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Parenteral and Ophthalmic Drug Products Leachables and Extractables Working Group

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Study Protocol – Stage 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

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 1999¹. In addition, the European Medicines Agency (EMA) 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 OINDP¹: (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 has developed this experimental protocol as an means of establishing Best Demonstrated Practices for the performance of Controlled Extraction Studies, specifically relevant for PODP container closure systems and dosage forms. This protocol considers the processes by which a Controlled Extract is generated, the processes by which a Controlled Extract is analyzed and processes by which the test results are evaluated and interpreted, specifically within the context of the Working Group's approved Work Plan and experimental hypothesis.

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

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 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

- 138 1. Threshold concepts that have been developed for safety qualification of leachables in
139 OINDP can be extrapolated to the evaluation and safety qualification of leachables in
140 PODP, with consideration of factors and parameters such as dose, duration, patient
141 population and product dependent characteristics unique to various PODP types.
142
- 143 2. The science-based best demonstrated practices established for the OINDP pharmaceutical
144 development process can be extrapolated to PODP container closure systems.
145
- 146 3. Threshold and best practices concepts can be integrated into a comprehensive process for
147 characterizing container closure systems with respect to leachable substances and their
148 associated impact on PODP safety.
149

150 Controlled Extraction Studies will be performed following the general methodologies contained
151 in this protocol. Test articles will be subjected to different extraction conditions to establish how
152 different experimentally controlled parameters affect the resulting extractables profiles. Of
153 specific interest to the Working Group are the parenteral and ophthalmic dosage forms,
154 particularly Small Volume Parenterals (SVP), Large Volume Parenterals (LVP), Pre-filled
155 Syringes (PFS) and Blow-Fill-Seal systems (BFS). This Stage 1 Protocol specifically focuses on
156 the SVP and PFS dosage forms and on the generation of qualitative extractables profiles. Future
157 Stages will focus on additional dosage forms and/or quantitative aspects of extractables profiling.
158 The intent of this Stage 1 assessment is to generate the fundamental information from which Best
159 Demonstrated Practices can be derived; it is not the intent of this Stage 1 assessment to
160 prospectively establish the practices used in this study as the Best Demonstrated Practices
161 themselves.
162

163 As no single analytical technique can be used to identify and quantify all unknown extractables,
164 a variety of methods will be utilized in this protocol to maximize the likelihood that all
165 predominant extractable compounds associated with the test articles are accounted for and
166 appropriately evaluated. Overlap between methods will supply corroborating data that
167 demonstrate the validity of the procedures. To provide a full analytical survey of possible
168 analytes the following strategy will be employed:
169

- 170 1. Gas Chromatography with appropriate sampling/injection and detection strategies
171 e.g. Flame Ionization Detection (GC/FID) and Mass Spectrometry (GC/MS)] for
172 identification and assessment of volatile and semi-volatile extractables.
- 173 2. High Performance Liquid Chromatography with appropriate detection strategies
174 [e.g. Diode Array Detection (HPLC/DAD), Mass Spectrometry (LC/MS)] for
175 identification and assessment of relatively polar and non-volatile extractables.
- 176 3. Inductively Coupled Plasma/Mass Spectrometry (ICP/MS) and/or Inductively
177 Coupled Plasma/Atomic Emission Spectroscopy (ICP/AES) to detect single
178 elements in the extracts (i.e. metals).
179

180 While analytical tests and measurements, such as pH, UV absorbance, and total organic carbon
181 (TOC), can provide insight into the general chemical nature and amount of extracted substances,

182 they do not directly provide information for the identification and/or quantitation of individual
183 extractables and thus will not be utilized in this study.

184
185 Studies designed to assess recovery (i.e. mass balance) for individual extractables relative to the
186 known formulations of chemical additives in the various test articles, or reproducibility of
187 extractables profiles for multiple “batches” of any particular test article are not within the scope
188 of this Stage of the test protocol. Additionally, the extraction procedures, analytical
189 techniques/methods, and analysis conditions described in this experimental test protocol will not
190 be fully and rigorously validated. Nevertheless, the scientific credibility of the data generated in
191 this study shall be established via the utilization of system suitability testing with all the analysis
192 methods and by the expert review of the generated data. Finally, “special case” classes of
193 extractables that have defined and highly specific analytical methods that are generally accepted
194 and commonly used for their identification and quantitative assessment will not be considered in
195 this study.

