Parenteral and Ophthalmic Drug Products (PODP) Leachables and Extractables Working Group

Issued and Effective
September, 2011

Study Protocol – Stage 2

Experimental Protocol for Simulation Study of Blow-Fill-Seal (BFS) PODP Container Closure Systems

TABLE OF CONTENTS

I. Introduction

It has been well established that substances extracted by drug products from their container closure systems can affect the drug product's safety and efficacy. Regulatory guidance has provided some recommendations regarding the analysis and toxicological safety assessment (*i.e.,* qualification) of such substances. Thus, for example, the FDA issued *Container Closure Systems for Packaging Human Drugs and Biologics – Chemistry, Manufacturing and Controls (CMC) documentation Guidance for Industry* in May 1999[1]. In addition, the European Medicines Agency (EMEA) issued its *Guideline on Plastic Immediate Packaging Materials* in May 2005.[2] Specific Guidance for Orally Inhaled and Nasal Drug Products (OINDP) is contained in two CMC Guidances addressing OINDP[1]: (i) the draft *Guidance for Industry, Metered Dose Inhaler (MDI) and Dry Powder Inhaler (DPI) Drug Products, Chemistry, Manufacturing, and Controls Documentation* (November, 1998); and (ii) the *Guidance for Industry, Nasal Spray and Inhalation Solution, Suspension, and Spray Drug Products Chemistry, Manufacturing, and Controls Documentation* (July, 2002).

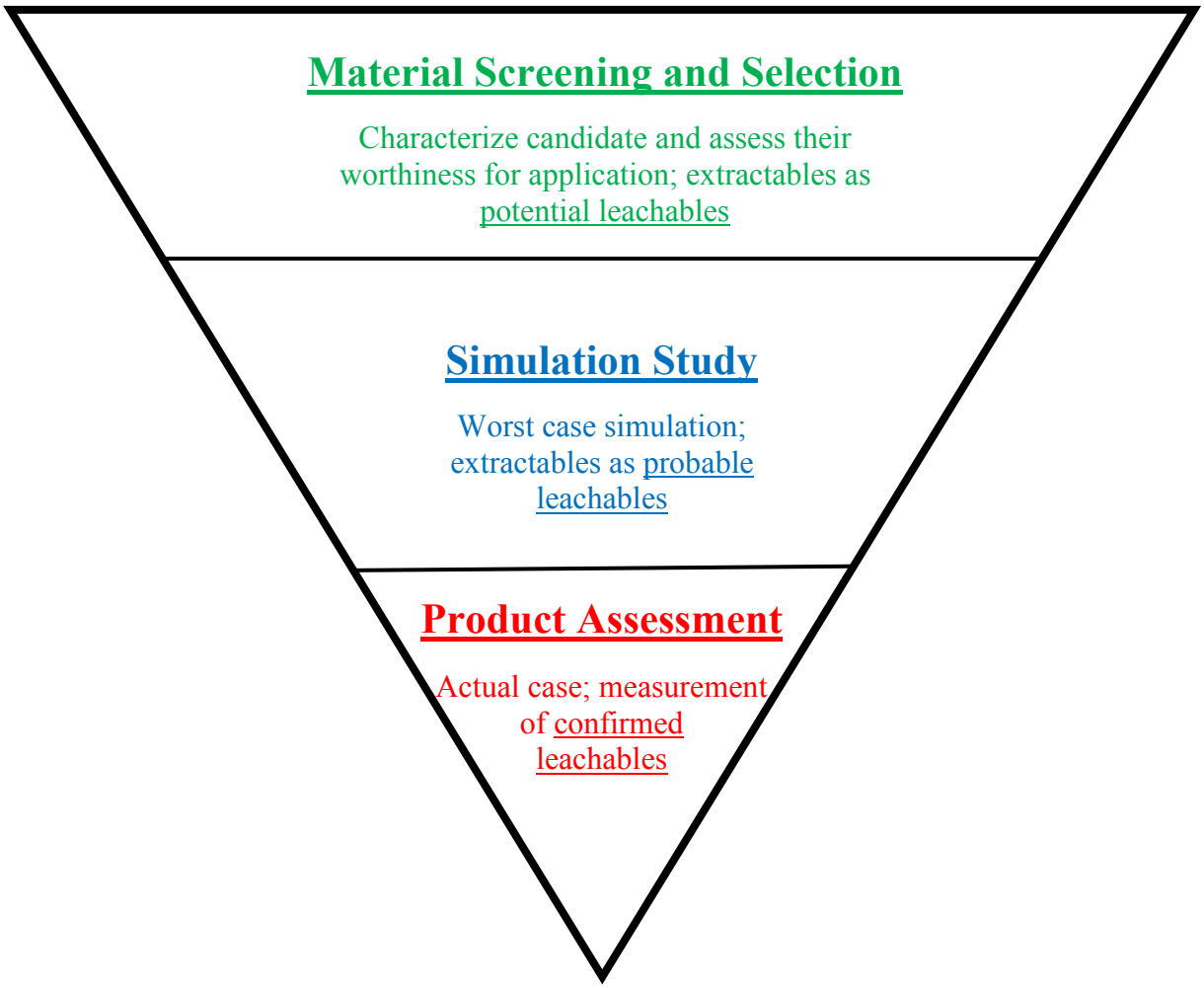
In September 2006, the Product Quality Research Institute (PQRI) issued a Recommendation entitled "Safety Thresholds and Best Practices for Extractables and Leachables in Orally Inhaled and Nasal Drug Products"[3]. This Recommendation provided a scientific rationale and process to identify, quantify and establish the biological safety (i.e. qualify) of leachables and/or extractables where appropriate, in OINDP. Included in this Recommendation were experimental protocols, and the results thereof, for establishing Best Demonstrated Practices for the performance of Controlled Extraction Studies, specifically relevant of the OINDP dosage forms.

The PQRI Parenteral and Ophthalmic Drug Products (PODP) Leachables and Extractables Working Group is developing, executing and reporting experimental studies as the means of establishing Best Demonstrated Practices for the performance of Chemical Assessments specifically relevant for PODP container closure systems and dosage forms. Figure 1, The Chemical Assessment Triad, illustrates the Chemical Assessment Process. The PODP Stage 1 study considered the process of Material Characterization; specifically the processes by which a Controlled Extract is generated, by which a Controlled Extract is analyzed and by which the test results are evaluated and interpreted. This Stage 2 study considers the process of performing a Simulation Study, specifically establishing the extractables profile of an experimental container closure system constructed from some of the materials that were characterized in the Stage 1 study. This experimental container closure system specifically mimics a Blow-Fill-Seal (BFS) packaging system, such as those used with many ophthalmic products, consisting of a BFS bottle, its associated cap, a closure gasket and an affixed printed label.

