commercial company.
Comment Summary #71: Regarding the paragraph describing another option for HCP enrichment using immobilized polyclonal-anti-HCP antibodies, the commenter stated that increasing HCPs for measuring on MS by using the antibodies designed for the ELISA does not seem beneficial as one can simply perform the common ELISA method, which will visualize the same HCPs.
Response: Comment not incorporated. The paragraph clearly stated that the method here will lose orthogonality to ELISA, and main application is coverage determination. In ELISA vs MS portion of this chapter, there is also description of these two methods.
Comment Summary #72: Regarding the paragraph describing another option for HCP enrichment using immobilized polyclonal-anti-HCP antibodies, the commenter suggested mentioning other common options for antibody affinity enrichments of HCPs. The commenter also suggested removing the mentioning of the HCP coverage as it is described in <1132>.
Response: Comment partially incorporated. The common options suggested by the commenter were added to the sentence as follows: "Another option for HCP enrichment is to use immobilized polyclonal-anti-HCP antibodies created for the HCP-ELISA assay to immunocapture HCPs. Antibodies are immobilized on affinity columns, on beads, or in 96-well ELISA plates". The Expert Committee decided to keep the HCP coverage because of the unique application of LC-MS/MS.
Comment Summary #73: For the ProA column caveat in *Table 2*, the commenter suggested revising the sentence as follows: "HCPs interacting with product protein or affinity ligand may not flow through and be detected."
Response: Comment incorporated. The sentence was revised as follows: "Consequently, HCPs bound to the product protein may be captured and not present in the flow through, potentially being missed during LC-MS/MS analysis."
Comment Summary #74: For the HCP antibody enrichment caveat in *Table 2*, the commenter suggested adding "it is not orthogonal to ELISA."
Response: Comment incorporated.
Comment Summary #75: Regarding the paragraph describing the sample fractionation prior to LC-MS/MS analysis, the commenter suggested re-phrasing the statement to avoid any misleading information because not only additional fractionation makes it difficult to quantify HCPs, but even using a proteomics approach, HCP quantification should also be used carefully due to the technical properties of MS.
Response: Comment not incorporated. The Expert Committee found the challenges of the proteomics approach have been adequately described in this general chapter.

Comment Summary #76: The commenter suggested adding details about reproducibility of the sample preparation because it is crucial to prepare samples side-by-side and compare/analyze them together.
Response: Comment incorporated. Add the following sentences at the end of Section 4.1: "Given that the use of different sample preparation strategies can lead to very different HCP identification results and there is inherent variability in the sample preparation step even if the same strategy is being used, it is highly recommended to standardize the sample preparation and /digestion procedure. Also, including replicates will help to understand the variability introduced by this step and gain more reproducible results from run-to-run. For trending or comparability purposes, side-by-side preparation of samples using the same procedure can help reduce the variability introduced in this step."
Comment Summary #77: The commenter suggested providing a reference regarding enriching HCPs using beads coated with members of a combinatorial library of peptides or aptamers that theoretically bind to HCPs.
Response: Comment not incorporated. This general chapter is not intended as a comprehensive review article.
Comment Summary #78: For clarity, the commenter suggested adding "e.g." as follows: "In addition, the concentration of drug must also be measured because the HCP levels measured will eventually be reported as a ratio to the protein product (**e.g.**, nanogram of HCP per milligram of protein product)."
Response: Comment incorporated.
Comment Summary #79: The commenter recommended adding the drawbacks of the "total digestion" approach to the chapter, for example, advanced instrumentation with high sensitivity is required, and very low abundance HCPs may not be detected.
Response: Comment not incorporated. The challenges of this approach are adequately described. A better instrument system could be beneficial for all methods. The second point of the comment is already listed in *Table 2* under the caveats of denatured digestion.
Comment Summary #80: The commenter suggested revising "Standard digestion" to "Total digestion" in Table 2 for consistency throughout the chapter.
Response: Comment incorporated. "Standard digestion" was changed to "Denatured digestion" and "total digestion" in the context was changed to "denatured digestion" as well.

4.2. Chromatographic Methods
Comment Summary #81: The commenter suggested correcting a typo of the capillary flow rate from "~8-10mL/min" to "~8-10µL/min."
Response: Comment incorporated.
Comment Summary #82: The commenter indicated that ~8-10mL/min flow rate seems excessive for 300µm ID columns and suggested changing it to ~8-10µL/min flow rate.
Response: Comment incorporated.
Comment Summary #83: The commenter asked if the units are correct for nanoflow vs capillary flow columns (250-300 nL/min vs. 8-10 mL/min).
Response: Comment incorporated. The capillary flow rate was changed to ~8-10µL/min.
Comment Summary #84: The commenter suggested adding "Ideally" in front of the sentence "Column temperature should be maintained constant …"
Response: Comment not incorporated. Maintaining a constant column temperature is a requirement for good HPLC practice.
Comment Summary #85: The commenter stated that the coming USP MS standard (including multiple proteins and peptides) would be helpful to set up system suitability for a LC-MS method for HCP analysis.
Response: Comment acknowledged. The statement is true, but this is not a comment that needs to be addressed.

Comment Summary #86: The commenter suggested adding HILIC to the second paragraph of Section 4.2 because HILIC is also used for peptide mapping, although C18 reversed phase is the most used one.
Response: Comment not incorporated. The text says a reversed-phase C18 column is the most frequently used column for LC-MS/MS proteomics but does not exclude HILIC.
Comment Summary #87: The commenter suggested including other volatile ion pairing agents in the paragraph describing the LC parameters which have an impact on the sensitivity of MS analysis.
Response: Comment not incorporated. Other ion pairing agents can be used, but they are not as common.

4.3. Mass Spectrometry Analysis
Comment Summary #88: The commenter suggested widening the m/z window of the precursor ion selection based on different vendor's isolation windows.
Response: Comment incorporated. The sentence was revised as follows: "the precursor ion selection m/z window is small (usually about 1 m/z) …" The "<" sign was removed to make the statement more general, but the Expert Committee does not want to get into instrument-specific differences.
Comment Summary #89: The commenter suggested widening the m/z window of the precursor ion selection because the m/z window of an Orbitrap instrument is usually set at 1.4 m/z instead of <1 m/z.
Response: Comment incorporated. Please see the response of comment summary #88.
Comment Summary #90: The commenter suggested including HCD into the alternative fragmentation modes. In addition, the commenter stated that it is not accurate to say, "these methods currently are not efficient enough for peptide fragmentation or are not commonly found in commercial systems."
Response: Comment incorporated. HCD was added and the mentioned sentence was deleted from the general chapter.
Comment Summary #91: The commenter suggested defining the error of high mass accuracy < 5ppm.
Response: Comment not incorporated. It could be different from instrument to instrument. Therefore, the Expert Committee does not think it should be defined.
Comment Summary #92: The commenter suggested changing the sentence as follows: "In DDA, one precursor ion is selected for fragmentation at a time following an initial precursor scan."
Response: Comment incorporated.
Comment Summary #93: The commenter suggested providing more description for DIA.
Response: Comment not incorporated. The commenter did not specify what needs more discussion. The Expert Committee found the current description to be sufficient.
Comment Summary #94: The commenter suggested revising the sentence as follows: "The selected precursor is then fragmented in a collision cell, and fragment ions are…"
Response: Comment incorporated.
Comment Summary #95: The commenter suggested indicated that there are other parameters that can influence separation dependent on the instrument type. Therefore, the commenter revising the sentence as follows: "This is the case of IMS, which can separate molecules based on molecular size and shape (radius) as well as other parameters, depending on the instrument model, and adds a further separation principle to the classical m/z separation of an MS instrument."
Response: Comment incorporated.

Comment Summary #96: The commenter indicated that “simple” MS system such as single-quadrupole and ESI-TOF do not provide sufficient information for identification of the HCP peptides but permit HCP peptides to be detected.
Response: Comment not incorporated. MS alone should not be used for HCP analysis. Even in targeted cases, fragment ions should be part of the analysis.
Comment Summary #97: The commenter suggested replacing “and so” with “therefore” in paragraph 5 under Section 4.3.
Response: Comment incorporated.
Comment Summary #98: The commenter recommended removing the data analysis descriptions for DDA and DIA. DDA and DIA have differences in data collection, not necessarily in data analysis. Data analysis can be completed via any number of overlapping means.
Response: Comment incorporated. Deleted the sentence “The resulting MS/MS spectra are more complex, but data analysis software tracks the elution profiles of the fragment and precursor ions and then identifies peptides by either a database search or comparison to a spectral ion library.” This sentence is redundant, and the concepts are covered in the last sentence of this paragraph.
Comment Summary #99: The commenter suggested adding HCD to the fragmentation methods as follows: “Most commercial MS/MS instruments commonly use CID or HCD to induce fragmentation of precursor ions, which has been a popular method used in HCP analysis.”
Response: Comment incorporated.

4.4. Data Analysis
Comment Summary #100: The commenter indicated that it is “unive” in paragraph 1 and recommended changing it to “universal.”
Response: Comment not incorporated. It is “universal” in the *PF* proposal.
Comment Summary #101: The commenter suggested including fixed modifications (e.g., cysteine carbamidomethylation) in the amino acid modification to better inform readers who may have less experience in the field.
Response: Comment not incorporated. The Expert Committee believes such details are not necessary.
Comment Summary #102: The commenter suggested clearly stating that MS-based quantitation can typically take either relative or absolute quantitation. The difference between these should be detailed and the quantitation methods in this general chapter should be clearly stated where they fall in this spectrum. The differentiation would aid better understanding for the less experienced reader.
Response: Comment not incorporated. This question has been adequately addressed in this general chapter.

QUANTITATION OF HCPs
Comment Summary #103: The commenter suggested adding “sum all” as an alternative MS quantitation method.
Response: Comment incorporated. The text was revised as follows: “One of the more common methods for MS quantitation is the Hi3 method developed by Silva et al. (2), where it was shown that a protein’s abundance (in molar units) is proportional to the signals obtained from its three most abundant peptides. Alternatively, summing the signals from all identified peptides (“sum all”) has also been shown to be proportional to HCP abundance (in mass units) by Krey et al. (3).”
Comment Summary #104: The commenter stated that the section is missing a major application of semi-quantitative MS HCP, which is for assessing the comparability of samples for the identity and relative intra-assay abundance of HCPs.

