Parenteral and Ophthalmic Drug Products Leachables and Extractables Working Group

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Study Protocol – Stage 1
Amendment #1

Experimental Protocol for Qualitative Controlled Extraction Studies on Material Test Articles Representative of Prefilled Syringe (PFS) and Small Volume Parenteral (SVP) Container Closure Systems

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I. Introduction; Purpose of Amendment #1

The original Protocol for this study included five Test Articles, as specified in Table I of that document. Three additional Test Articles, a label and a low-density polyethylene bottle and its associated polypropylene cap, are added to this study as such Articles may be relevant to the types of packaging systems utilized with PODP drug products.

II. Purpose and Scope of Work (Study Protocol Stage I)

The purpose of the experiments outlined in this protocol is to generate data from Controlled Extraction Studies, which the Working Group will use to investigate its hypotheses:

1. Threshold concepts that have been developed for safety qualification of leachables in OINDP can be extrapolated to the evaluation and safety qualification of leachables in PODP, with consideration of factors and parameters such as dose, duration, patient population and product dependent characteristics unique to various PODP types.

2. The science-based best demonstrated practices established for the OINDP pharmaceutical development process can be extrapolated to PODP container closure systems.

3. Threshold and best practices concepts can be integrated into a comprehensive process for characterizing container closure systems with respect to leachable substances and their associated impact on PODP safety.

Controlled Extraction Studies will be performed following the general methodologies contained in this protocol. Test articles will be subjected to different extraction conditions to establish how different experimentally controlled parameters affect the resulting extractables profiles. Of specific interest to the Working Group are the parenteral and ophthalmic dosage forms, particularly Small Volume Parenterals (SVP), Large Volume Parenterals (LVP), Pre-filled Syringes (PFS) and Blow-Fill-Seal systems (BFS). The Stage 1 Protocol specifically focuses on the SVP and PFS dosage forms and on the generation of qualitative extractables profiles. Future Stages will focus on additional dosage forms and/or quantitative aspects of extractables profiling. The intent of the Stage 1 assessment is to generate the fundamental information from which Best Demonstrated Practices can be derived; it is not the intent of this Stage 1 assessment to prospectively establish the practices used in this study as the Best Demonstrated Practices themselves.

As no single analytical technique can be used to identify and quantify all unknown extractables, a variety of methods will be utilized in this protocol to maximize the likelihood that all predominant extractable compounds associated with the test articles are accounted for and appropriately evaluated. Overlap between methods will supply corroborating data that demonstrate the validity of the procedures. To provide a full analytical survey of possible analytes the following strategy will be employed:

1. Gas Chromatography with appropriate sampling/injection and detection strategies e.g. Flame Ionization Detection (GC/FID) and Mass Spectrometry (GC/MS)] for identification and assessment of volatile and semi-volatile extractables.
2. High Performance Liquid Chromatography with appropriate detection strategies [e.g. Diode Array Detection (HPLC/DAD), Mass Spectrometry (LC/MS)] for identification and assessment of relatively polar and non-volatile extractables.
3. Inductively Coupled Plasma/Mass Spectrometry (ICP/MS) and/or Inductively Coupled Plasma/Atomic Emission Spectroscopy (ICP/AES) to detect single elements in the extracts (i.e. metals).

While analytical tests and measurements, such as pH, UV absorbance, and total organic carbon (TOC), can provide insight into the general chemical nature and amount of extracted substances, they do not directly provide information for the identification and/or quantitation of individual extractables and thus will not be utilized in this study.

Studies designed to assess recovery (i.e. mass balance) for individual extractables relative to the known formulations of chemical additives in the various test articles, or reproducibility of extractables profiles for multiple "batches" of any particular test article are not within the scope of this Stage of the test protocol. Additionally, the extraction procedures, analytical techniques/methods, and analysis conditions described in this experimental test protocol will not be fully and rigorously validated. Nevertheless, the scientific credibility of the data generated in this study shall be established via the utilization of system suitability testing with all the analysis methods and by the expert review of the generated data. Finally, "special case" classes of extractables that have defined and highly specific analytical methods that are generally accepted and commonly used for their identification and quantitative assessment will not be considered in this study.

III. Regulatory Status

This experimental test protocol will be conducted in the spirit of Good Laboratory Practices and Good Manufacturing Practices (GXP) requirements. All experiments shall be documented based on the appropriate GXP compliance systems in a participating laboratory. Any changes or clarifications that a participating laboratory makes to this test protocol shall be documented as appropriate, and discussed/approved by the Study Coordination as appropriate.