This experimental protocol will be used by all participating laboratories and investigators.

[1] Available at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070551.pdf.
[2] Available at http://www.emea.europa.eu/pdfs/human/qwp/435903en.pdf
[3] Available at http://pqri.org/pdfs/LE_Recommendations_to_FDA_09-29-06.pdf

Figure 1. Generalized Chemical Assessment, The Chemical Assessment Triad. A simulation study may be an appropriate and effective bridge between the material characterization process, which establishes extractables that are tentative leachables and product assessment, which measured confirmed leachables. As the name suggests, a simulation study seeks to mimic the product assessment by using simulating solvents to facilitate the analytical tasks and using extraction conditions which accelerate the product contact conditions. The simulation study may be the basis of a preliminary toxicological assessment and can be used to establish target leachables to measure during product assessment.



II. Purpose and Scope of Work (Study Protocol Stage 2)

The purpose of the experiments outlined in this protocol is to generate data from a Simulation 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.

The Simulation Study will be performed following the general methodologies contained in this protocol. The Test System, designed to mimic a Blow-Fill-Seal (BFS) packaging system, will be filled with various simulating extraction solvents which are intended to mimic aqueous PODP drug products. The Test System will be exposed to accelerated storage conditions that mimic a post-filling shelf life of 2 years at ambient temperature. Consistent with the BFS process, the filled Test System will not be exposed to terminal or auxiliary sterilization. At certain times during accelerated storage, the simulating extraction solvents will be characterized to reveal organic and inorganic extractables, thus establishing the Test System's Extractables Profile. The intent of this Stage 2 assessment is to generate the fundamental information from which Best Demonstrated Practices can be derived; it is not the intent of this Stage 2 assessment to prospectively establish the practices used in this study as the Best Demonstrated Practices themselves. It can be reasonably expected that this Extractables Profile will include tentative extractables revealed in the Stage 1, Material Characterization, study,

As no single analytical technique can be used to identify and quantify all 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. Gas chromatography with headspace sampling (HS-GC) for identification and assessment of volatile extractables.
4. 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 SYSTEM

The Test System is designed to mimic a Blow-Fill-Seal (BFS) packaging system, such as that used for ophthalmics and small volume parenterals (SVP). In general such a system consists of the

BFS container (in this case a bottle), one or more closures (in this case the bottle's associated cap and a gasket/liner) and some type of labeling (in this case a printed label). Due to procurement limitations and confidentiality issues, a Test System that truly represents commercial BFS packaging systems was not obtained. Rather, a test system was loosely constructed from materials which had been characterized in the Stage 1 study. Specifically, the Test System consists of:

1. A low density polyethylene (LDPE) bottle, with a PP screw cap,
2. A rubber gasket which is used as a liner inside the plastic cap, and
3. A printed adhesive label, which is affixed to the bottle's outer surface.

Identifying information related to these Test System Components is contained in Table 1.

TABLE 1. TEST ARTICLE.

MATERIAL TYPE	MATERIAL APPLICATION	MATERIAL FORMAT	DESCRIPTION
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 on Container Surface	Label 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
Rubber (Elastomer) (RE)	Closures	Gasket/liner	Brominated isobutylene isoprene copolymer (57.3%); calcined aluminum silicate, 38.2%, titanium dioxide, 1.2%; paraffinic oil, 1.2%; zinc oxide, 0.6%; polyethylene, 0.6%; SRF Carbon block mixture, 0.4%; calcined magnesium oxide, 0.3%; 4,4'-dithiodi-morpholine/polyisobutylene, 0.3%

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

Chemicals required for the use as, or preparation of, extraction solvents, are as follows:

- Laboratory research grade water or Water for Injection (WFI), appropriately sourced, collected and stored to minimize background levels of extraneous substances.
- Potassium chloride
- Hydrochloric acid, 0.1 N
- Sodium phosphate monobasic
- Sodium phosphate dibasic
- Sodium hydroxide, 1 N
- Isopropyl alcohol (glass bottled; IPA)
- pH calibration buffers; pH 1.68, 4.01, 9.18 and 12.48 (saturated calcium hydroxide)

The preparation of several of these extraction solvents is as follows:

- Water at pH 2.5 (HCl/KCl mixture): The KCl solution is prepared at 0.01M. Weigh 1.5 grams of KCl into a 2.0 L vol flask containing 1500 mL water. Add 60 mL 0.1 N HCl. Dilute to volume with water. This final solution is 0.01 M KCl and 0.003 M HCl, which should have a pH of 2.5.

- Water at pH 9.5: Weigh 1.24 grams sodium phosphate monobasic and 18.7 grams of sodium phosphate dibasic, transfer to an appropriate vessel, and dissolve in 2 liters of water. The pH of this solution is reported to be 8.0 Titrate with 1 N NaOH to get a pH of 9.5. This solution is 0.0045 M monobasic and 0.066 M dibasic.

- IPA/Water (1/1): Mix equal volumes of IPA and water.

B. Additional Chemicals

- Analytical reagents required to perform the analytical testing.
- Reference and/or Internal standards required to perform the analytical testing.

C. Extraction Equipment

- Oven with operating range of 30 to 50 °C; explosion proof

D. 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, preferred) and/or Inductively Coupled Plasma Atomic Emission Spectrometer (ICP/AES)

VII. EXTRACTION PROCEDURE

A. Simulating Extraction Solvents

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 replaced by aqueous extraction media. These 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.
* Water alone.

B. Accelerated Extraction Conditions

It is generally well-established that storage at 40°C for 6 months accelerates a product shelf life of 2 years at ambient temperature. Such acceleration will be utilized in this study. In order to allow for trending of the extractables profile versus time, this study will utilize three test intervals; after 1, 2 and 6 months of storage. Thus individual test systems will be filled, stored for either 1, 2 or 6 months and then profiled fro extracted substances. The filling will be staggered so that all the filled Test Articles “mature” at the same time, facilitating the analysis of the fill solutions. This will be accomplished by filling the test articles at three different times. One group of test articles will be filled at the initiation of the study and placed into storage.

These would be the 6 month samples. After four months of storage has occurred, a second group of test articles will be filled and placed into storage. These would be the 2 months samples. After five months of storage, a third group of samples will be filled and put into storage. These would be the 1 month samples. After 6 months of storage, all units would be removed from storage and tested.