Response: Comment not incorporated. This has been covered in other parts of this general chapter.
Comment Summary #105: The commenter suggested this general chapter must clearly and openly present the issues that impact accurate MS quantitation of total HCPs, therefore recommended moving up the Kreimer reference (3) to the introductory section.
Response: Comment not incorporated. The Expert Committee reviewed again and believes the location of this reference belongs where it is. This will provide a fuller context and better understanding. It is also tied to the data table which illustrates the point.
Comment Summary #106: The commenter suggested adding one sentence at the end of this section as follows: "…plus the quantitative relationship of MS response factors of any internal standards or controls compared to the MS response factor of the intended HCP analyte(s)."
Response: Comment incorporated.
Comment Summary #107: The commenter requested to clarify the number of peptides from an HCP that should be used to assign a concentration to the HCP. The commenter further requested to clarify how close the peptides need to be to each other because if 3 peptides are being used, each of those 3 peptides will yield a quantitative value.
Response: Comment acknowledged. Top 3 peptides is using average and alternative "sum all" is also provided in the general chapter.

5.1. Methods for HCP Quantitation
Comment Summary #108: The commenter suggested clarifying that Section 5.1 relates to relative quantitation as opposed to absolute quantitation.
Response: Comment not incorporated. The Expert Committee found the current description to be clear. Three scenarios have explained in detail how the quantitation should be done.
Comment Summary #109: The commenter indicated that it is highly critical to assure that readers clearly understand which of the options are speculative estimates of total HCP quantity, and which are accurate quantitation of known HCPs. Therefore, the commenter suggested revising the entire section as follows: "Below are several MS methods that have been used to quantitively estimate either total or specified HCPs. However, some of them require assumptions about the relationship of the MS signal response factor of known protein(s) to that of multiple unknown host cell proteins with very different molecular properties. These approaches should not be used when the accuracy of total HCP quantitation is required. As with HCP ELISA, MS cannot detect all HCPs. But with HCP ELISA, the HCP reference standard can be characterized to confirm the presence of all HCPs detected by the multiplex immunoreagents. Therefore, if the HCP test samples contain the same HCPs in the ELISA reference standard, each of their individual ELISA response factors will be the same. As discussed in <1132> and above, it is not possible to quantify individual HCPs with a multiplex HCP ELISA. But it is possible to quantify the total population of detectable HCPs present when the ELISA reference standard contains the same set of HCPs. The discussions below will highlight which of the MS options utilize internal reference standards of known HCPs to generate accurate quantitative results, and which do not. Each method has advantages and disadvantages, and it is up to analysts to determine how to implement an appropriate method for their samples."
Response: Comment partially incorporated. The Expert Committee adopted some of the ideas but did not adopt all because some points do not reflect the views of the Expert Committee. The section was revised as follows: "Below are several MS methods that can be used to quantitatively estimate the amounts of individual HCPs or of a specific list of HCPs. MS quantitation options are limited either to a relative comparison with the drug product protein amount or the internal HCP reference standards to allow more accurate quantitation. Each method has advantages and disadvantages, and it is up to analysts to determine how to implement an appropriate method for their samples."

5.1.1 Relative to Product Protein

Comment Summary #110: The commenter indicated that this strategy would not be suitable with MS1-level due to the dynamic range of MS instruments and excess of product protein. Therefore, the commenter suggested adding a sentence at the end of this section as follows: “In addition, since many biopharmaceutical proteins are highly purified, and most MS instruments only have ~2-3 orders of intra-scan dynamic range at MS1 level, this strategy may not be suitable for MS1-level quantitation.”

Response: Comment incorporated. One sentence was added at the end of this section as follows: “In addition, because many biopharmaceutical proteins are highly purified (typically > 4 orders more abundant than HCPs), and most Orbitrap MS instruments have only 2-3 orders of magnitude of intra-scan dynamic range at the MS1 level, this strategy may not be suitable for MS1-level.”

Comment Summary #111: The commenter suggested revising this section to make it clear that this is not suitable for accurate quantitation of total HCPs. The proposed revision is as follows: “If the product protein’s peptide signals in the LC-MS/MS experiment are confirmed to be in the working range of the instrument’s detector via generation of a linear or nonlinear product calibration curve, this approach may be used for relative estimation of HCPs based solely on the response factor of the product’s peptides. A known amount of product protein digest is injected onto the column, and the quantity of HCPs calculated from the signal ratio between HCPs and the product protein peptides. Although this method is easy to implement with no additional reagents required, it leverages the assumption that the HCP peptides from a diverse population of HCPs will have the same ionization and fragmentation efficiencies (i.e. response factors) compared to those of the product protein's peptides. Since the peptides from different HCPs likely have different MS response factors from each other as well as from the product protein, it is not possible to confirm how accurately this MS approach estimates the quantity of total detectable HCPs.”

Response: Comment partially incorporated. The Expert Committee acknowledged the commentor’s concern and revised this section to clarify that this approach will be a good estimate on the quantity of HCPs if applied consistently. It could achieve relative accuracy for any given HCP but may not be accurate for total HCPs. The section was revised as follows: “If the product protein’s peptide signals in the LC-MS/MS experiment are in the working range of the instrument’s detector (or a nonlinear calibration curve has been generated), they can be used as a relative quantitation standard. A known amount of product protein digest is injected onto the column, and the quantity of HCPs will be estimated from the signal ratio between HCPs and the product protein peptides. This method is easy to implement with no additional reagents required. However, the accuracy of this quantitation approach for total detectable HCPs is impacted by the difference in ionization and fragmentation efficiencies when comparing individual HCP peptides to product protein peptides.”

5.1.2 Relative to Spiked-in Proteins

Comment Summary #112: The commenter suggested including 2 more examples of typical analyses based on B) all observed peptides with SumAll quantification, and C) MRM on single target peptide and external calibration curve on a protein standard.

Response: Comment partially incorporated. “Sum all” approach has been added into the general chapter per other comments USP received. External calibration curve should be used cautiously, and the Expert Committee does not think additional examples based on (C) are necessary.

Comment Summary #113: The commenter suggested adding several sentences at the end of this section to indicate that known quantities of one or a few proteins cannot be used to quantitatively determine the quantities of hundreds of other proteins. They do not have the same

response factors as each other. The commenter suggested revising the section as follows: “A second approach used to estimate the quantity of total HCPs based on the response factors of other proteins is to spike one or more intact proteins at known concentrations as protein standard(s) into the product protein sample before enzyme digestion. Protein standards used for such studies should be well characterized for purity and of known concentration and should be expected to generate unique peptides compared to the expected HCP peptides. Analysts should demonstrate that proteins used in this approach do not result in false-positive HCP identification by generating ambiguous peptides. If a set of known protein standards is employed, a median response factor based on the average responses is calculated. In a typical analysis, the top three highest intensity peptide signals from protein standard(s) are compared to those from each HCP, in terms of peak area or peak intensity, and a signal ratio is calculated. As with the method above (5.1.1.) this approach leverages the assumption that the MS response factors of the peptides from one or more known protein standards will quantitatively reflect those of every other detectable HCP. This is observed with the spiked protein standards themselves, which generate different MS response factors from each other in the method and requires a median response factor to be used in calculations of recovery. With known variations in signal among spiked protein standards and unknown signal variations in total HCPs, it is not possible to confirm the accuracy of estimates in the quantity of total detectable HCPs.”
Response: Comment partially incorporated. The quantification accuracy has been discussed in the introduction to Section 5. The Expert Committee added in language to clarify that this method is providing a relative quantification for individual HCP as follows, “As with the method in 5.1.1 Relative to Product Protein, this approach provides a relative quantification for each HCP.”
Comment Summary #114: The commenter suggested deleting the last sentence of this section. The information on calculations of mass balance should all be provided in one complete section because this mathematical approach is not unique to method 5.1.2.
Response: Comment incorporated. Moved the sentence below up a level to 5.1 to be applicable to all 3 methods of quantitation: “In each case, the known concentration of product protein, spiked protein, or spiked peptide is used to calculate the amount of HCP peptide being measured. Moles of individual HCPs can thus be converted to nanograms per milligram, taking into consideration specific molecular weights of unknowns (HCPs) and product protein or spiked entities.”

5.1.2 Relative to Spiked-in Peptides
Comment Summary #115: The commenter suggested providing clarification on the statement, “the quantitation can differ by two- to three-fold, which was attributed to a variety of factors”.
Response: Comment incorporated. The clarification was added in paratheses after “two- to three- fold” as follows: “(estimated by comparing calculated peptide concentrations from the calibration curve - generated using protein standards - to spiked peptide concentrations, or by comparing calculated protein concentrations from the spiked SIL peptides to those obtained through label-free quantitation).”
Comment Summary #116: The commenter suggested deleting the last two sentences of this section.
Response: Comment not incorporated. The Expert Committee believes the location of this reference belongs where it is. It is also tied to the data table which illustrates the point.

5.2 Method Validation
Comment Summary #117: The commenter suggested expanding the method validation section, for example, to clarify whether the validation should follow ICH Q2 or M10, and why there is a need for product-specific LC-MS method or a matching standard if a platform-based non-targeted proteomics workflow is used.

Response: Comment partially incorporated. This general chapter is not intended to give specific guidance on method validation. Text was clarified to state only individual HCPs require product specific methods and a matching standard. The sentence was revised as follows: "Product-specific methods for measurement of individual HCP(s) for release testing are to be validated per ICH guidance and require a matching standard or stable, characterized reference material (or SIL peptide) for each HCP quantified."
Comment Summary #118: The commenter suggested editing this section by adding the full reference of ICHQ2 and a CMC forum reference paper and an FDA analytical method reference.
Response: Comment not incorporated. The proposed edit of how to apply ICHQ2 is a level of detail beyond this general chapter's scope. The Expert Committee also seeks to avoid using non-peer reviewed materials.

5.3 System Suitability
Comment Summary #119: The commenter indicated that some readily available commercial products can serve as useful standards. Commercial reagents can be used to ensure the MS sensitivity is maintained prior to any experiments, especially quantitation.
Response: Comment not incorporated. The text as written does not exclude the use of commercially available cocktails of HCP or standard.
Comment Summary #120: The commenter suggested that this section could be expanded to include the system suitability checks for sample prep, LC, MS, and corresponding criteria.
Response: Comment not incorporated. There is a lot of guidance already. This general chapter is not intended to give guidance on this.
Comment Summary #121: The commenter suggested deleting the last sentence of this section, "This concept is explained in the following section."
Response: Comment incorporated.