IV. Safety and Environmental Impact

Chemicals and reagents used in this study (e.g. organic solvents commonly used to enhance solubility of lipophilic targets and to increase transport of small molecules out of complex matrices) may be flammable and/or pose short-term and long-term environmental health risks. Care must be exercised with their use. Consult the Material Safety and Data Sheet (MSDS) for appropriate personal protection and disposal. Safety risks associated with the various processes and procedures performed in this study may exist and should be understood and managed using such strategies as environmental control and personal protection.

V. TEST ARTICLES

A list of the test articles available for use in this study is provided in Table 1. Test articles will be provided in an appropriate form for use as test articles. Certain, but not necessarily all, details of the additive formulations and manufacturing conditions for these test articles are known and are captured in Table 1.

TABLE 1. TEST ARTICLES.

MATERIAL TYPE	MATERIAL APPLICATION	MATERIAL FORMAT	COMPOSITION
Low density polyethylene (LDPE)	Bottle/ Vial	Bottle	4 oz LDPE, part B347A (Container & Packaging Supply)
Polypropylene (PP)	Cap	Cap	PP, Part L764(Container & Packaging Supply)
Adhesive Label	Label applied to outside of PODP Packaging Systems	Sheets	Substrate: Unknown Adhesive: Acrylic polymer(s), residual monomers, water, ammonia (99.55%); wetting agent, Surfynol 336, at 0.4% containing CAS 577-11-7 (> 25%), CAS 9014-85-1 (> 25%); Biocide, Kathon LX, at 0.05% containing Chloro-2-methyl-4-isothiazolin-3-one (CAS 26172-55-4), 1.1-1.4%,2-Methyl-4-isothiazolin-3-one (CAS 2682-20-4), 0.3 - 0.5%, Magnesium Chloride (CAS 7786-30-3), 1.0 - 1.2%, Magnesium nitrate (CAS 10377-60-3), 1.4 - 2.0% Copper nitrate (CAS 3251-23-8) 1,500 - 1,700 ppm, Water, 95 - 97% Printing ink: Irgacure 369 (CAS 119313-12-1) and Irgacure 1173 (CAS 7473-98-5), photoinitiators; Trimethylolpropane triacrylate (TMPTA, CAS 15625-89-5), Tripropylene glycol diacrylate (TPGDA, CAS 42978-66-5), Glycerol propoxy triacrylate (GPTA, CAS 52408-84-1), monomers; HQME/Mequinol (CAS 150-76-5), stabilizer; Carbon black (CAS 1333-86-4),Phthalo blue (CAS 147-14-8),Carbazole violet (CAS 215247-95-3), pigments Varnish : Unknown

VI. CHEMICALS AND EQUIPMENT

Extraction and analytical methods were chosen and designed to utilize chemicals, apparatus, and instrumentation available in typical laboratories routinely involved with this type of study.

A. Extraction Solvents and Additional Chemicals

The chemicals required for use as, or in preparation of, extraction solvents, as well as the directions for the preparation of several of these extraction solvents, were outlined in the original Protocol and such information is directly relevant to the testing to be performed as a result of this Protocol Amendment #1.

B. Extraction Equipment

1. Soxhlet Extraction
   - Soxhlet apparatus.
   - All glass labware for these extractions must be acid-washed prior to use.
   - The use of any lubricants, such as vacuum grease on ground glass joints, should be avoided.

2. Reflux
   - Reflux apparatus [e.g. round bottom flask (200 mL or larger), condenser with ground glass joints, hot plate or heating mantle].
   - All glass labware for these extractions must be acid-washed prior to use.
   - The use of any lubricants, such as vacuum grease on ground glass joints, should be avoided.