C. Exaggerating Factors

The weights and surface areas of the Test System components will be measured so that the Test System can be compared to commercial BFS packaging systems either on the basis of mass or surface area.

VIII. Analytical Methods

A. General

The analytical screening methods that will be utilized to discover, identify and quantitate extracted substances will be similar to the same analytical methods that we used to provide the data in the Stage 1 study. As considerable experience was gained with these methods during the Phase 1 study, the actual operating conditions used to support this Phase 2 study may be somewhat different that the conditions enumerated in this Protocol. Such differences will be noted in the Final Report associated with this study.
It is never-the-less expected than all analyses performed in this study will be performed with systems and operating parameters that meet the system suitability requirements given herein.

It is the general objective that all organic extractables that are present in the simulating extracts at a concentration of 0.1 µg/mL (ppm) will be confidently identified and effectively quantitated. It is the general objective that all extracted metals and trace elements that are present in the simulating extracts at a concentration of 0.01 µg/mL (ppm) will be confidently identified and effectively quantitated. It is noted that testing of extraction blanks (portions of simulating solvent stored in inert vessels) allows one to differentiate between analytical artifacts (which are present in both the blanks and the extracts at roughly similar levels) and extractables, which are present in the extracts at levels significantly higher than they are in the blanks.

B. System Suitability

All testing performed in support of this Protocol shall include appropriate system suitability assessment. Demonstration of system suitability will be accomplished according to the following three-step approach:

Step 1: Each participating laboratory will ensure that analytical instrumentation is in proper condition and will demonstrate instrument suitability by following its proprietary (in-house) procedures.

Step 2: Each participating laboratory will follow the procedures defined in this Protocol which involve the characterization of specified test mixtures by GC, HS-GC, LC and ICP. The test mixtures are suitable to demonstrate adequate and effective analytical performance (for example, separation efficiency, selectivity and sensitivity). All generated system suitability data will be evaluated with regard to the required specifications/acceptance criteria.

Step 3: Internal Standardization. Specifically for the GC methodology, the extracts will be supplemented by introducing a surrogate internal standard and an injection standard. Analysis of these standards complements system suitability testing by providing a means of establishing the effectiveness of sample preparation/sample introduction processes. The use of internal standards is discussed in the section describing the actual GC analysis of the extracts.

Table 2 presents a list of system suitability analytes for GC and HPLC based analytical techniques.

System suitability testing for the ICP trace element analysis shall include the preparation and testing of a system suitability test mixtures that contains all the targeted elements listed previously at a concentration of 0.25 µg/ml. System suitability testing shall consist of the demonstration that all elements can be detected at the prepared concentration.

All system suitability testing performed during the course of this study and all system suitability test results thereof shall be reported to, and reviewed by, the PODP study coordinator before any analytical data is accepted by the PODP Working Group. Failure to meet acceptance criteria will be the basis for rejecting analytical data provided by the participating laboratory and frequent failures by a participating laboratory can be the basis for the disqualification of that laboratory.

Table 2. Composition of the System Suitability Test Mixtures.

Compounds for HPLC Analysis:

Custom-made test mixture to be prepared by the participating laboratories from standard grade reference materials:

Compound	Abbreviation	LC Test Mixture Concentration (µg/ml, ppm)
Caprolactam	CAP	1
Butylatedhydroxytoluene	BHT	5
Diphenylamine	DPA	5
Mono-(2-ethylhexyl) phthalate	MEHP	1
Stearic acid	SA	5
Di-(2-ethylhexyl phthalate)	DEHP	1
Bisphenol A	BPA	1

The test mix should be prepared by appropriate dilution of more concentrated stock solutions, prepared using solvents appropriate for the individual reagents. The final composition of the test mixture should be similar to, or compatible with, the mobile phase used in the LC analysis.

Table 2. Composition of the System Suitability Test Mixtures (continued).

Compounds for GC Analysis, Grob Mixture:

Commercial Sources:
e. g.: "Grob-Test-Mix", Cat# 11373, Restek

Reference:
K. Grob, Jr.., G. Grob and K. Grob, "Testing Capillary Gas Chromatographic Columns", Journal of Chromatography, 219, p. 13-20, (1981)

Combined solution of the following substances in methylene chloride:	**Concentration, µg/ml (ppm)**
	GC Test Mixture: (Grob Mixture diluted 1/20 in methylene chloride)
L(+)-2,3-butanediol	27
n-decane	14
2,6-dimethylaniline	16
2,6-dimethylphenol	16
methyl decanoate (C10:0)	21
methyl docecanoate (C12:0)	21
methyl undecanoate (C11:0)	21
nonanal	20
1-octanal	18
n-undecane (C11)	14

Compounds for Headspace GC Analysis:

Custom-made test mixture to be prepared by the participating laboratories from standard grade reference materials:

Combined solution of the following substances in polyethylene glycol 200[1] (PEG 200):	**HSGC Test Mixture I**	
	µg/ml	**µg/vial**
Methanol	200	2
Acetic Acid	200	2
Cyclohexanone	100	1
Toluene	100	1
Trimethylsilanol[2]	200	2
2-Ethyl hexanol	200	2

[1] Preparation of SST-Sample:
- add 10 µl of the HS-Test-Mixture-I to a 20 ml crimp-cap vial
- add 10 µl of internal standard solution (2 mg of 1,4-Dioxane/ml PEG 200)

[2]The material used is actually the sodium salt (sodium trimethylsilanolate).

The test mixture for headspace analysis can be prepared to contain the internal standard (1,4-Dioxane) at the discretion of the testing laboratory.

Composition of the ICP Test Mixture:

System suitability testing for the ICP trace element analysis shall include the preparation and testing of a system suitability test mixture that contains all the targeted elements listed previously at a concentration of 0.25 mg/L.

The system suitability mixtures are minimally analyzed twice in the analytical runs, at the beginning and at the end, thus establishing that adequate system performance is achieved and maintained.

The evaluation of the system suitability results is as follows:

LC Analysis: The chromatograms for the system suitability test mixture are examined for the presence of peaks corresponding to each analyte in the mix. While all analytes may not produce responses in all detection methods, all analytes should produce peaks in at least one detection method. All peaks should have a response with a signal to noise ratio (S/N) of 10 or greater. The closest elution peak pair shall exhibit a resolution of greater than 1.5. All peaks should be well-shaped, with a tailing factor less than 2.0. There should be no significant differences in the chromatograms obtained at the beginning and the end of the chromatographic run. See Figure 2 for a sample chromatogram of the suitability test mixture.