REPORTING RESULTS
Comment Summary #122: For the statement regarding assay sensitivity, "the lowest level of HCP (nanogram per milligram) that can be detected at least 90% of the time is fairly conventional", the commenter suggested clarifying the minimal times that the system suitability sample should be run to claim the sensitivity. The commenter indicated that 90% may be too high.
Response: Comment not incorporated. The text states that 90% is typical. And this is for information only, not a requirement.
Comment Summary #123: The commenter suggested providing recommendations and/ or further guidance of when to produce data to the regulatory agency and in which format this data should be presented alongside the expectation of reportable results.
Response: Comment not incorporated. The regulatory expectations vary from case to case. The Expert Committee believes the basic information on reporting is described adequately in the general chapter (e.g., units).
Comment Summary #124: For *Table 3* and *Table 4*, the commenter indicated that a sample is rarely tested more than once or twice when using MS for HCP characterization. The commenter suggested reporting total HCP (ng/ml >LLOQ), total HCP number and total HCP number >LLOQ, along with a list of the identified and quantified HCPs. For duplicate and triplicate analysis, average numbers with CVs can be reported as for other analytical methods.
Response: Comment not incorporated. There is no suggestion that samples should be routinely tested by any replication number. The data is presented as an example that users can use to choose their own replication strategy for various applications (e.g., validation, comparability, release).

Comment Summary #125: The commenter suggested deleting the 2nd sentence through to the last sentence of the first paragraph. The commenter indicated that it is not appropriate to present highly quantitative treatment of data that is acknowledged to be semi-quantitative for each HCP.
Response: Comment not incorporated. While the Expert Committee agreed that the HCP results obtained by LC-MS/MS may be semi-quantitative, the description of the differences between molar ratio-based ppm and mass ratio-based ng/mg is of critical importance in understanding the levels of individual HCPs identified by LC-MS/MS.
Comment Summary #126: The commenter suggested deleting everything starting from "Various approaches may be used …." to the very end of this section because it represents mathematical conclusions derived from assigning highly quantitative values to non-quantitative data.
Response: Comment not incorporated. This is the most common application of LC-MS/MS for drug substances, and it is common for HCPs to be present right at the detection and/or quantitation level. The data came from a well-qualified system and is typical. It is essential to explain to readers how to interpret batch and process data when an infinite number of analytes are present and close to the limits. It also suggests the experimental design and replication that users may need to apply to obtain quantitative results and interpret data batch to batch when HCPs are close to the limit.
Comment Summary #127: Regarding the sentence below *Table 4*, "This is helpful for interpreting batch analysis results. These data also illustrate the semiquantitative nature of LC-MS/MS in discovery mode," the commenter requested to clarify why such detailed mathematical calculation for 18 HCPs is acknowledged as semiquantitative.
Response: Comment not incorporated. All methods, quantitative or not, have degrees of accuracy that are known. In the HCP testing, the difficulty of measuring trace HCPs at the limits of testing (this is of interest always) needs illustration. The data provides this and the readers may use it to explain data. LC-MS/MS method does have the power to report a quantitative value, the example illustrated that the quantification has run-to-run variabilities and it is only semi-quantitative in discovery mode. Knowing this limitation will help us better understand the suitability of using the LC-MS/MS based method for HCP identification and quantitation.

BEST PRACTICES FOR REPORTING DATA FROM ELISA VERSUS LC-MS/MS
Comment Summary #128: The commenter suggested deleting the introduction section, Section 7.1, and Section 7.2.
Response: Comment not incorporated. The Expert Committee believes these sections are an essential part of the general chapter and they are critical and necessary.

7.2 Units of Measurement
Comment Summary #129: The commenter indicated that the standard reporting unit for resolution of mass spectrometers and measurement error is ppm. As such the commenter suggested revising the text as follows: "Because LC-MS/MS commonly reports values in mole-based units, or ppm..."
Response: Comment not incorporated. Because ppm can also refer to the ELISA convention of ng of HCP per mg of product, the Expert Committee found that this distinction is necessary.

7.3 Comparison of ELISA and LC-MS/MS HCP Results
Comment Summary #130: The commenter suggested providing clarity on the statement, "However, the comparison for specific HCPs between ELISA and LC-MS/MS may be appropriate in those rare cases when specific known HCPs are available as standards for both assays", whether the statement implies using an ELISA and MS assay specific to a single HCP to perform quantitation or using total population ELISA and traditional abundance-based MS.

Response: Comment incorporated. The sentence was revised as follows: “However, in cases where single HCPs are being monitored, the comparing ELISA specific for these given HCPs to LC-MS/MS may be appropriate. In such cases, individual HCPs or SIL peptides must be available as standards in both assays.”
Comment Summary #131: The commenter suggested providing clarity on the statement, “One might be tempted to sum up the individual HCP amount quantified by an LC-MS/MS approach and to compare it with the total HCP ELISA values measured. In many cases the results (quantity of HCPs) agree between methods,” whether the agreement refers to absolute values or just trending.
Response: Comment partially incorporated. The statement was revised as follows: “In many cases the results follow the same trends between methods but should not be strictly compared for the following reasons.” The Expert Committee found the revised statement to be clear and the term “absolute” values would add confusion. The point is that summing is fraught when HCPs below the detection limit cannot be summed.
Comment Summary #132: The commenter requested clarity on how to justify if ELISA and MS results are different.
Response: Comment acknowledged. The Expert Committee has provided examples of why this is the case that can be used to explain the situation where they do not trend together. The description is thorough and needs no elaboration.

7.4 Complementarity of LC-MS/MS and Total HCP ELISA
Comment Summary #133: For the statement, “If both methods indicate low amounts of residual HCPs, then one can be more confident on the purity of a drug substance”, the commenter suggested adding another statement regarding assessment of overall clearance across unit operations. The comparison of trending is not only useful for testing DS/DP but also across the processing train.
Response: Comment incorporated. One sentence was added before the statement: “Both methods are suitable for process trending, to follow the progressive clearance of HCP along downstream processing steps to ensure there are no copurified HCPs.”
Comment Summary #134: The commenter suggested providing clarity on the statement**,** “Of most value is the use of LC-MS/MS to confirm the absence of HCPs in samples by ELISA to be free of detectable HCPs,” and indicated that it seems like some words are missing.
Response: Comment incorporated. The sentence was revised as follows: “LC-MS/MS is useful to confirm the absence of HCPs in samples where HCPs are not detected by ELISA. This orthogonality provides more confidence that the ELISA is not missing any HCPs at significant levels.”

Monograph/Section(s): <1243> Wetting Properties of Pharmaceutical Systems / Multiple Sections
Expert Committee(s): General Chapters-Physical Analysis Expert Committee
No. of Commenters: 4

Raw Materials
Comment Summary #1: The commenter suggested deleting the sentence, “The problem can be resolved by selecting a solid form with better wetting properties.” Wetting properties are not resolved by selection, but rather by physical and chemical alteration of the surface characteristics through either chemical bonding or solid dispersion approaches.
Response: Comment incorporated.
Comment Summary #2: The commenter suggested the following text change: “*It also may be found that the poor wetting of the powder is due to the crystal morphology, with a predominant ~~crystal face~~* ***molecular moiety within the crystal lattice*** *that is hydrophobic, in which case it*

may be possible to develop a crystallization process that produces a particle morphology with a hydrophilic face as prominent to improve wetting (e.g., Ritonavir)."
Response: Comment not incorporated. The Expert Committee members agreed that "crystal face" is a better term in the text than the suggested change.
Comment Summary #3: The commenter suggested the following change: "*When wetting properties are critical, ~~tests to assess~~ assessment of wetting* ***characteristics*** *may be part of the specification of the materials."*
Response: Comment incorporated.
Comment Summary #4: The commenter expressed the concern of applying acceptance criteria to raw materials as well as recommended USP develop a standard method for contact angle analysis as guidance for the stakeholders.
Response: See comment summary #3. The above 1000 chapters are for guidance only and are not mandatory requirements. The stakeholders should conduct their own risk analysis to fit their specific situation.

Manufacturing
Comment Summary #5: Under the Film Coating subtitle, for clarity, the commenter suggests the following edit: "*The choice of excipients in the tablet formulation can improve wetting ~~and produce~~ a robust film ~~coating process~~* ***coat****."*
Response: Comment not incorporated. The Expert Committee members think the original text is clear and agree to keep the text as is.
Comment Summary #6: The commenter recommended revising "*Milling*" to "*Levigation*" as the subtitle for the final bullet as well as revising the text under the subtitle. The commenter stated that agglomeration may occur in nanomilling due to failed stabilization of the surface energy at new surfaces formed during the milling process. Wetting alone may not achieve this without adding a stabilizer (e.g., PEG).
Response: Comment partially incorporated. The Expert Committee decided to keep the subtitle as is because wet milling is discussed in the first sentence. The text will be changed to the following: "…if they are not sufficiently wetted **and stabilized** by the medium…."

Dosage Form Performance and Stability
Comment Summary #7: The commenter noted the following statement only applies to matrix tablets: "*Water must be able to penetrate into the porous capillary network, which requires spontaneous wetting*."
Response: Comment incorporated. The text is changed as follows: "***For matrix tablets,*** *water must be able to penetrate into the porous capillary network, which requires spontaneous wetting."*

Comment Summary #8: The commenter stated that the wetting properties are not resolved or "improved" by surfactant selection, but rather by the physical and chemical alteration of the surface characteristics through either chemical bonding or solid dispersion approaches. The commenter suggested revising the following text for accuracy: "*Pharmaceutical formulators must stabilize suspensions using ingredients, such as surfactants, to improve wetting in order to promote long term stability and an acceptable shelf life."*
Response: Comment incorporated. The text is revised as follows: "*Pharmaceutical formulators must stabilize suspensions using ingredients, such as surfactants, ~~to improve wetting~~ in order to promote long term stability and an acceptable shelf life*."
Comment Summary #9: For clarity, the commenter suggested the following revision: "*In topical, intranasal, and ocular drug delivery, wetting properties of formulations are important to increase ~~contact area~~* ***the area of exposure****.*" (Alternatively, "drug availability at the site of

action" could also be considered an acceptable replacement for "contact area" in the statement.).
Response: Comment incorporated. The text is revised as follows: "*In topical, intranasal, and ocular drug delivery, wetting properties of formulations are important to ~~increase~~ ~~contact area~~* ***optimize the formulation target contact area***."

Device and Packaging
Expert Committee initiated change #1: Device was added in the following sentence: "For example, in a metered dose inhaler suspension of micronized drug, poor wetting of the particles by the medium can lead to deposition of the particles on the device or primary packaging components, leading to unacceptable dose delivery from the device." because the deposition of particles could also happen on the device.
Comment Summary #10: The commenter recommended including additional discussion on relevant packaging concepts. For example, leachable and extractables increase as the wetting of the container closure system increases. This could be included in the discussion here.
Response: Comment incorporated. The following text is added at the end of the section: "*Leachable and extractables may also be affected by the wetting of the device or packaging materials*." More detailed information may be added in later revision.