3. Sealed Container
   - Teflon [Savillex (6133 Baker Road, Minnetonka, MN 55345-5910 USA, Phone: 952-935-4100, E-mail: info@savillex.com), Part # 0108, 8 fl. Oz. Teflon Jar]
   - Pyrex [VWR (Customer Service: 1-800-932-5000), Catalog # 89000-236, Media / Storage Bottles with Standard GL45 Polypropylene Cap, 250 mL] containers
   - All glass labware for these extractions must be acid-washed prior to use. Teflon vessels are used with the high pH extractions to avoid any leaching from glass, especially for samples for ICP analysis
   - Autoclave
   - Oven with operating range of 30 to 75 °C; explosion proof

C. Analytical Instrumentation

- Gas chromatograph equipped with a Flame Ionization Detector (GC/FID)
- Gas chromatograph equipped with a Mass Spectrometer (GC/MS). GC systems that employ flow splitting to accomplish FID and MS detection in tandem could be used in this study.
- Headspace Sampler/Injector (HS) for GC/MS Instrumentation.
- Liquid chromatograph equipped with a photodiode array detector
- Liquid chromatograph equipped with an APCI (Atmospheric Pressure Chemical Ionization) capable Mass Spectrometer (LC/MS). Preference is given to LC systems that are capable of both DAD and MS detection. Additional detectors (e.g. corona assisted discharge detectors, evaporative light scattering) may be used as appropriate.
- Inductively Coupled Plasma Mass Spectrometer (ICP-MS)

VII. EXTRACTION PROCEDURES

A. General

In the PQRI OINDP studies, extractions were performed on each test article using three solvents representing a range of polarity, specifically

- methylene chloride (dichloromethane)
- 2-propanol (isopropanol, IPA)
- hexane (n-hexane, not hexanes).

This was appropriate in the case of OINDP given the nature of the drug vehicles used in those types of products (organic solvents) and the conditions of contact between the drug vehicles and the container closure system (continuous direct contact over shelf life).

While the use of such extraction solvents may be relevant for PODP products, a significant portion of PODP products are water-based and the three solvents previously employed do not address the unique solubilizing properties of water and aqueous buffer systems. Thus in the case of PODP, the OINDP solvents will be augmented by aqueous extraction media. These additional aqueous extraction media, and their associated justification, include

* Water at pH 2.5 (HCl/KCl mixture); justification, few therapeutic products are lower than pH 2.5.
* Water at pH 9.5 (Phosphate buffer); justification, few therapeutic products are higher in pH than 9.5.
* 1/1 IPA/water; justification; simulates aqueous formulations containing solubilizing agents, provides for trend analysis (with IPA and water alone).

Thus, the five extraction media to be used in this Stage 1 Protocol are the three aqueous systems listed above, IPA and hexane.

Similarly, the extractions performed in the PQRI OINDP study, including Soxhlet and reflux, were consistent with the nature of the test materials, the extraction solvents and the nature of OINDP products. Because a significant portion of PODP products are water-based, extractions performed in this study will be include the OINDP methods and extraction methods compatible with aqueous extraction media, specifically sealed vessel extraction.

The specific operational details associated with performing these extractions are outlined in the following sections. Note that the outlined extraction parameters and conditions maybe subject to modification and the details of any modified extraction process will be established in consultation with study coordinator prior to initiation of experimental work in any particular laboratory. Additionally, all extractions should be performed with appropriate extraction blanks.

B. Extraction Maps

The number of potential test situations, defined as the coupling of a test material, an extraction solvent and an extraction process, is large and addressing each individual test situation is not necessary to generate relevant information upon which best demonstrated practice recommendations may be based. Additionally, some experience has already been gained during the characterization of the initial set of five test Articles. Finally, the nature of the label itself is such that it is clear that under certain extraction procedures, the label would dissolve. Thus not all extraction conditions utilized in the intial phase of this Study will be used to characterize the two new test materials. Test situations that are within the scope of this study are delineated in the following Extraction Maps. The intent of this Stage 1 assessment is to generate the fundamental information from which best demonstrated practices can be derived; it is not the intent of this Stage 1 assessment to establish the practices used in this study as best demonstrated practices themselves.

1. Test Material Versus Extraction Solvent Map

Table 2 establishes which extraction solvents will be utilized with which materials.

Table 2. Material Versus Extraction Solvent Map (1, 3)

	Aqueous		Mixed	Organic		Thermal
	pH 2.5	pH 9.5	IPA/Water	IPA	Hexane	(2)
LDPE and PP	X	X	X[4]	X	X	X
Label	X	X	X	---	---	X

Notes: (1) An X denotes a material/solvent couple that will be performed, an --- denotes a couple that will not be performed.
(2) By Headspace analysis.
(3) During the course of this study it may be the case that certain material – solvent couples will be incompatible. Such incompatibilities should be reported the PODP study coordinator and incompatible extracts should not be tested.
(4) Both reflux and sealed vessel with the IPA/Water mixture

2. Extraction Method Versus Extraction Solvent Map

Table 3 establishes which extraction methods will be utilized with which extraction solvents.