196 197 **III. REGULATORY STATUS**

198
199 This experimental test protocol will be conducted in the spirit of Good Laboratory Practices and
200 Good Manufacturing Practices (GXP) requirements. All experiments shall be documented based
201 on the appropriate GXP compliance systems in a participating laboratory. Any changes or
202 clarifications that a participating laboratory makes to this test protocol shall be documented as
203 appropriate, and discussed/approved by the Study Coordination as appropriate.

204 205 **IV. SAFETY AND ENVIRONMENTAL IMPACT**

206
207 Chemicals and reagents used in this study (e.g. organic solvents commonly used to enhance
208 solubility of lipophilic targets and to increase transport of small molecules out of complex
209 matrices) may be flammable and/or pose short-term and long-term environmental health risks.
210 Care must be exercised with their use. Consult the Material Safety and Data Sheet (MSDS) for
211 appropriate personal protection and disposal. Safety risks associated with the various processes
212 and procedures performed in this study may exist and should be understood and managed using
213 such strategies as environmental control and personal protection.

214 215 **V. TEST ARTICLES**

216
217 A list of the test articles available for use in this study is provided in Table 1. Test articles will
218 be provided in an appropriate form for use as test articles. Certain, but not necessarily all, details
219 of the additive formulations and manufacturing conditions for these test articles are known and
220 are captured in Table 1.

221
222

223

TABLE 1. TEST ARTICLES.			
MATERIAL TYPE	MATERIAL APPLICATION	MATERIAL FORMAT	COMPOSITION
Low density polyethylene (LDPE)	Overpouch	Blown Film	Dow 640-I LDPE resin; Irganox B 215 (2:1 blend of Irgafos 168 and Irganox 1010) 1000 ppm, BHT 200 ppm, Calcium Stearate 500 ppm, Erucamide 500 ppm, Chimassorb 944 2000 ppm
Cyclic Olefin (COC)	Syringe barrels, vials	Plaques	Irganox 1010, Ultramarine Blue
Polycarbonate (PC)	Port Tubes	Injection molded plaques	0.05 PHR Irganox 1076, 0.1 PHR Irgafos 168
Poly (vinyl chloride) (PVC)	Solution Bags, tubing	Pellets	PVC resin; DEHP 30%; Epoxidized oil 7%, Zn stearate 0.5%; Ca stearate 0.5%; Stearamide 1%
Rubber (Elastomer) (RE)	Gaskets, stoppers, closures	Sheets	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'-dithiodimorpholine/polyisobutylene, 0.3%

224

225 VI. CHEMICALS AND EQUIPMENT

226

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

229

230 A. Extraction Solvents

231

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

233

- 234 • Laboratory research grade water or Water for Injection (WFI), appropriately sourced,
 235 collected and stored to minimize background levels of extraneous substances.
- 236 • Potassium chloride
- 237 • Hydrochloric acid, 0.1 N
- 238 • Sodium phosphate monobasic
- 239 • Sodium phosphate dibasic
- 240 • Sodium hydroxide, 1 N
- 241 • Isopropyl alcohol (glass bottled; IPA)
- 242 • Hexane (glass bottled)

- 243 • pH calibration buffers; pH 1.68, 4.01, 9.18 and 12.48 (saturated calcium hydroxide)

244

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

246

- 247 • Water at pH 2.5 (HCl/KCl mixture): The KCl solution is prepared at 0.01M. Weigh
248 1.5 grams of KCl into a 2.0 L vol flask containing 1500 mL water. Add 60 mL 0.1 N
249 HCl. Dilute to volume with water. This final solution is 0.01 M KCl and 0.003 M
250 HCl, which should have a pH of 2.5.

251

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

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

257

258 **B. Additional Chemicals**

259

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

262

263 **C. Extraction Equipment**

264

265 1. Soxhlet Extraction

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

270 2. Reflux

- 271 • Reflux apparatus [e.g. round bottom flask (200 mL or larger), condenser with ground
272 glass joints, hot plate or heating mantle].

- 273 • All glass labware for these extractions must be acid-washed prior to use.

- 274 • The use of any lubricants, such as vacuum grease on ground glass joints, should be
275 avoided.