WETTING PROPERTIES BACKGROUND
Comment Summary #11: The commenter suggested to delete the first sentence "*Very few surface properties can be directly measured*." It is unclear what this is referring to in the context of wetting properties.
Response: Comment incorporated.
Comment Summary #12: For clarity, the commenter suggested revising the next sentence in the section as follows: "*The main experimental ~~observable~~* ***parameter*** *for quantifying wetting is the contact angle (θ)…"*
Response: Comment incorporated. The text is revised as follows: "*The main experimental ~~observable~~* ***measurable parameter*** *for quantifying wetting is the contact angle (θ)…."*

Comment Summary #13: The commenter recommended reframing the discussion in the second paragraph to describe the interactions (forces) managing the wetting phenomenon in terms of cohesion and adhesion forces between the solvent molecules and the solid surface molecules. Adhesion forces encompass the attraction force between the solvent and the solid surface molecules to form new bonds for the wetting to occur.
Response: Comment incorporated. The text in this section is revised to use the terms of cohesion forces and adhesion forces when applicable.

SURFACE FREE ENERGY FROM CONTACT ANGLE MEASUREMENTS
Comment Summary #14: The commenter pointed out some typographical errors in Table 8. (1) The 1st column of data has the header for surface free energy with a superscript p, but it should be a superscript d for the dispersive component. (2) The last column header looks like a Y, but it should be a gamma just like the previous column headers. (3) In the row for Water-Glycerol, the value for the total surface free energy (last column) is 36.5, but this value is the sum of the dispersive and polar components which are 19.3 and 7.2 which sums to 26.5. This error is in the original article. Given the uncertainty of which number is wrong, the commenter suggested deleting this row from the table.
Response: Comment incorporated.

SURFACE ENERGY OF SOLID BY INVERSE GAS CHROMATOGRAPHY

Comment Summary #15: The commenter suggested adding more explanation and schematic of the Inverse Gas Chromatography (IGC) technique under this section.
Response: Comment incorporated. More detailed information is provided.

Monographs

Monograph/Section(s): Adapalene and Benzoyl Peroxide Gel/Multiple Sections
Expert Committee: Small Molecules 1
No. of Commenters: 3

Comment Summary #1: The commenter indicated that the acceptance criteria for *pH* <791> in the *Specific Tests* section are different from what has been approved and recommends revising the acceptance criteria to be consistent with what has been approved.
Response: Comment incorporated. The *Acceptance criteria* for *pH* <*791*> in the *Specific Tests* section are widened from "3.2–4.7" to "3.0 to 4.7" to accommodate other approved products.
Comment Summary #2: The commenter indicated that they were not able to detect an end point for the Benzoyl Peroxide standard using the titration procedure outlined in the *Assay, Benzoyl Peroxide*.
Response: Comment not incorporated. USP did not identify concerns within our laboratories with the recommendations listed in the note for the recommended electrode. Upon follow up, the commenter indicated that a silver ring electrode was used initially instead of a platinum ring electrode as recommended in the proposal.
Expert Committee initiated change #1: Based on data received, the acceptance criterion for "Any unspecified degradation product" is widened from "NMT 0.1% to "NMT 0.21%" in the test for *Organic Impurities, Adapalene* to accommodate other FDA-approved products.
Expert Committee initiated change #2: Based on data received, the acceptance criterion for "Any unspecified degradation product" is widened from "NMT 0.1% to "NMT 0.20%" in the test for *Organic Impurities, Benzoyl Peroxide* to accommodate other FDA-approved products.

Monograph/Section(s): Aspirin/Multiple Sections
Expert Committee: Small Molecules 2
No. of Commenters: 2

Comment Summary #1: The commenter indicated that the maximum daily dose of Aspirin may be >2g based on DailyMed. Therefore, the commenter recommends setting the limit of "Any unspecified impurity" to be in line with ICH Q3A guidelines.
Response: Comment not incorporated. The limit for "Any unspecified impurity" is based on the widest specifications available to USP from approved products.
Comment summary #2: The commenter recommended removing the reporting threshold in the test for Organic Impurities as it will vary based on product-specific factors.
Response: Comment not incorporated. A new USP general chapter, User-Determined Reporting Thresholds ⟨477⟩, supports a flexible reporting threshold to accommodate product-specific factors. The Expert Committee will consider incorporating this new approach in future revisions, as applicable.
Comment summary #3: The commenter indicated that the *Assay* method does not have sufficient resolution between Salicylic Acid related compound B and Aspirin and there is not a resolution requirement in the method. Assay methods are required to show specificity for components that are likely to be present. As such, resolution between Aspirin and Salicylic Acid related compound B should be improved to ensure separation from Salicylic Acid and to allow for typical laboratory variations.

Response: Comment not incorporated. The Expert Committee determined, based on validation data, that Salicylic Acid related compound B is fully resolved from the main peak of Aspirin.
Comment summary #4: The commenter observed in the test for *Organic impurities* a resolution of 1.1 between Salicylic acid Related compound B and Aspirin while the method resolution requirements were between Aspirin and Salicylic Acid (NLT 6.0). Commenter recommends using Salicylic acid related compound B and Aspirin as the resolution critical pair.
Response: Comment not incorporated. The Expert Committee determined, based on validation data, that Salicylic Acid related compound B is sufficiently resolved from the main peak of Aspirin. The proposed resolution requirement of NLT 6.0 between Aspirin and Salicylic Acid addresses a potential non-characterized impurity which may be present and interfere with salicylic acid.
Comment summary #5: The commenter recommends the inclusion of relative response factors (RRF) for the specified impurities in the test for *Organic impurities*. Preliminary determination of RRFs for Salicylic Acid related compounds A and B indicate that accuracy of the quantitation would be improved with the inclusion of RRFs.
Response: Comment not incorporated. The validation data shows that the relative response factor is close to 1.0 in each case. Therefore, the Expert Committee determined it is appropriate to use a RRF of 1.0 throughout.
Comment summary #6: The commenter recommended, in the test for *Organic Impurities,* including a statement in the *Sample solution* preparation to use freshly prepared samples due to potential issues resulting from extended runs.
Response: Comment not incorporated. The Expert Committee determined that the Note proposed in the current proposal is sufficient to alert users of any potential issue.

Monograph/Section(s): Bendamustine Hydrochloride/Organic Impurities
Expert Committee: Small Molecules 3
No. of Commenters: 1

Comment summary #1: The commenter recommended removing the “reporting thresholds” in the test for Organic impurities as they will vary based on product-specific factors.
Response: Comment not incorporated. A new USP general chapter, *User-Determined Reporting Thresholds* ⟨477⟩, supports a flexible reporting threshold to accommodate product-specific factors. The Expert Committee will consider incorporating this new approach in future revisions, as applicable.

Monograph/Section(s): Caffeine/Multiple Sections
Expert Committee: Small Molecules 5
No. of Commenters: 2

Comment summary #1: The commenter indicated that an L1 column of 4.6 mm x 15 cm;5-µm dimensions is sufficient for the proposed *Assay* and *Organic Impurities* tests and is less expensive than the proposed L60.
Response: Comment not incorporated. The Expert Committee determined that the proposed L60 column is suitable and consistent with the *Assay* and *Organic Impurities* validation data. Use of alternate procedures is discussed in *General Notices 6.30*. *Alternative and Harmonized Methods and Procedures*.
Comment summary #2: The commenter recommended omitting the proposed autosampler temperature setting of 5°C because there is no impact to the chromatography in the tests for *Assay* and *Organic impurities*.
Response: Comment partially incorporated. Solution stability data indicates enhanced stability with an autosampler temperature of 5° versus 25°. The Expert Committee determined that an

autosampler temperature of 5° may be appropriate for longer runs and recommended adding a note as follows: [Note —This solution may be stable for 12 h at room temperature. An autosampler temperature of 5° may be appropriate for runs longer than 12 h.]
Comment summary #3: The commenter recommended in the tests for *Assay* and *Organic impurities* to remove the run time requirement as the commenter did not experience any peaks eluting after the Caffeine peak.
Response: Comment partially incorporated. USP typically includes run time information for isocratic runs to help users. The Expert Committee found that the proposed run time is consistent with the validation data but determined that the run time can be shortened from NLT 2 to NLT 1.5 times the retention time of caffeine.
Comment summary #4: The commenter recommended that the *Sample* and *Standard solution* concentration in the *Assay* be reduced from 0.2 mg/mL to 0.1 mg/mL for ease of preparation.
Response: Comment not incorporated. The Expert Committee determined that the proposal is consistent with validation data.
Comment summary #5: The commenter recommended removing the "reporting threshold" in the test for *Organic impurities* as it will vary based on product-specific factors.
Response: Comment not incorporated. A new USP general chapter, *User-Determined Reporting Thresholds* ⟨477⟩, supports a flexible reporting threshold to accommodate product-specific factors. The Expert Committee will consider incorporating this new approach in future revisions, as applicable.
Expert Committee initiated change #1: Update the calculation section in the test for *Organic Impurities* by replacing "Calculate the percentage of any unspecified impurity in the portion of Caffeine taken:" with "Calculate the percentage of each impurity in the portion of Caffeine taken."
Expert Committee initiated change #2: In the *Assay*, correct the reagent hyperlink for "acetic acid" to "acetic acid, glacial" and update the text from "acetic acid, glacial" to "glacial acetic acid" in the *Buffer*.

Monograph/Section(s): Cefoxitin for Injection/Organic Impurities
Expert Committee: Small Molecules 1
No. of Commenters: 1

Comment Summary #1: The commenter recommended that USP work with approved manufacturers to ensure that the marketed products will be able to meet the requirements in the proposed monograph to avoid a drug shortage.
Response: Comment incorporated. Comment summary #2 outlines changes made to accommodate additional approved specifications.
Comment Summary #2: The commenter indicated that in the test for *Organic impurities* the acceptance criteria for "Decarbamoylcefoxitin", "Cefoxitin 3-ene", "Cefoxitin lactone," and "Any unspecified impurity" are different from what has been approved and recommends revising the acceptance criteria to be consistent with what has been approved.
Response: Comment partially incorporated. The limits for Decarbamoylcefoxitin, Desmethoxycefoxitin, Methoxycefoxitin *R*-isomer, Methoxycefoxitin *S*-isomer and Cefoxitin 3-ene and are revised from NMT 0.50% to NMT 0.5%. The limit for Cefoxitin lactone is revised from NMT 0.50% to NMT 0.7%. The limit for Total impurities is revised from NMT 4.0% to NMT 4.5%. The limit for Any unspecified impurity is unchanged.