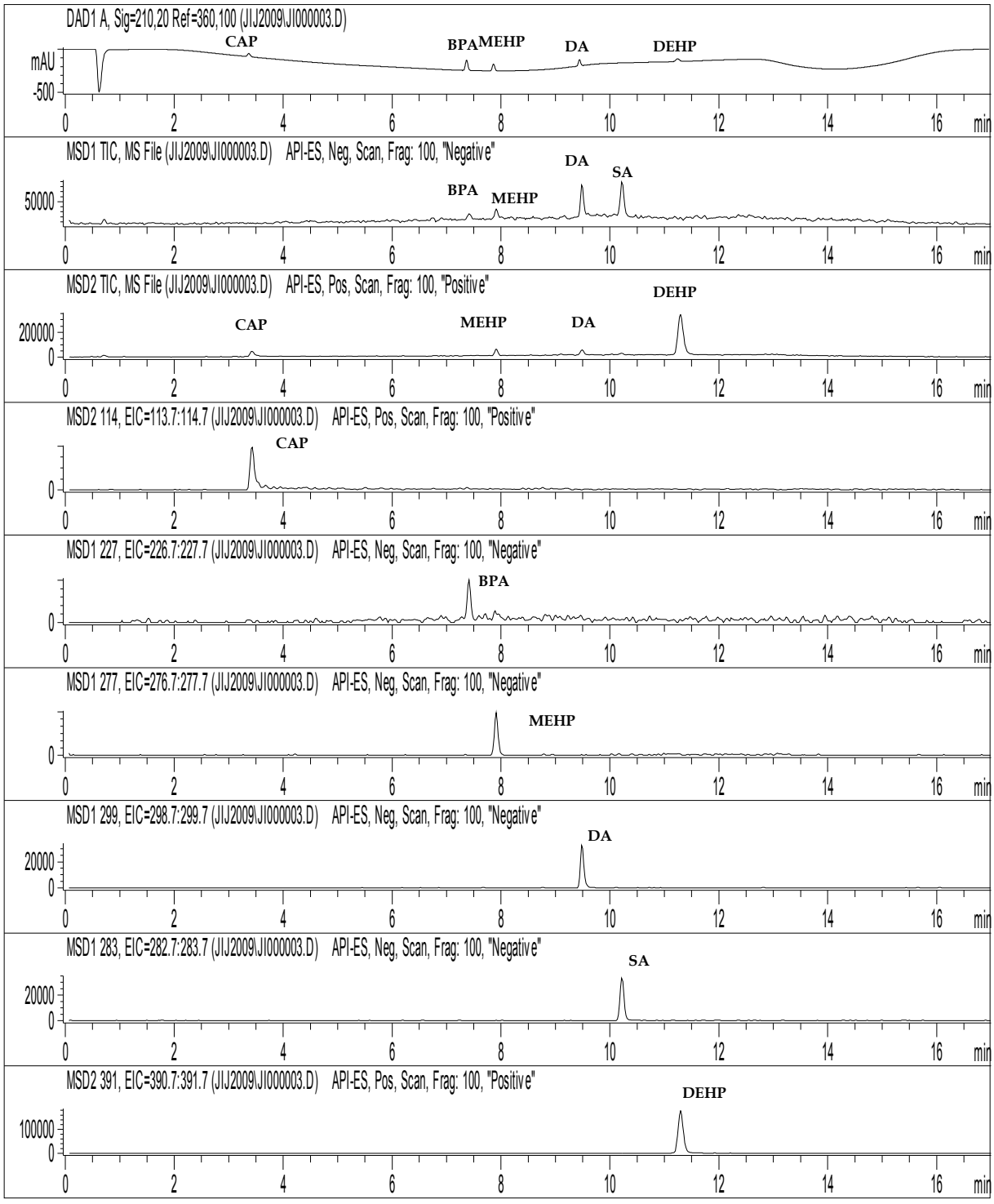
GC Analysis: The chromatograms for the system suitability test mixture are examined for the presence of peaks corresponding to each analyte in the mix. While all analytes may not produce responses in all sample work-up methods (derivatized and non-derivatized), all analytes should produce peaks in at least one work-up method. All peaks should have a response with a signal to noise ratio (S/N) of 10 or greater. The closest elution peak pair shall exhibit a resolution of greater than 1.5. All peaks should be well-shaped, with a tailing factor less than 2.0. There

should be no significant differences in the chromatograms obtained at the beginning and the end of the chromatographic run. See Figure 3 for a sample chromatogram of the suitability test mixture.

HS-GC Analysis: The chromatograms for the system suitability test mixture are examined for the presence of peaks corresponding to each analyte in the mix. All analytes should produce peaks that have a response with a signal to noise ratio (S/N) of 10 or greater. The closest elution peak pair shall exhibit a resolution of greater than 1.5. All peaks should be well-shaped, with a tailing factor less than 2.0. There should be no significant differences in the chromatograms obtained at the beginning and the end of the chromatographic run. See Figure 4 for a sample chromatogram of the suitability test mixture.

Figure 2. LC/UV/MS Chromatograms of the Suitability Mixture.

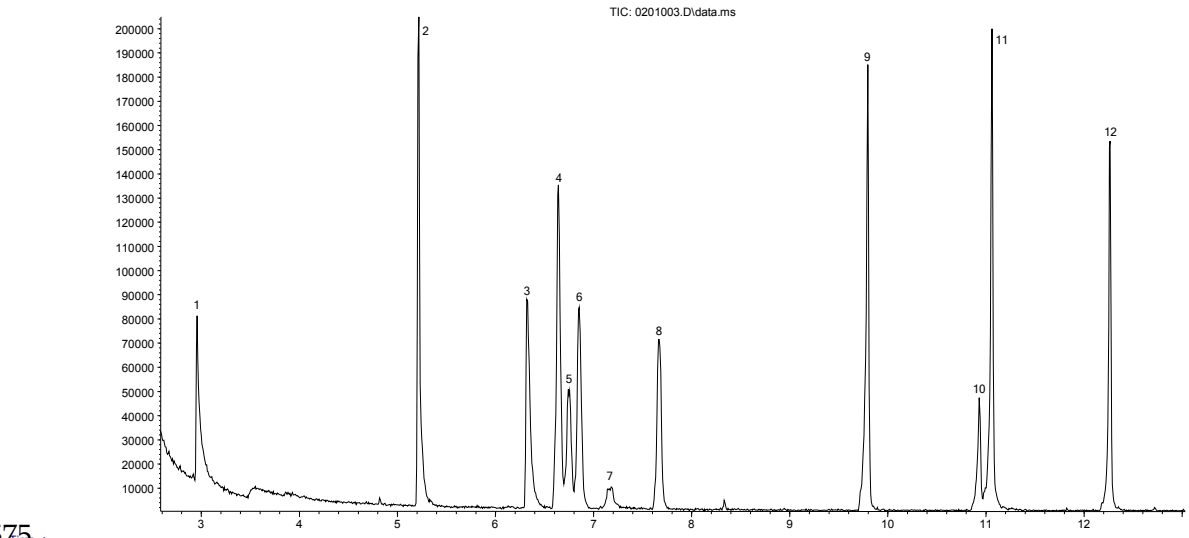
CAP = caprolactam; BPA = Bisphenol A; MEHP = mono-(ethylhexyl) phthalate; SA = stearic acid; DA = dehydroabietic acid; DEHP = di-(2-ethylhexyl) phthalate. Peaks for BHT and DPA were not obtained in this run.