Table 3. Extraction Method Versus Extraction Solvent Map (1, 4)					
	Aqueous		Mixed	Organic	
	pH 2.5	pH 9.5	IPA/Water	IPA	Hexane
Testing for the LDPE and PP					
Soxhlet	---	---	---	X	X
Reflux	---	---	--	X	X
Sealed Vessel	X (2)	X (2)	X (3)	---	--
Testing for the Label					
Sealed Vessel	X (2)	X (2)	X (3)	---	--

Notes: (1) An X denotes a method/solvent couple that will be performed, an --- denotes a couple that will not be performed.
(2) Under autoclave conditions (121°C for 1 hr).
(3) Storage at 55°C for 3 days.
(4) During the course of this study it may be the case that certain material – solvent couples will be incompatible. Such incompatibilities should be reported the PODP study coordinator and incompatible extracts should not be tested.

Soxhlet and reflux extractions will not be performed on the label as it is envisioned that such extractions would essentially dissolve the label. Sonication extractions will not be used in this phase of the study as the previous study results suggest that this method does not produce useful extractables profiles.

C. General Considerations

Care in experimental approach should be exercised in terms of producing extracts that are free from analytical artifacts. Glass is the appropriate vessel for samples intended for organic analysis, while Teflon is recommended for inorganic (metals) analysis. Glass is a problem in metal analysis especially at higher pHs due to leaching of glass (*e.g.* Si, B, Al, Na). Teflon is a problem with organics due to adsorption of extractables.

Extraction vessels shall be cooled and the materials separated from the liquid, by an appropriate means. The extracts shall be collected and stored in an appropriate vessel with minimal headspace. Retain the extract for analysis in such a way as to preserve their compositional integrity (protect from light, heat and evaporation losses).

For all extractions, the weight of test article sample, extracting solvent volume, and sample extract concentration factors should be established and adjusted so that it is possible to detect and identify individual extractables present at the 10 µg/g (ppm) level. Individual extractables may be detected and identified at lower levels if the analytical method employed is readily capable of achieving such sensitivity.

For each extraction technique and solvent type, appropriate blanks (no test article sample) must be prepared. These must be prepared concurrently using a different extraction apparatus (same type) under the same conditions, or by using the same apparatus prior to charging with sample. The extraction conditions represent the censuses opinion of the PODP chemistry subteam.

All extracts should be visually inspected prior to analysis to ensure that they are free from obvious particulate matter. Should such an inspection reveal particulate matter, this finding should be reported to the Study Coordinator prior to proceeding with sample analysis. In most cases it is likely that the Study Coordinator will request that the sample be processed in such a way that the particulate is removed from the extract prior to its testing. Collection of the removed particulate may be requested so that the material itself can be analyzed and identified.

D. Soxhlet Extraction

The conditions for performing Soxhlet extractions were specified in the original Protocol. Any modifications appropriate to the conditions specified in the original Protocol, based on the experiences gained during the characterization of the five original Test Articles, will be documented in the Final Report associated with this study.

E. Reflux

The conditions for performing Reflux extractions were specified in the original Protocol. Any modifications appropriate to the conditions specified in the original Protocol, based on the experiences gained during the characterization of the five original Test Articles, will be documented in the Final Report associated with this study.

F. Sealed Vessel Extraction

The conditions for performing sealed vessel extractions were specified in the original Protocol. Any modifications appropriate to the conditions specified in the original Protocol, based on the experiences gained during the characterization of the five original Test Articles, will be documented in the Final Report associated with this study.

VIII. ANALYTICAL METHODS

A. General

Considerable experience was gained during the characterization of the original five Test Articles, specifically related to the analytical methods employed. While in general the same analtytical techniques outlined in the original Protocol will be used with the additional two Test Articles, the specific operating details of the methods used may be somewhat different from those specified in the original Protocol. Any modifications appropriate to the conditions specified in the original Protocol, based on the experiences gained during the characterization of the five original Test Articles, will be documented in the Final Report associated with this study.

The system suitability requirements contained in the original Protocol are relevant to and required for the analyses performed to characterize the two additional Test Articles.

B. Gas Chromatography (GC)

The conditions for performing GC analyses of the extracts were specified in the original Protocol. Any modifications appropriate to the conditions specified in the original Protocol, based on the experiences gained during the characterization of the five original Test Articles, will be documented in the Final Report associated with this study.