Name	RRT	PF49(1) Proposal	Revised Limits
Decarbamoylcefoxitin	0.84	0.50	**0.5**
3-Thienyl Cefoxitin	0.98	1.0	1.0
Cefoxitin	1.00	-	-
Desmethoxycefoxitin	1.04	0.50	**0.5**
Methoxycefoxitin *R*-isomer	1.11	0.50	**0.5**
Methoxycefoxitin *S*-isomer	1.15	0.50	**0.5**
Cefoxitin 3-ene	1.18	0.50	**0.5**
Cefoxitin lactone	1.61	0.50	**0.7**
Any unspecified impurity		0.20	0.20
Total impurities		4.0	**4.5**

Comment Summary #3: The commenter recommended, in the test for *Organic impurities*, removing Methoxycefoxitin R-isomer and Methoxycefoxitin S-isomer from the impurity table as they are sufficiently controlled in the drug substance.
Response: Comment not incorporated. Methoxycefoxitin R-isomer and Methoxycefoxitin S-isomer are controlled in the drug substance monograph. However, these impurities are also included in the drug product specifications and included in the limit for Total impurities available to USP.
Comment Summary #4: The commenter recommended adding a reference to *Injections and Implanted Drug Products (Parenterals) <1> – Product Quality Tests* in the *Specific Tests* section.
Response: Comment not incorporated. This proposed change is beyond the intended scope of this PF proposal and may be considered in a future revision.
Expert Committee initiated change #1: Update *Identification A* to include "or *Sample Solution 2*, as appropriate."
Expert Committee initiated change #2: Update *Identification B* to include "or *Sample Solution 2*, as appropriate."
Expert Committee initiated change #3: Update the *Sample solution* under *Identification C* to read "Nominally 50 mg/mL of cefoxitin."
Expert Committee initiated change #4: Update the Sample solution under test for *pH* to read "Nominally 100 mg/mL of cefoxitin."

Monograph/Section(s): Cefoxitin Sodium/Multiple Sections
Expert Committee: Small Molecules 1
No. of Commenters: 2

Comment summary #1: Commenter indicated that EDQM has updated their analytical monograph introducing essentially the co-elution of Impurity K with Impurity F (official since 1st Jan. 2023). The commenter suggests aligning the inclusion of Impurity K with EP.
Response: Comment not incorporated. The Expert Committee determined that the procedure is consistent with the sponsor's validation and is suitable for the intended use. Future revisions to the monograph can be considered upon receipt of the necessary supporting data.
Comment summary #2: The commenter expressed concern that Cefoxitin Sodium in water can decompose and release methanol potentially resulting in methanol test results that are artificially higher by GC-FID with headspace auto sampler. The commenter requested that the Expert Committee postpone the proposed method change effective date while they finalize development of a GC-MS direct injection method that does not require adding acid to precipitate Cefoxitin Sodium and having to filter it from sample solution which has better repeatability. The commenter would like their method to be considered as an alternative method for the acetone and methanol limit test before Cefoxitin Sodium monograph is revised.
Response: Comment not incorporated. The comment is beyond the intended scope for this proposal since the *Limit of Acetone and Methanol* method was not proposed for revision. Future revisions to the monograph can be considered upon receipt of the necessary supporting data. Use of alternate procedures is discussed in *General Notices 6.30*. *Alternative and Harmonized Methods and Procedures*.

Monograph/Section(s): Chlorhexidine Gluconate and Isopropyl Alcohol Topical Solution/ Organic Impurities
Expert Committee: Small Molecules 3
No. of Commenters: 1

Comment summary #1: The commenter recommended adding requirement for "Any unspecified degradation products" with acceptance criteria consistent with ICH Q3B identification threshold in the test for *Organic Impurities*.
Response: Comment not incorporated. The proposed acceptance criteria are consistent with the sponsor's approved specifications. The Expert Committee may consider a future revision upon the receipt approved specifications and supporting data.
Comment summary #2: The commenter recommended removing the "reporting threshold" in the test for *Organic impurities* as it will vary based on product-specific factors.
Response: Comment not incorporated. A new USP general chapter, *User-Determined Reporting Thresholds* ⟨477⟩, supports a flexible reporting threshold to accommodate product-specific factors. The Expert Committee may consider incorporating this new approach in future revisions, as applicable.

Monograph/Section(s): Clioquinol/Organic Impurities
Expert Committee: Small Molecules 1
No. of Commenters: 1

Comment summary #1: The commentor indicated that the Gas Chromatographic (GC) based *Assay* procedure is replaced by an HPLC procedure based on the EP. The commenter recommended tightening the acceptance criteria because the acceptance criteria remain wider than the generally acceptable range for HPLC procedures for a drug substance.
Response: Comment not incorporated. The acceptance criteria for the proposed HPLC Assay procedure are consistent with the *Definition* and the currently official *Assay* limits based on GC. The Expert Committee may consider a future revision upon the receipt approved specifications and supporting data.

Comment summary #2: The commenter recommended tightening the acceptance criteria in the test for *Residue on Ignition* to an acceptable level because there is no inorganic salt or heavy metal present in the drug substance. Therefore, a limit of 0.5% is too wide.
Response: Comment not incorporated. The test for *Residue on Ignition* is out of scope for this *PF* proposal. The Expert Committee may consider a future revision upon the receipt approved specifications and supporting data.
Comment summary #3: The commenter is concerned the *Assay* and *Residue on Ignition* tests currently proposed are inadequate to assure drug substance quality and recommends referring to the *European Pharmacopoeia* monograph for Clioquinol for relevant information.
Response: Comment not incorporated. The Expert Committee may consider future revisions to the *Assay* and *Residue on Ignition* tests upon the receipt approved specifications and supporting data.
Comment summary #4: The commenter recommended removing the "reporting threshold" in the test for *Organic impurities* as it will vary based on product-specific factors.
Response: Comment not incorporated. A new USP general chapter, *User-Determined Reporting Thresholds* ⟨477⟩, supports a flexible reporting threshold to accommodate product-specific factors. The Expert Committee may consider incorporating this new approach in future revisions, as applicable.

Monograph/Section(s): Daunorubicin Hydrochloride/Organic Impurities
Expert Committee: Small Molecules 1
No. of Commenters: 1

Comment summary #1: The commenter recommended removing the "reporting threshold" in the test for *Organic impurities* as it will vary based on product-specific factors.
Response: Comment not incorporated. A new USP general chapter, *User-Determined Reporting Thresholds* ⟨477⟩, supports a flexible reporting threshold to accommodate product-specific factors. The Expert Committee may consider incorporating this new approach in future revisions, as applicable.

Monograph/Section(s): DL-Lactide and Glycolide (50:50) Copolymer 12000 Acid/Multiple Sections
Expert Committee(s): Complex Excipients
No. of Commenters: 2

Comment Summary #1: The commenter requested to change "12000 Mw" to "IV 0.2" in the monograph *Title*, the *Chemical Information*, and the *Definition* sections because an analytical method for the determination of the molecular weight is not included in the monograph.
Response: Comment not incorporated. The "12000 Mw" is from the FDA Inactive Ingredient Database (IID) listing; USP obtained supporting data for the molecule weight and its range requirement included in the Definition section; as an iterative approach, USP is working on introducing an optimized and validated gel permeation chromatography (GPC) molecular weight method through a test method general chapter.
Comment Summary #2: The commenter recommended changing "peaks at approximately 4.9 and 5.1 ppm" to "peaks between approximately 4.9 and 5.1 ppm" in the *Ratio of DL-Lactide to Glycolide in Copolymer* section for accuracy.
Response: Comment incorporated.
Comment Summary #3: The commenter recommended changing the diluent volume from 60 mL to 90 mL in the *Acid Number* section to ensure a smooth analytical working.
Response: Comment incorporated.

Comment Summary #4: The commenter recommended deleting “Method I” from “Method I, Method Ic” in the *Water Determination* section.
Response: Comment not incorporated. The format is consistent with language used throughout the *USP-NF*.
Comment Summary #5: The commenter recommended deleting the sentences regarding the use in injectable dosage forms in the *Labelling* section to remove an undue burden and the sentences were confusing.
Response: Comment not incorporated. This text and format is consistent with language used throughout the USP-NF.
Comment Summary #6: The commenter recommended lowering the acceptance criteria from NMT 200ppm to NMT 60ppm in the *Limit of Tin* section.
Response: Comment not incorporated. The limit was based on data from multiple manufacturers that provide pharmaceutical grade of this excipient.
Comment Summary #7: The commenter recommended adding a system suitability test in the *Viscosity* section to ensure that the instrument is suitable for this test.
Response: Comment incorporated.
Expert Committee initiated change #1: In the *Chemical Information* section, removed the CAS number because it does not accurately represent this specific excipient.

Monograph/Section(s): DL-Lactide and Glycolide (50:50) Copolymer 12000 Ethyl Ester
Expert Committee(s): Complex Excipients
No. of Commenters: 0

Expert Committee initiated change #1: In the *Chemical Information* section, removed the CAS number because it does not accurately represent this specific excipient.

Monograph/Section(s): Hard Gelatin Capsule Shells/Multiple Sections
Expert Committee(s): General Chapters-Dosage Form Expert Committee
No. of Commenters: 3

DEFINITION
Comment Summary #1: The commenter made the following suggestions.

1. Add gliding agent in the following sentence “They may contain processing aids such as surfactants, lubricants, and dispersing agents.”
2. Remove “externally coated” in the last sentence in this section as after coating it will not be hard gelatin capsule shells.

Response: Comment not incorporated. The Capsule Expert Panel agreed that the current text is complete and clear in the definition section. Coating does not change the polymer.

IDENTIFICATION A
Comment Summary #2: The commenter stated that the acceptance criterion for *Identification A* is unclear as written. It could be interpreted as only applying to capsule shells containing colorants, which may not be the intent. The Commenter suggested to revise the text as the following: "a violet color is produced in the absence of colorant, or a more-intense-than-violet color is produced if the capsules are colored" or "for uncolored capsules, a violet color is produced, and for colored capsules, a color darker than violet is produced".
Response: Comment not incorporated. The Capsule Expert Panel discussed the comment and found the current text to be clear.