572 **Figure 3. GC/FID Chromatograms of the Grob Mixture.**

573

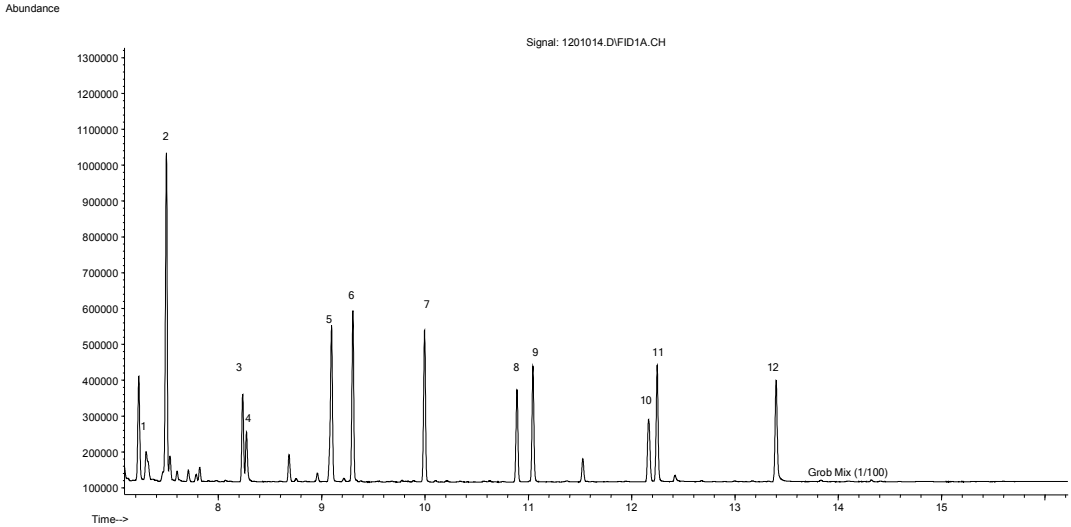
574 A. Underivatized.



575

Peak ID	Compound	Peak ID	Compound
1	2,3-Butanediol	7	2-ethyl hexanoic acid
2	Decane	8	2,6-Dimethyl aniline
3	1-Octanol	9	Methyl decanoate
4	Undecane	10	Dicyclohexylamine
5	1- Nonanal	11	Methyl undecanoate
6	2,6-Dimethyl phenol	12	Methyl dodecanoate