C. High Performance Liquid Chromatography (HPLC)

The conditions for performing HPLC analyses of the extracts were specified in the original Protocol. Any modifications appropriate to the conditions specified in the original Protocol, based on the experiences gained during the characterization of the five original Test Articles, will be documented in the Final Report associated with this study.

D. Inductively Coupled Plasma – Mass Spectrometry (ICP-MS)

The conditions for performing ICP-MS analyses of the extracts were specified in the original Protocol. Any modifications appropriate to the conditions specified in the original Protocol, based on the experiences gained during the characterization of the five original Test Articles, will be documented in the Final Report associated with this study.

E. Headspace GC/MS

The conditions for performing Headspace GC/MS analyses of the Test Articles themselves were specified in the original Protocol. Any modifications appropriate to the conditions specified in the original Protocol, based on the experiences gained during the characterization of the five original Test Articles, will be documented in the Final Report associated with this study.

IX. Data Evaluation and Reporting

A. Qualitative Analysis

- A list of all identified entities (compounds, elements) that were not detected in the corresponding blank. This list should include the recognized compound name, CAS Registry number, chemical formula, and chemical structure.
- A list of all unidentified chromatographic peaks that were not detected in the corresponding blank at signal to noise ratios greater than 10. The participating laboratory should determine and report the analyte concentration that corresponds to this signal to noise ratio (typically defined as the limit of quantitation, LOQ).
- Copies of chromatograms, spectra, etc.
- Complete methodological information for both the extraction and analysis processes.
- The required system suitability results, which should include an assessment of detectablility.

- The identification status for all compounds shall be established and reported as follows:

  - A *Confirmed* identification means that collaborating information has been obtained including mass spectrometric fragmentation pattern, confirmation of molecular weight (or elemental composition), match in retention time and spectrum with authentic standard.
  - A *Confident* identification means that sufficient data to preclude all but the most closely related structures have been obtained
  - A *Tentative* identification means that data have been obtained that are consistent with a class of molecule only.

B. Semi-Quantitative Analysis

While it is not the primary intent of this Stage 1 Protocol to produce quantitative data, some of the test methods employed may be amenable to concentration estimation (e.g. ICP, GC with internal standards). In the case that a participating laboratory reports concentration estimates, the means by which such estimates were obtained must be indicated. Additionally, all such estimates shall be reported with a convention (e.g. significant figures) which effectively reflects the uncertainty in the determination. As was noted previously, the threshold for reporting semi-quantitative results is 10 µg/g.

X. GLOSSARY OF ABBREVIATIONS

GC/FID	Gas Chromatography with Flame Ionization Detector
GC/MS	Gas Chromatography with Mass Spectrometric Detection
HPLC/DAD	High Pressure Liquid Chromatography-Diode Array Detection
LC/MS	Liquid Chromatography Mass Spectrometric Detection
ICP-MS	Inductively Coupled Plasma Atomic Emission Spectroscopy
TIC	Total Ion Chromatogram
API-ES	Atmospheric Pressure Ionization - Electrospray
HS	Headspace
PQRI	Product Quality Research Institute
OINDP	Orally Inhaled and Nasal Drug Products
PODP	Parenteral and Ophthalmic Drug Products
LDPE	Low density polyethylene
PP	Polypropylene
LVP	Large volume parenteral
SVP	Small volume parenteral
BFS	Blow-fill-seal
PFS	Pre-filled syringe

XI. REFERENCES

PQRI Research Project Proposal: *Development of Scientifically Justifiable Thresholds and Best Demonstrated Characterization Practices for Leachables and Extractables in Parenterals and Ophthalmic Drug Products (PODP).* (March 2008).

FDA Guidance for Industry: *Container Closure Systems for Packaging Human Drugs and Biologics-Chemistry Manufacturing, and Controls Documentation,* (May 1999).

European Medicines Agency (EMEA): *Guideline on Plastic Immediate Packaging Materials,* (May, 2005).

Parenteral and Ophthalmic Drug Products Leachables and Extractables Working Group. *Experimental Protocol for Qualitative Controlled Extraction Studies on Material Test Articles Representative of Prefilled Syringe (PFS) and Small Volume Parenteral (SVP) Container Closure Systems.* Study Protocol – Stage 1. Issued and Effective, December, 2009.