276 3. Sealed Container

- 277 • Teflon [Saville (6133 Baker Road, Minnetonka, MN 55345-5910 USA, Phone: 952-
278 935-4100, E-mail: info@saville.com), Part # 0108, 8 fl. Oz. Teflon Jar]

- 279 • Pyrex [VWR (Customer Service: 1-800-932-5000), Catalog # 89000-236, Media /
280 Storage Bottles with Standard GL45 Polypropylene Cap, 250 mL] containers

- 281 • All glass labware for these extractions must be acid-washed prior to use. Teflon
282 vessels are used with the high pH extractions to avoid any leaching from glass,
283 especially for samples for ICP analysis

- 284 • Autoclave

- 285 • Oven with operating range of 30 to 75 °C; explosion proof
286
287 4. Sonication
288 • General laboratory ultrasonic bath
289 • Calibrated thermometer
290 • Extraction vessel
291 • Must have wide enough neck to allow addition of test article
292 • Must be of minimum capacity 100 mL
293 • Must be sealable

294 • All glass labware for these extractions must be acid-washed prior to use.
295 Alternatively, Teflon vessels may be used to avoid any leaching from glass
296

297 **D. Analytical Instrumentation**

- 298
299 • Gas chromatograph equipped with a Flame Ionization Detector (GC/FID)
300 • Gas chromatograph equipped with a Mass Spectrometer (GC/MS). GC systems that employ
301 flow splitting to accomplish FID and MS detection in tandem could be used in this study.
302 • Headspace Sampler/Injector (HS) for GC/MS Instrumentation.
303 • Liquid chromatograph equipped with a photodiode array detector
304 • Liquid chromatograph equipped with an APCI (Atmospheric Pressure Chemical Ionization)
305 capable Mass Spectrometer (LC/MS). Preference is given to LC systems that are capable of
306 both DAD and MS detection. Additional detectors (e.g. corona assisted discharge detectors,
307 evaporative light scattering) may be used as appropriate.
308 • Inductively Coupled Plasma Mass Spectrometer (ICP/MS, preferred) and/or Inductively
309 Coupled Plasma Atomic Emission Spectrometer (ICP/AES)
310

311 **VII. EXTRACTION PROCEDURES**

312 **A. General**

313
314
315 In the PQRI OINDP studies, extractions were performed on each test article using three solvents
316 representing a range of polarity, specifically
317

- 318 • methylene chloride (dichloromethane)
319 • 2-propanol (isopropanol, IPA)
320 • hexane (n-hexane, not hexanes).
321

322 This was appropriate in the case of OINDP given the nature of the drug vehicles used in those
323 types of products (organic solvents) and the conditions of contact between the drug vehicles and
324 the container closure system (continuous direct contact over shelf life).
325

326 While the use of such extraction solvents may be relevant for PODP products, a significant
327 portion of PODP products are water-based and the three solvents previously employed do not

328 address the unique solubilizing properties of water and aqueous buffer systems. Thus in the case
329 of PODP, the OINDP solvents will be augmented by aqueous extraction media. These additional
330 aqueous extraction media, and their associated justification, include

- 331
- 332 * Water at pH 2.5 (HCl/KCl mixture); justification, few therapeutic products are lower than pH
 - 333 2.5.
 - 334 * Water at pH 9.5 (Phosphate buffer); justification, few therapeutic products are higher in pH
 - 335 than 9.5.
 - 336 * 1/1 IPA/water; justification; simulates aqueous formulations containing solubilizing agents,
 - 337 provides for trend analysis (with IPA and water alone).
- 338

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

341

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

347

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

353

354 **B. Extraction Maps**

355

356 The number of potential test situations, defined as the coupling of a test material, an extraction
357 solvent and an extraction process, is large and addressing each individual test situation is not
358 necessary to generate relevant information upon which best demonstrated practice
359 recommendations may be based.. Test situations that are within the scope of this study are
360 delineated in the following Extraction Maps. The intent of this Stage 1 assessment is to generate
361 the fundamental information from which best demonstrated practices can be derived; it is not the
362 intent of this Stage 1 assessment to establish the practices used in this study as best demonstrated
363 practices themselves.