LOSS ON DRYING

Comment Summary #3: The commenter suggested adding the text “or in pre-locked position” after "with body and cap separated" in the preparation step of the test method. For large volume production sites, the separation of cap and body would be cumbersome, and does not have significant impact on the outcome of the test method.
Response: Comment incorporated.
Comment Summary #4: The commenter suggested to add a note to the acceptance criteria to explain that a specification set within the currently proposed range is acceptable, and may be used to reflect specific capsule applications (low moisture, dry power inhalation, etc.)
Response: Comment not incorporated. The Capsule Expert Panel discussed the comment and agreed the current text is clear and concise.
Comment Summary #5: The commenter suggested adding “or to constant weight” in *Analysis* subsection.
Response: Comment not incorporated. At least 5 hours drying is enough to achieve constant weight. The EP agreed that the text and test should be as simple as possible.
Comment Summary #6: The commenter suggested changing the *Acceptance criteria* to 13% - 16% to maintain the elasticity of the capsules and avoid its rupture during processing.
Response: Comment not incorporated. The current range of 9.0% -16.0% covers all kinds of capsule shells from low moisture as well as conventional capsule shells.

DISINTEGRATION
Comment Summary #7: One commenter suggested removing the requirement for disintegration for delayed-release capsules. The reference to *Dissolution* may create confusion for acceptance criteria. The other commenters made the same suggestion and stated that these capsules cannot be manufactured as such.
Response: Comment not incorporated. The Capsule Expert Panel discussed the comment and agreed the current text is clear.
Comment Summary #8: The commenter pointed out that there might be a typographical error when referencing the method in <711> for delayed release capsules.
Response: Comment not incorporated. This is not a typographical error. The procedure is described in General Chapter <711>.
Comment Summary #9: The commenter suggested to add the following text in the acceptance criteria “Observe tube visually for small fragments and release of filled material”.
Response: Comment not incorporated. The Expert Panel agreed that the current text is clear and concise to fulfill the purpose.

MICROBIAL ENUMERATION TESTS <61> and TESTS FOR SPECIFIED MICROORGANISMS <62>
Comment Summary #10: The commenter recommended to exclude microbial enumeration tests and tests for specified microorganism for nasal use and vaginal use from this monograph, stating that they have no industrial experience for nasal and vaginal use of Hard Gelatin capsule Shells.
Response: Comment not incorporated. The Capsule Expert Panel agreed to keep text as is because the rationale provided for the proposed change was not found to be sufficient.

ADDITIONAL REQUIREMENTS
Comment Summary #11: The commenter suggested changing the relative humidity range to 40-65%.
Response: Comment partially incorporated. The text was modified to allow the use of other storage conditions based on stability studies.

Monograph/Section(s): Hard Hypromellose Capsule Shells/Multiple Sections

Expert Committee(s): General Chapters-Dosage Form Expert Committee
No. of Commenters: 4

DEFINITION
Comment Summary #1: Under Definition, the commenter suggested adding “gelling aids” and removing “externally coated.”
Response: Comment not incorporated. The Capsule Expert Panel agreed that the current text is complete and clear as stated.

IDENTIFICATION A
Comment Summary #2: The commenter requested to add “centrifuge” before “decant” to take care of interference of color of capsule to obtain clear aqueous part of the slurry.
Response: Comment incorporated.

LOSS ON DRYING
Comment Summary #3: The commenter suggested adding the text “or in pre-locked position” after "with body and cap separated" in the preparation step of the test method. For large volume production sites, the separation of cap and body would be cumbersome, and does not have significant impact on the outcome of the test method.
Response: Comment incorporated.
Comment Summary #4: The commenter suggested to add a note to the *Acceptance criteria* to explain that a specification set within the currently proposed range is acceptable, and may be used to reflect specific capsule applications (low moisture, dry power inhalation, etc.)
Response: Comment not incorporated. The Capsule Expert Panel discussed the comment and agreed the current text is clear and concise.
Comment Summary #5: The commenter suggested adding “or to constant weight” in Analysis subsection.
Response: Comment not incorporated. At least 5 hours drying is enough to achieve constant weight. The EP agreed that the text and test should be as simple as possible.

DISINTEGRATION
Comment Summary #6: One commenter suggested removing the requirement for disintegration for delayed-release capsules. The reference to *Dissolution* may create confusion for acceptance criteria. The other commenters made the same suggestion and stated that these capsules cannot be manufactured as such.
Response: Comment not incorporated. The Capsule Expert Panel discussed the comment and agreed the current text is clear.
Comment Summary #7: The commenter pointed out that there might be a typographical error when referencing the method in <711> for delayed release capsules.
Response: Comment not incorporated. This is not a typographical error. The procedure is described in GC <711>.
Comment Summary #8: The commenter suggested adding the following text in the acceptance criteria “Observe tube visually for small fragments and release of filled material”.
Response: Comment not incorporated. The Expert Panel agreed that the current text is clear and concise to fulfill the purpose.

MICROBIAL ENUMERATION TESTS <61> and TESTS FOR SPECIFIED MICROORGANISMS <62>
Comment Summary #9: The commenter stated that they have no industrial experience for nasal and vaginal use of Hard Gelatin Capsule Shells, so they recommended to exclude

microbial enumeration tests and tests for specified microorganism for nasal use and vaginal use from this monograph.
Response: Comment not incorporated. The Capsule Expert Panel agreed to keep text as is because the rationale provided for the proposed change was not found to be sufficient.

ADDITIONAL REQUIREMENTS
Comment Summary #10: The commenter suggested changing the relative humidity range to 40-65%.
Response: Comment partially incorporated. The text was modified to allow the use of other storage conditions based on stability studies.

Monograph/Section(s): Hard Pullulan Capsule Shells/Multiple Sections
Expert Committee(s): General Chapters-Dosage Form Expert Committee
No. of Commenters: 3

DEFINITION
Comment Summary #1: Under Definition, the commenter suggested adding “gelling aids” and removing “externally coated”.
Response: Comment not incorporated. The Capsule Expert Committee agreed that the current text is complete and clear as stated.
The commenter made the following suggestions.

1. Add “gel promoter” in the following sentence “They may contain processing aids such as surfactants, lubricants, and dispersing agents.”
2. Remove “externally coated” in the last sentence in this section as after coating it will not be hard pullulan capsule shells.

Response: Comment not incorporated. The Capsule Expert Committee agreed that the current text is complete and clear in the definition section. Coating does not change the polymer.

IDENTIFICATION C
Comment Summary #2: The commenter suggested revising the second sentence in Analysis to the following “Transfer 10 mL of solution to 2 mL of polyethylene glycol 600…”.
Response: Comment partially incorporated. The text revised to read “Transfer 10 mL of the above solution to a test tube, …”

LOSS ON DRYING
Comment Summary #3: The commenter suggested adding the text “or in pre-locked position” after "with body and cap separated" in the preparation step of the test method. For large volume production sites, the separation of cap and body would be cumbersome, and does not have significant impact on the outcome of the test method.
Response: Comment incorporated.
Comment Summary #4: The commenter suggested to add a note to the *Acceptance criteria* to explain that a specification set within the currently proposed range is acceptable, and may be used to reflect specific capsule applications (low moisture, dry power inhalation, etc.)
Response: Comment not incorporated. The Capsule Expert Committee discussed the comment and agreed the current text is clear and concise.
Comment Summary #5: The commenter suggested adding “or to constant weight” in Analysis subsection.
Response: Comment not incorporated. At least 5 hours drying is enough to achieve constant weight. The EP agreed that the text and test should be as simple as possible.

DISINTEGRATION

Comment Summary #6: One commenter suggested removing the requirement for disintegration for delayed-release capsules. The reference to *Dissolution* may create confusion for acceptance criteria. The other commenters made the same suggestion and stated that these capsules cannot be manufactured as such.
Response: Comment not incorporated. The Capsule Expert Committee discussed the comment and agreed the current text is clear.
Comment Summary #7: The commenter pointed out that there might be a typographical error when referencing the method in <711> for delayed release capsules.
Response: Comment not incorporated. This is not a typographical error. The procedure is described in GC <711>.
Comment Summary #8: The commenter suggested adding the following text in the acceptance criteria "Observe tube visually for small fragments and release of filled material".
Response: Comment not incorporated. The Expert Committee agreed that the current text is clear and concise to fulfill the purpose.

MICROBIAL ENUMERATION TESTS <61> and TESTS FOR SPECIFIED MICROORGANISMS <62>
Comment Summary #9: The commenter recommended exclude microbial enumeration tests and tests for specified microorganism for nasal use and vaginal use from this monograph, stating that they have no industrial experience for nasal and vaginal use of *Hard Gelatin Capsule Shells*.
Response: Comment not incorporated. The Capsule Expert Committee agreed to keep text as is because the rationale provided for the proposed change was not found to be sufficient.

ADDITIONAL REQUIREMENTS
Comment Summary #10: The commenter suggested changing the relative humidity range to 40-65%.
Response: Comment partially incorporated. The text was modified to allow the use of other storage conditions based on stability studies.

Monograph/Section(s): Isradipine Compounded Oral Suspension
Expert Committee: Compounding
No. of Commenters: 1

Comment Summary #1: A commenter recommended including some text to indicate that the glycerin used in the preparation should be tested for diethylene glycol and ethylene glycol before use.
Response: Comment not incorporated. Consistent with the requirements in <795>, compounders must verify their glycerin meets the criteria of the USP *Glycerin* monograph that requires performance of these tests already.
Comment Summary #2: A commenter notes the preparation indicates use of DynaCirc 5-mg capsules, Sandoz Pharmaceuticals, East Hanover, NJ. This manufacturer does not appear in the Orange Book and thus may not be available in the US market. It is unclear whether compounders will be able to obtain this necessary drug product to create the oral suspension. If DynaCirc 5-mg capsules manufactured by Sandoz Pharmaceuticals are not currently available on the US market, the commenter objects to the availability of this monograph and recommends that it be deleted from the *USP-NF*.
Response: Comment not incorporated. USP Compounded Preparation Monographs (CPMs) are also used outside of the United States, where it may be the case that components are available from a manufacturer specified in a monograph. This formula also allows compounding from isradipine powder.

Comment Summary #3: A commenter notes if the product is to be used in pediatric patients, they recommend including testing for USP *Microbiological Examination of Nonsterile Products Tests for Burkholderia Cepacia Complex* <60>, *Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests* <61>, and <62> *Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms* in the *Specific Tests* section.
Response: Comment not incorporated. Requiring compliance with USP <60>, USP <61>, and USP <62> would not be consistent with the requirements in *Pharmaceutical Compounding – Nonsterile Preparations* <795>.
Comment Summary #4: A commenter notes it is unclear how the *Acceptance criteria* for *pH* were determined. They recommend reviewing the scientific studies upon which this range is based.
Response: Comment not incorporated. The *pH Acceptance criteria* are based on an average of the data and considerations of a suitable range for preparation.
Comment Summary #5: A commenter recommends specifying the containers used (e.g., glass type) to support the Beyond-Use Date criteria in the *Packaging and Storage* section.
Response: Comment not incorporated. The Compounding Expert Committee concluded the language in the monograph stating to, "package in tight, light-resistant glass containers" is sufficient.
Comment Summary #6: A commenter recommends revising the *Labeling* as follows: "Label shake well before use, and to state the Beyond-Use Date."
Response: Comment not incorporated. The *Labeling* section is written according to the *USP* Style Guide.