576 B. Derivatized.

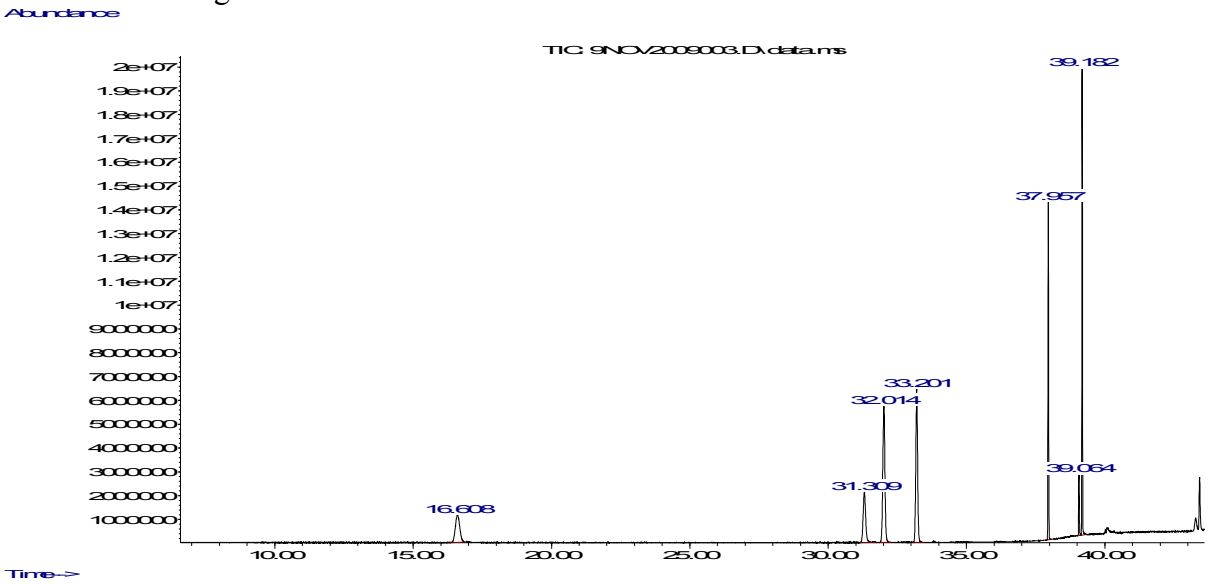


577

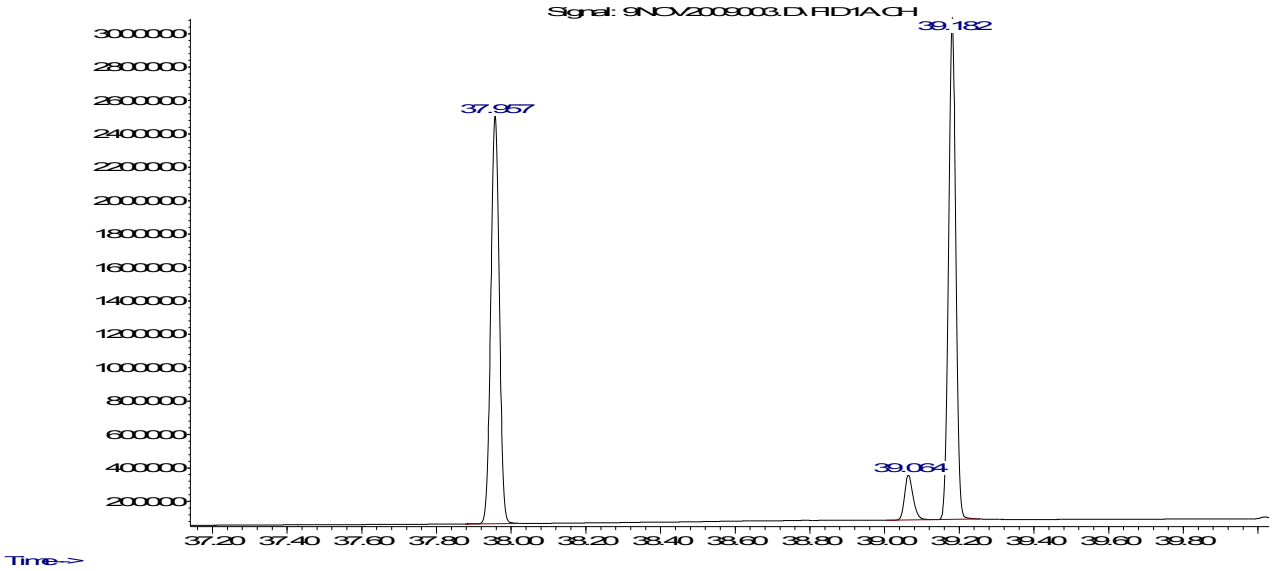
Peak ID	Compound	Peak ID	Compound
1	Decane	7	2,6-Dimethyl phenol [TMS]
2	2,3-Butanediol [2TMS]	8	2,6-Dimethyl aniline [TMS]
3	Undecane	9	Methyl decanoate
4	1-Nonanal	10	Dicyclohexylamine
5	2-Ethyl hexanoic acid [TMS]	11	Methyl undecanoate
6	1-Octanol [TMS]	12	Methyl dodecanoate

Figure 4. GC/MS Chromatograms of the Headspace Suitability Mix.

Entire Chromatogram



Expanded view of 37 – 40 minutes.



Retention Time (min)	Compound
16.1	Methanol
31.3	Trimethylsilanol
32.0	Toluene
33.2	1,4-dioxane
38.0	Cyclohexanone
39.1	Acetic acid
39.2	2-ethyl-1-hexanol

ICP Analysis: It shall be demonstrated that all elements can be detected at the prepared concentration.

The performance expectations enumerated previously are general guidelines. All system suitability data shall be reviewed by the Protocol's Study Coordinator and it is the responsibility of the Coordinator to evaluate the system suitability data and establish its acceptability.

C. Gas Chromatography (GC)

1. General

Relatively volatile and semi-volatile compounds will be analyzed by Gas Chromatography (GC) using a predominantly non-polar capillary column with wide (40 °C to 300 °C) temperature programming. As noted previously, appropriate detection strategies will be employed (e.g. FID, MS). Each GC analysis will produce an extractables "profile" in the form of a Total Response Chromatogram (e.g. TIC for MS detection). As a first pass, identifications of individual extractables will be accomplished with manual interpretation of the Electron Ionization (EI) spectra assisted by computerized mass spectral library searching. Beyond this, more difficult identifications may require the collection of additional data (such as Chemical Ionization GC/MS for molecular weight confirmation and High Resolution Mass Spectrometry for elemental composition), the purchase of reference compounds, *etc*. The PODP study coordinator shall be consulted before a participating laboratory pursues the more difficult identifications.

2. Sample Preparation

The resulting extracts will usually contain low-level amounts of extractables. Sample concentration and/or solvent switching may be necessary to provide compatible samples for the analytical instrumentation. While it is possible to manipulate extracts to provide very large concentration ratios, this has the undesirable effect of concentrating normal solvent impurities. Therefore, extracts will be concentrated no more than 100X, which is reasonable given normal ACS reagent purities of 99+%. The process for preparing (working-up) the aqueous extracts for GC analyses is shown in Table 3. Similar evaporative sample concentration strategies may be utilized with the organic extracts.

632

Table 3. Sample Work-up for Aqueous Extracts, GC Analysis	
Sample Preparation, Liquid-liquid Extraction; pH 2.5 and pH 9.5 Solutions.	1 A 50-mL portion of each of the solutions is transferred to a 125 mL separatory funnel. 2 A 1.0-mL aliquot of the surrogate internal standard solution is added to each sample. 3 25 mL of Dichloromethane (DCM) is added to each funnel. 4 Each funnel is shaken for 1 minute. 5 The layers are allowed to separate and the lower (DCM) layer is collected. 6 Steps 3 through 5 are repeated. The collected DCM layers are combined. 7 The pH of each pH 2.5 sample is adjusted to ≈10 with 5 N NaOH. The pH of the pH 9.5 sample is adjusted to ≈ 2 with 5 N HCl. 8 Steps 3 through 5 are repeated twice for the pH adjusted samples. The collected DCM layers from all extractions are combined. 9 The DCM extracts are dried by adding anhydrous sodium sulfate to each collection flask. 10 Each DCM extract is transferred from the collection flask to a different Turbovap concentration tube with DCM rinses, and concentrated to less than 0.5 mL. A 0.5 mL aliquot of the injection internal standard is then added to the Turbovap tube. The final volume is adjusted to approximately 1 mL with DCM. 11 0.5 mL of each concentrated extract is transferred from the Turbovap tube to an autosampler vial. 12 The remaining 0.5 mL aliquot of each of dichloromethane extract described above is transferred to separate amber autosampler vials.for TMS derivatization (see below)
Sample Preparation, Liquid-liquid Extraction; IPA/Water Solutions	The same basic process as noted above will be followed for the IPA/water samples. In the first extraction step, these samples will be pH adjusted to ≈ pH 2 and extracted twice. In the second extraction step, the samples will be adjusted to ≈ pH 10 and extracted twice. The resultant DCM extracts will be combined, dried and concentrated per steps 9 through 11 above.
TMS Derivatization of Residues	1 Approximately 100 µL dimethyl formamide is added to each amber autosampler vial prepared under step 12 above. 2 The contents of each vial are evaporated nearly to dryness using nitrogen. 3 To each of the sample extracts, and the standard solutions is added 100 µL of BSTFA w/ 1% TMCS (Pierce) 4 Each vial is capped and allowed to stand for one hour at approximately 70°C. 5 DCM is added to each auto-sampler vial to make a final volume of approximately 0.5 mL, and is mixed.