[*The Ketamine Compounded Cream commentary was updated on January 23, 2025 to correct the previous error indicated that Comment Summary #3 was partially incorporated. The comments summarized were not incorporated into the approved text of the monongraph.]*
Monograph/Section(s): Ketamine Compounded Cream
Expert Committee: Compounding
No. of Commenters: 1

Comment Summary #1: A commenter notes that they have general concerns given the limited evidence regarding the bioavailability of ketamine when used as a compounded topical product and the potential safety concerns associated with compounded ketamine products and the communication of the risks to patients due to compounded products being exempt from section 502(f)(1) concerning the labeling of drugs with adequate directions for use. They have published a compounding risk alert warning patients and health care providers about the potential risks associated with compounded ketamine products.
Response: Comment not incorporated. This monograph is being published to provide compounders with a preparation to compound when the drug product is unavailable, such as when it is in shortage. Topical ketamine targets localized neuropathic pain, as opposed to systemic administration, and the Compounding Expert Committee is aware of studies supporting the existence of N-methyl-D-aspartate receptors in superficial layers of the skin and basal keratinocytes.
Commentary Summary #2: A commenter notes that the *Definition* section directs the compounder to prepare the product following *Pharmaceutical Compounding – Sterile Preparations* <797>, however, this is a non-sterile preparation. As such, they recommend revising the definition to follow *Pharmaceutical Compounding – Nonsterile Preparations* <795>.
Response: Comment incorporated.
Commentary Summary #3: A commenter recommends clearly stating the calculation (ratio) used to determine the amount of Ketamine Hydrochloride from Ketamine Hydrochloride powder

to avoid confusion and potential mistakes in dosing that could potentially lead to serious adverse events.
Response: Comment not incorporated. The proposed monograph already included "Ketamine (as Ketamine Hydrochloride) powder, equivalent to," "1 g (1.15 g of Ketamine Hydrochloride)", and "Ketamine (as Ketamine Hydrochloride) powder, equivalent to", "10 g (11.53 g of Ketamine Hydrochloride)", which is consistent with the *USP* Style Guide. The Compounding Expert Committee concluded to maintain this information as was already included.
Commentary Summary #4: A commenter notes that the preparation protocol states to use Lipoderm, permeation-enhancing vehicle manufactured by PCCA, Houston, Texas. To help the public evaluate the clinical risks/benefits associated with the use of the final compounded product prepared with this proprietary vehicle, we recommend that the ingredients of these excipients be included in the monograph.
Response: Comment not incorporated. This information was obtained from the manufacturer.
Commentary Summary #5: A commenter recommends revising the text to state the specific type of API used when preparing the 100 mg/g cream as follows: "Ketamine (as Ketamine Hydrochloride) powder equivalent to…"
Response: Comment incorporated.
Commentary Summary #6: A commenter recommends revising the text to clarify the type of API in the method protocol. For example: "Wet the Ketamine powder with…" and "…place the Ketamine powder and …"
Response: Comment incorporated.
Commentary Summary #7: A commenter notes the *Assay Acceptance criteria* range is given as 90.0%-110.0%. This is inconsistent with their understanding of the data. They recommend tightening this range to be consistent with the raw data.
Response: Comment not incorporated. The Expert Committee also bases its acceptance criteria on existing *USP* monographs and expert opinion, not solely on laboratory testing criteria.
Commentary Summary #8: A commenter notes it is unclear how the *Acceptance criteria* for *pH* were determined. They recommend reviewing the scientific studies that were performed to support the proposed range.
Response: Comment not incorporated. The *pH Acceptance criteria* are based on an average of the data and a suitable window for preparation.
Commentary Summary #9: A commenter recommends specifying the type of metered-dose container (glass type, plastic, etc.) used to support the Beyond-Use Date criteria to ensure all compounded ketamine cream can be stored appropriately without degradation.
Response: Comment incorporated.

Monograph/Section(s): Mannose/Multiple Sections
Expert Committee(s): Simple Excipients
No. of Commenters: 2

Comment Summary #1: The commenter recommended revising the *Definition* from "Mannose is a sugar derived from the chemical synthesis or biotransformation of D-fructose or D-glucose." to "Mannose is a sugar derived from chemical synthesis or biotransformation starting with D-fructose or D-glucose." to clarify the process by which mannose is produced, consistent with the information in scientific literature.
Response: Comment incorporated.
Comment Summary #2: The commenter requested that the error in the acceptance criterion for the Residues on Ignition (ROI) test identified in the *PF* proposal be corrected by changing it from NMT 0.02% to NMT 0.2%.
Response: Comment incorporated.

Monograph/Sections: Menthol/Multiple sections
Expert Committee: Botanical Dietary Supplements and Herbal Medicines
No. of Commenters: 5

Assay
Comments Summary #1: The commenter indicated that the default tailing specification of 0.8 - 1.8 based on the current *Chromatography* <621> is adequate, and requested the proposed specification of 0.5 - 1.0 is aggressive, likely to needlessly reduce GC setups with passing system suitability performance.
Response: Comment not incorporated. Proposed GC condition in the *PF* produced tailing factor around 0.6.
Comments Summary #2: The commenter mentioned that, historically, they have obtained tailing factors of 0.6 for the Assay method. This is an indication of column overload and suggests that the column specified in the monograph does not have adequate capacity for the method as written. While adding a tailing factor range of 0.5 to 1.0 will make it possible to meet suitability with the method as written, the commenter mentioned it does not address the real problem of column overload. The commenter suggested that a change be made to the method that addresses the column overload problem (i.e., different column, lower standard/sample concentrations, increased split ratio, etc.). The commenter was verifying the *Assay* and *Related Compounds* methods with two adjustments that are within the allowable ranges specified in <621>. They proposed to change the column from a "0.18-mm x 20-m fused silica; coated with a 0.18-µm film of stationary phase G16" to a "0.25-mm x 15-m fused silica; coated with a 0.25-µm film of stationary phase G16" and increased the split ratio from "50:1" to "125:1". They claimed that this yielded a tailing factor of 0.8 for the menthol peak in the *Standard solution* and a *Signal-to-noise* ratio of ~ 15 at the reporting threshold. While not optimal, they claimed that would meet the *System Sensitivity* and *Peak Symmetry* requirements in <621>.
Response: Comment not incorporated. The proposed range of tailing factor is sufficient with the current method. The proposed method may be incorporated at a later time if issues arise.
Comments Summary #3: The commenter indicated that the tailing factor: 0.5–1.0. The commenter asked whether this applied for the menthol peak? They mentioned the method up to *USP 40* had the tailing factor requirement only for the menthol peak. They have difficulty in applying it for the peak of the internal standard: 1-butanol in the current condition. The commenter requested to specify that it is applied for the peak of Menthol.
Response: Comments not incorporated. The tailing factor is applied for menthol peak only.

Related Compounds
Comments Summary #4: The commenter indicated that *Related Compounds* under the *Menthol* monograph are specified to use the same sample solution for both the assay and the related compound test. However, this solution is prepared using an internal standard solution containing 10 mg/mL of 1-butanol in hexanes. The commenter was concerned that the peak from the internal standard in the related compound test might interfere with any related compound (or impurity) peak sharing the same retention time. The commenter requested whether it is advisable to address this potential issue by preparing a separate sample solution for the related compound test, using exclusively hexanes as the solvent.
Response: Comments not incorporated. Peaks from internal standard do not interfere with any related compounds (impurities).
Comments Summary #5: The commenter indicated that calculation for the *Related Compounds* test in the *Menthol* monograph appears to be incorrect. They mentioned calculation is performed using area percent and "r_T" is defined as "sum of the peak areas from the Sample solution." Since the sample solution contains internal standard, the commenter believes the

internal standard area is included in the sum, but the internal standard areas should not be included. Commenter requested for comment.
Response: Comments not incorporated. Internal standard should not be included in the calculation for the *Sample solution*.

Monograph/Section(s): Nisoldipine/Organic Impurities
Expert Committee: Small Molecules 2
No. of Commenters: 1

Comment summary #1: The commenter recommended removing the "reporting threshold" in the test for *Organic impurities* as it will vary based on product-specific factors.
Response: Comment not incorporated. A new USP general chapter, *User-Determined Reporting Thresholds* ⟨477⟩, supports a flexible reporting threshold to accommodate product-specific factors. The Expert Committee may consider incorporating this new approach in future revisions, as applicable.

Monograph/Section(s): Permethrin/Organic Impurities
Expert Committee: Small Molecules 3
No. of Commenters: 1

Comment summary #1: The commenter recommended removing the "reporting threshold" in the test for *Organic impurities* as it will vary based on product-specific factors.
Response: Comment not incorporated. A new USP general chapter, *User-Determined Reporting Thresholds* ⟨477⟩, supports a flexible reporting threshold to accommodate product-specific factors. The Expert Committee may consider incorporating this new approach in future revisions, as applicable.

[*Sodium Nitroprusside monograph commentary was updated on 23-Jan-2025 to correct numbering]*
Monograph/Section(s): Sodium Nitroprusside/Multiple Sections
Expert Committee: Small Molecules 2
No. of Commenters: 4

Comment summary #1: The commenter requested that the *Assay* titration test be retained indicating that the titration is easier to perform and is less prone to error.
Response: Comment not incorporated. The Expert Committee determined the proposed HPLC based *Assay* procedure is suitable for its intended use.
Comment summary #2: The commenter indicated that the *USP* and *European Pharmacopoeia* (*EP*) monographs differ in small detail for almost every test requiring testing to be done twice when releasing Sodium Nitroprusside per *USP* and *EP*. The commenter requests harmonization between USP and EP to reduce workload. . Specifically, with this revision proposal, the tests for *Identification A* and *Insoluble matter* differ slightly from the *EP*.
Response: Comment not incorporated. The *Identification A* and *Insoluble matter* tests are out of scope for this proposal. The Expert Committee may consider future revisions to the *Identification A* and *Insoluble matter* tests or other tests upon the receipt of supporting data.
Comment summary #3: The commenter recommended removing the "reporting threshold" in the test for *Organic impurities* as it will vary based on product-specific factors.
Response: Comment not incorporated. A new USP general chapter, *User-Determined Reporting Thresholds* ⟨477⟩, supports a flexible reporting threshold to accommodate product-specific factors. The Expert Committee may consider incorporating this new approach in future revisions, as applicable.