633

634 The procedure contained in this Table is an example only and it is not required that participating
635 laboratories adopt this procedure in either whole or in parts. However, any and all sample
636 preparation procedures that will be used by a participating laboratory must be discussed with the
637 PODP study coordinator prior to their utilization so that appropriate testing methodologies are
638 utilized and harmonization between laboratories working on the same test articles can be
639 achieved.

640

641 The procedure calls for the addition of a surrogate and injection internal standard, consistent with
642 the system suitability assessment strategy enumerated previously. A surrogate internal standard
643 is used to monitor the performance of the total procedure and is added to each extract in the intial

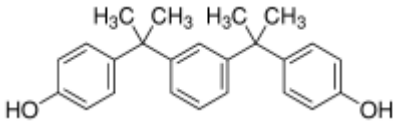
stage of its work-up. Requirements for such an internal standard are:

- sufficiently stable
- sufficiently soluble in all extraction solvents
- amenable to back-extraction from aqueous extracts by organic solvents
- semi-volatile
- amenable to all detection principles
- selectively detectable
- amenable to TMS-derivatization

The surrogate internal standard compound that meets these criteria has been identified as 4,4'-(m-Phenylenediisopropylidene)diphenol (Bisphenol M):

CAS-no.: 13595-15-0
Molecular weight: 346.46
Molecular formula: $C_6H_4[C(CH_3)_2C_6H_4OH]_2$

Structure:



Source: e. g. Aldrich #450464

The Surrogate Standard Solution is prepared by dissolving 100 mg of Bisphenol M in 100 ml of methanol, resulting in a concentration of 1000 µg/ml. This stock is further diluted 1 to 20 with methanol to produce the surrogate internal standard solution containing 50 µg/mL Bisphenol M.

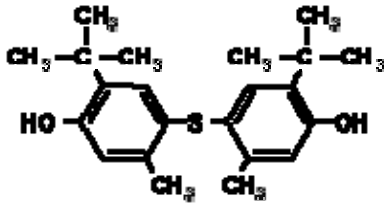
An injection internal standard is used to monitor the performance of the instrumental process only and is added to each sample at the last stage of its work-up. Such an internal standard must be:

- sufficiently stable
- sufficiently soluble in final extract
- semi-volatile
- amenable to all detection principles
- selectively detectable

The injection internal standard compound that meets these criteria has been identified as 4,4'-(m-4,4'-Thiobis(3-methyl-6-t-butylphenol), Irganox 415:

CAS-no.: 96-69-5
Molecular weight: 358.538
Molecular formular: $C_{22}H_{30}O_2S$

Structure:



Source: e. g. Aldrich #366285

The Injection Standard Solution is prepared as follows: 100 mg of Irganox 415 are dissolved in 20 ml of methanol, concentration = 5000 µg/ml. This stock is further diluted 1 to 100 with methanol to produce the surrogate internal standard solution containing 50 µg/mL Irganox 415.

The surrogate and injection internal standards are added to all samples to ensure that they are properly worked-up and injected. Two internal standards are used to isolate the analytical processes of sample work-up and instrumental analysis. The minimum performance expectation for the internal standards is that they be present in the sample chromatograms with a response whose signal to noise ratio is 10 or greater.

3. Operating Conditions

The following GC conditions (Table 4) serve as an illustration of a methodology which is suitable for testing the prepared samples. The procedure contained in this Table is an example only and it is not required that participating laboratories adopt this procedure in either whole or in parts. However, any and all sample analysis procedures that will be used by a participating laboratory must be discussed with the PODP study coordinator prior to their utilization so that appropriate testing methodologies are utilized and harmonization between laboratories working on the same test articles can be achieved.

Data cannot be collected while the injection solvent is in the ion source.

Table 4. Example Operating Parameters, GC Analysis of the Extracts.

Operating Parameter	Operating Value
Column	J&W DB-5HT, 30m x 0.25mm, 0.1 µm film thickness
Oven Program	Start at 50°C, hold for 5 min; ramp at 10°C/min to 300°C, hold for 5 min
Carrier Gas	He at 1 mL/min
Injection	Splitless; 2 µL.
Injector Temperature	310°C
FID Detector Temperature:	250°C
MS Transfer Line Temperature	310°C
MS Detection Details	70 eV (+), mass range of 33 – 650 amu (3.0 min or 6.0 min solvent delay used for un-derivatized or derivatized samples)

4. General Comments.

Note that the presented GC sample preparation and/or instrumental conditions are target conditions for all participating laboratories and investigators. The actual conditions employed by any participating laboratory should be reviewed by the PODP study coordinator prior to their utilization so that appropriate testing methodologies are utilized and harmonization between laboratories working on the same test articles can be achieved. In any event, the analyses performed by the participating laboratory must meet system suitability criteria, as established in Section VIII.A.

Any additional identification work beyond the first pass analysis will be performed only after consultation with the PODP study coordinator.

Chromatograms of the extracts should be compared to chromatograms of the extraction blanks so that peaks due to extractables can be delineated from peaks that reflect analytical artifacts.

D. High Performance Liquid Chromatography (HPLC)

1. General

Extracts and extraction blanks will be analyzed by High Performance Liquid Chromatography with appropriate detection strategies, including DAD and MS as noted previously. The method will use reversed-phase chromatography with a wide (gradient) range of solvent strengths. Each LC analysis will produce several extractables "profiles" in the form of a Total Ion Chromatogram (TIC), Extracted Ion Chromatograms (EIC) and UV chromatograms (total response and/or specific UV wavelengths). As a first pass, identifications of individual extractables will be accomplished with manual interpretation of the Atmospheric Pressure Ionization Electrospray (API-ES) information. The LC and GC chromatograms will be correlated to facilitate compound identification.

2. Sample Preparation

Unlike the GC analysis, the extracts and extraction blanks will typically not require extensive sample preparation prior to HPLC analysis as the extraction matrices are generally compatible with common HPLC mobile phases, thereby eliminating the need for solvent switching, and the detection methods are sufficiently sensitive that sample concentration is not required. However, some "solvent switching" may be necessary to produce samples that are HPLC-compatible.

3. Operating Conditions

The LC conditions in Table 5 serve as an illustration of a methodology which is suitable for testing the prepared samples. The procedure contained in this Table is an example only and it is not required that participating laboratories adopt this procedure in either whole or in parts. However, any and all sample analysis procedures that will be used by a participating laboratory must be discussed with the PODP study coordinator prior to their utilization so that appropriate

testing methodologies are utilized and harmonization between laboratories working on the same test articles can be achieved.