Comment summary #4: The commenter observed a retention time (RT) of 25 min for ferricyanide rather than 17 min as indicated in the briefing for the test for *Limit of Ferricyanide*.
Response: Comment not incorporated. Review of the validation data confirms a RT of 21 min for ferricyanide. The typical retention time for ferricyanide is only mentioned in the PF Briefing. Therefore, no change is required.
Expert Committee initiated change #1: In the test for *Limit of Ferrocyanide and Other Related Compounds* revise the calculation to refer to potassium ferrocyanide with a molecular weight of 368.34 for M_{r2} to align with the labelled value of the reference standard

Monograph/Section(s): Sodium Nitroprusside Injection/Multiple Sections
Expert Committee: Small Molecules 2
No. of Commenters: 1

Comment summary #1: The commenter indicated that the acceptance criteria for Ferrocyanide in the test for *Limit of Ferrocyanide and Other Related Compounds* is different from what has been approved and recommends revising to be consistent with the approved acceptance criteria.
Response: Comment incorporated. The acceptance criteria for Ferrocyanide is widened from 0.05% to 0.1%.
Comment summary #2: The commenter recommended removing the "reporting threshold" in the test for *Organic impurities* as it will vary based on product-specific factors.
Response: Comment not incorporated. A new USP general chapter, *User-Determined Reporting Thresholds* ⟨477⟩, supports a flexible reporting threshold to accommodate product-specific factors. The Expert Committee may consider incorporating this new approach in future revisions, as applicable.
Expert Committee initiated change #1: In the test for *Limit of Ferrocyanide and Other Related Compounds* revise the calculation to refer to potassium ferrocyanide with a molecular weight of 368.34 for M_{r2} to align with the labelled value of the reference standard.

Monograph/Section(s): Tolterodine Tartrate/Multiple Sections
Expert Committee: Small Molecules 3
No. of Commenters: 3

Comment summary #1: The commenter indicated that the proposed *Assay* method is not suitable for their drug substance because the Diol impurity and Diol acetate impurity are not eluted in the stipulated run time.
Response: Comment not incorporated. The Expert Committee indicated that while a run time of NLT 3 times the retention time of tolterodine is proposed for the *Assay,* the user is not restricted from increasing the run time as needed.
Comment summary #2: The commenter recommends listing the chemical names of the impurities that have been removed from the *USP Reference Standards* section below *Table 2* for the relative retention times in the test for *Organic Impurities*.
Response: Comment incorporated.
Comment summary #3: The commenter recommended removing the "reporting threshold" in the test for *Organic impurities* as it will vary based on product-specific factors.
Response: Comment not incorporated. A new USP general chapter, *User-Determined Reporting Thresholds* ⟨477⟩, supports a flexible reporting threshold to accommodate product-specific factors. The Expert Committee may consider incorporating this new approach in future revisions, as applicable.
Comment summary #4: The commenter observed that tolterodine dimer impurity elutes in the gradient and resulted in poor response posing difficulty in establishment of LOD, LOQ values.

Response: Comment not incorporated. The Expert Committee determined the method supports the detection of the impurity based on the validation data.
Comment summary #5: The commenter observed retention time variation for 6-methyl-4-phenylchromanone, 6-Methyl-4-phenylchromanol and Diol acetate impurity. In addition, the 6-methyl-4-phenylchromanone impurity and the commenter's In-House Impurity-C eluted at the same retention time. The commenter is also concerned with recovery of the Trans Cinnamic acid indicating that when spiked at 0.15% level 0.44% is observed.
Response: Comment not incorporated. The Expert Committee determined that the relative retention times in the proposal are consistent with the validation. The relative retention times listed in the monograph are intended for information. Use of alternate procedures is discussed in *General Notices 6.30. Alternative and Harmonized Methods and Procedures*. Future revisions to this monograph can be considered upon receipt of supporting information.
Comment summary #6: The commenter observed issues with separation between the Tolterodine S-Enantiomer and Tolterodine tartrate peaks in the test for *Enantiomeric Purity* and indicated that the method is not suitable for their drug substance.
Response: Comment not incorporated. The minor revisions to the *Enantiomeric Purity* test do not impact separation. The comment is beyond the intended scope of the *PF* proposal. If necessary, the Expert Committee may consider future revisions to this monograph upon receipt of supporting information.
Expert Committee initiated change #1: In *Table 2* under *Organic Impurities*, the note is revised as follows: [Note—The relative retention times in *Table 2* are provided as information that could aid in peak assignment.]
Expert Committee initiated change #2: In *Table 3* under *Organic Impurities*, the *Relative Retention Time* column is removed.

Monograph/Section(s): Tolterodine Tartrate Extended-Release Capsules/Organic Impurities
Expert Committee: Small Molecules 3
No. of Commenters: 1

Comment summary #1: The commenter indicated that, in the test for *Organic impurities*, the acceptance criteria for "Monoisopropyl tolterodine", "6-Methyl-4phenylchromanol" and "Total degradation products" are different from what has been approved and recommends revising the acceptance criteria to be consistent with what has been approved
Response: Comment not incorporated. The proposed acceptance criteria are consistent with the sponsor's approved specifications and represent the widest approved limits available to USP. If needed a future revision can be considered upon receipt of approved specifications and supporting data.
Comment summary #2: The commenter indicated that the test for *Organic impurities* is missing some key degradation products. The commenter recommends including other degradation products with limits consistent with what has been approved.
Response: Comment not incorporated. The proposed acceptance criteria are consistent with the sponsor's approved specifications. If needed a future revision can be considered upon receipt of approved specifications and supporting data.
Comment summary #3: The commenter recommended removing the "reporting threshold" in the test for *Organic impurities* as it will vary based on product-specific factors.
Response: Comment not incorporated. A new USP general chapter, *User-Determined Reporting Thresholds* ⟨477⟩, supports a flexible reporting threshold to accommodate product-specific factors. The Expert Committee may consider incorporating this new approach in future revisions, as applicable.

[*Ulipristal Acetate monograph commentary was updated on 23-Jan-2025 to correct numbering]*

Monograph/Section(s): Ulipristal Acetate/Organic Impurities
Expert Committee: Small Molecules 5
No. of Commenters: 1

Comment summary #1: The commenter recommended removing the "reporting threshold" in the test for *Organic impurities* as it will vary based on product-specific factors.
Response: Comment not incorporated. A new USP general chapter, *User-Determined Reporting Thresholds* ⟨477⟩, supports a flexible reporting threshold to accommodate product-specific factors. The Expert Committee may consider incorporating this new approach in future revisions, as applicable.

Monograph/Section(s): Vardenafil Tablets/Organic Impurities
Expert Committee: Small Molecules 5
No. of Commenters: 1

Comment summary #1: The commenter indicated that the acceptance criteria for vardenafil acid and vardenafil related compound E" are different from what has been approved and recommended to reach out to FDA-approved applicants to obtain relevant information.
Response: Comment incorporated. The acceptance criteria for Vardenafil acid and Vardenafil related compound E are revised from NMT 0.3% to NMT 0.4%.
Comment summary #2: The commenter indicated that the acceptance criteria for "Total degradation products" is different from what has been approved and recommended to reach out to FDA approved applicants to obtain relevant information.
Response: Comment not incorporated. The proposed acceptance criteria are consistent with what has been approved by the FDA. The Expert Committee may consider future revisions to this monograph upon receipt of supporting data.
Comment summary #3: The commenter recommended removing the "reporting threshold" in the test for *Organic impurities* as it will vary based on product-specific factors.
Response: Comment not incorporated. A new USP general chapter, *User-Determined Reporting Thresholds* ⟨477⟩, supports a flexible reporting threshold to accommodate product-specific factors. The Expert Committee may consider incorporating this new approach in future revisions, as applicable.

Monograph/Section(s): Xanthan Gum/Impurities
Expert Committee(s): Complex Excipients
No. of Commenters: 8

Comment Summary #1: The commenter requested to add the quantities of *System Suitability solution*, *Sensitivity solution*, and *Standard solution* transferred into headspace vials for clarification.
Response: Comment incorporated.
Comment Summary #2: The commenter requested to add a resolution requirement between Methanol and 2-Propanol from *System Suitability solution* since no requirement is specified.
Response: Comment not incorporated. The methanol listed was an error. The methanol in the *System Suitability solution* was deleted.
Comment Summary #3: The commenter requested to clarify the use of *Sensitivity solution* in the requirement of %RSD since a sensitivity solution is normally used to calculate the signal-to-noise ratio only.
Response: Comment incorporated. The %RSD in the *Sensitivity solution* was deleted.

Comment Summary #4: The commenter requested to modify the calculation formula to provide more details.
Response: Comment partially incorporated. The expert committee acknowledged the need for improvement and made changes based on the commenter's suggestions.
Comment Summary #5: The commenter requested to modify the *Stock Internal Standard solution* preparation for ease of preparation.
Response: Comment not incorporated. The preparation was not prescribed so users have the flexibility to choose their preparation and dilution steps.
Comment Summary #6: The commenter requested to add needle inject as an equivalent method to loop inject to accommodate their need.
Response: Comment not incorporated. There was no supporting data for the needle injection approach. Stakeholders can use alternative procedures after the validation of their procedure and show comparability.
Comment Summary #7: The commenter requested to change "Suitability requirements *Resolution*: NMT 2.0" to "NLT 2.0" to correct the error.
Response: Comment incorporated.
Comment Summary #8: The commenter requested to add a recommended liner information to the test for information.
Response: Comment incorporated to add a note to indicate the liner used.
Comment Summary #9: The commenter requested to clarify the parameter "Cycle" under the "Time" section since this was not commonly used as a parameter.
Response: Comment incorporated. The "cycle" line was deleted.
Comment Summary #10: The commenter requested to clarify the wording in parentheses for the "Carrier pressure" parameter whether it is a necessary requirement.
Response: Comment incorporated. The "Carrier pressure" line was deleted.
Comment Summary #11: The commenter requested to 1) retain the current GC-FID method and include the Limit of Isopropyl Alcohol using the GC-Headspace method proposed as an alternative method, or 2) have an extended implementation date with advanced warning of at least one year (versus the typical six months) to allow sufficient time to react to this change and reduce significant business impact.
Response: Comment incorporated. The Expert Committee acknowledged the commenter's concern and agreed to extend the implementation date by one year (1-Nov-2025) after publication.
Comment Summary #12: The commenter requested to confirm the oven temperature of 310° and a split ratio of 230:1 as they are not typical.
Response: Comment not incorporated. The parameters were not typical, however, are necessary to ensure reproducibility.