Table 5. Operating Parameters, LC/UV/MS Analysis of the Extracts.

Operating Parameter	Operating value	
Column	Agilent Zorbax Eclipse Plus C_{18}, 100 x 3.0 mm, 3.5µm particles	
Column Temperature	40°C	
Mobile Stage Components	A = 10 mM ammonium acetate, B = acetonitrile	
Mobile Stage Gradient	Time	% B
	0.0	5.0
	8.0	95.0
	11.0	95.0
	14.0	5.0
	17.0	5.0
Mobile Stage Flow Rate	0.8 mL/min	
Sample Size	60 µL	
Detection, UV	205 –300 nm	
Detection, MS	API-ES, positive ion and negative ion (mass range 80 – 1200)	
Sample Preparation	None, direct injection	

4. General Comments

Any additional identification work beyond the first pass analysis will be performed only after consultation with the PODP study coordinator.

Chromatograms of the extracts should be compared to chromatograms of the extraction blanks so that peaks due to extractables can be delineated from peaks that reflect analytical artifacts.

E. Headspace GC/MS (HS-GC)

1. General

Headspace analysis of extracts allows for an assessment of the volatile organic extractables. Volatiles present in the extract are thermally evolved into the headspace. The evolved volatile entities are "captured" in the headspace gas, which is transferred, in whole or in part, to an appropriate analytical technique. Since the headspace sample is a gas, gas chromatography is the analytical method of choice. Mass spectrometry is the detection method of choice because it facilitates the identification of evolved entities.

The headspace methodology is intended to uncover volatile entities that are present in the extract; it is not intended to produce "volatiles" by causing extractables present in the extracts to thermally decompose. Thus the headspace "extraction" is accomplished at relatively low temperatures (e.g. 120°C or less).

2. Sample Preparation

Place approximately 4 mL of sample (extract or extraction blank) into a 20 mL headspace autosampler vial containing approximately 10 grams anhydrous sodium sulfate. Seal the vial by crimping a cap onto it.

For semiquantitative evaluation and also to check for proper performance of the measurement, an 10 µL aliquot of a solution of 1,4-Dioxane in polyethylene glycol 200 (concentration 2 mg/mL) is added to each vial. This solution is prepared as follows: 20 mg of 1,4-Dioxane are dissolved in 10 mL of polyethylene glycol 200, resulting in a concentration of 2 mg/mL.

Note: A positive displacement pipetting system (e. g. Gilson Microman®) should be used for dosing this solution due to its high viscosity.

3. Operating Conditions

The operating conditions for the Headspace GC/MS are contained in Table 6.

Table 6. Operating Parameters, Headspace GC/MS Analysis for Volatiles.

Operating Parameter	Operating Value
A. Headspace Autosampler	
Oven Temperature	80°C
Needle Temperature	120°C
Transfer Line Temperature	155°C
Carrier gas	He at 5 psi
Equilibrium Time	120 min
B. GC/MS Analyzer	
Column	J&W DB-WAXETR, 60 m x 0.32 mm I.D., 1 µm film
Oven Program	Start at 35°C, hold for 7 minutes. Ramp at 1°C/min to 40°C, hold for 15 minutes. Ramp at 10°C/min to 100°C. Ramp at 25°C/min to 240 °C, hold for 5 min.
MS Ionization Mode	EI+, 70 eV
MS Transfer Line Temperature	240°C
MS Detection Mass Range	25 – 200 amu
Solvent Delay	0 min

4. General Comments

The analyses performed by the participating laboratory must meet system suitability criteria, as established in Section VIII.A.

The Headspace GC/MS analysis will produce an extractables "profile" in the form of a Total Response Chromatogram (e.g. TIC for MS detection). As a first pass, identifications of individual extractables will be accomplished with manual interpretation of the Electron

Ionization (EI) spectra assisted by computerized mass spectral library searching. More difficult identifications may require the collection of additional data (such as Chemical Ionization GC/MS for molecular weight confirmation and High Resolution Mass Spectrometry for elemental composition), should be discussed with the PODP study coordinator before a participating laboratory pursues these more difficult identifications.

Chromatograms of the extracts should be compared to chromatograms of the extraction blanks (Headspace vials containing no test material) so that peaks due to extractables can be delineated from peaks that reflect analytical artifacts.

The concentration of any extractables can be estimated via the use of the internal standard.

F. Inductively Coupled Plasma Atomic Spectroscopy (ICPAS)

1. General

Single elements (e.g. metals) in the extracts will be analyzed by Inductively Coupled Plasma Atomic Spectroscopy using appropriate methods and techniques for the determination of common analytes. Detection strategies such as optical emission and mass spectrometry shall be employed. ICP analyses should be performed consistent with USP practices.[4]

2. Sample Preparation

The resulting extracts will usually contain low-level amounts of extractables as well as the ionic constituents of the extracting media. The pH 2.5 and pH 9.5 extracts will contain large quantities of sodium and the pH 9.5 extracts will have large quantities of phosphorous. Thus these analytes cannot be determinable in these extracts.

The material extracts will need to be processed to some extent prior to testing. Aqueous samples will be acidified directly via addition of nitric acid. Place 10 mL of aqueous extract in a trace metal-free plastic vessel. Add 0.5 mL of concentrated nitric acid and mix well.

3. Operating Conditions

The ICP spectrometers shall be operated consistent with good laboratory practices and standard procedures in place in the participating testing facilities. The following is a list of elements that must be included in the ICP analysis: Al, As, Be, B, Cd, Cr, Co, Cu, Fe, Li, Mg, Mn, Mo, Ni, Pd, Pt, S, Sb, Se, Si, Sn, Sr, Ti, V, W, Zn, and Zr. Additional elements may be reported depending on the capabilities of the lab. The analysis conditions should be such that these elements can be measured at the appropriately low levels, typically 0.01 μg/mL or less in the material extracts.

[4] USP 30, <730> Plasma Spectroscopy.

4. General Comments

The analyses performed by the participating laboratory must meet system suitability criteria, as established in Section VIII.B.

Results for the extracts should be compared the results for the extraction blanks so that extractables can be delineated from analytical artifacts and solvent impurities/components.

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.

- A report format will be distributed to the participating laboratories.

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 0.1 ppm (μg/ml).

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/AES	Inductively Coupled Plasma Atomic Emission Spectroscopy
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
HS-GC	Gas Chromatography with Headspace gas Aampling
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
BFS	Blow-fill-seal

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; Amendment #1, September, 2011.