364

365 *1. Test Material Versus Extraction Solvent Map*

366

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

368

369

370

371

372

Table 2. Material Versus Extraction Solvent Map (1, 3)						
	Aqueous		Mixed	Organic		Thermal (2)
	pH 2.5	pH 9.5	IPA/Water	IPA	Hexane	
LDPE	X	X	X	---	X	X
PC (4)	X	X	X	X	X	X
PVC (4)	X	X	X	X	X	X
Rubber	X	X	X	X	X	X
COC	X	X	X	X	X	X

373
374 Notes: (1) An X denotes a material/solvent couple that will be performed, an --- denotes a couple that will not be
375 performed.
376 (2) By Headspace analysis.
377 (3) During the course of this study it may be the case that certain material – solvent couples will be
378 incompatible. Such incompatibilities should be reported the PODP study coordinator and incompatible
379 extracts should not be tested.
380 (4) Both reflux and sealed vessel with the IPA/Water mixture
381

382 2. Extraction Method Versus Extraction Solvent Map

383 Table 3 establishes which extraction methods will be utilized with which extraction solvents.
384
385

Table 3. Extraction Method Versus Extraction Solvent Map (1, 4)					
	Aqueous		Mixed	Organic	
	pH 2.5	pH 9.5	IPA/Water	IPA	Hexane
Soxhlet	---	---	---	X	X
Reflux	---	---	X (5)	X	X
Sonication	X	X	---	X	---
Sealed Vessel	X (2)	X (2)	X (3)	---	--

386
387 Notes: (1) An X denotes a method/solvent couple that will be performed, an --- denotes a couple that will not be
388 performed.
389 (2) Under autoclave conditions (121°C for 1 hr).
390 (3) Storage at 55°C for 3 days.
391 (4) During the course of this study it may be the case that certain material – solvent couples will be
392 incompatible. Such incompatibilities should be reported the PODP study coordinator and incompatible
393 extracts should not be tested.
394 (5) This testing will only be performed for the PC and PVC materials.
395

396 C. General Considerations

397
398 Care in experimental approach should be exercised in terms of producing extracts that are free
399 from analytical artifacts. Glass is the appropriate vessel for samples intended for organic
400 analysis, while Teflon is recommended for inorganic (metals) analysis. Glass is a problem in
401 metal analysis especially at higher pHs due to leaching of glass (e.g. Si, B, Al, Na). Teflon is a
402 problem with organics due to adsorption of extractables.
403

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

408
409 For all extractions, the weight of test article sample, extracting solvent volume, and sample
410 extract concentration factors should be established and adjusted so that it is possible to detect and
411 identify individual extractables present at the 10 µg/g (ppm) level. Individual extractables may
412 be detected and identified at lower levels if the analytical method employed is readily capable of
413 achieving such sensitivity.

414
415 For each extraction technique and solvent type, appropriate blanks (no test article sample) must
416 be prepared. These must be prepared concurrently using a different extraction apparatus (same
417 type) under the same conditions, or by using the same apparatus prior to charging with sample.
418 The extraction conditions represent the consensus opinion of the PODP chemistry subteam.

419
420 All extracts should be visually inspected prior to analysis to ensure that they are free from
421 obvious particulate matter. Should such an inspection reveal particulate matter, this finding
422 should be reported to the Study Coordinator prior to proceeding with sample analysis. In most
423 cases it is likely that the Study Coordinator will request that the sample be processed in such a
424 way that the particulate is removed from the extract prior to its testing. Collection of the
425 removed particulate may be requested so that the material itself can be analyzed and identified.

426

427 **D. Soxhlet Extraction**

428

429 *1. Sample Preparation*

430

431 Transport of extractables out of the complex matrix may be affected by the surface area and
432 thickness of the test article. Test articles may be “processed” (or “sized”) by appropriate
433 methods, cutting, not grinding into appropriately sized pieces in order to fit into the reflux
434 apparatus

435

436 *2. Extraction Conditions*

437

438 Under normal laboratory conditions, three physical extraction parameters may be modified,
439 turnover number, total extraction time and temperature. Temperature is the most difficult of the
440 three parameters to control as the sample holder is maintained above the vapor level (temperature
441 may be above the boiling point), but will be continuously bathed in freshly distilled solvent (coil
442 temperature). It is recommended that the coil temperature be kept as low as possible to avoid
443 heating above the solvent flashpoint.

444

445 Turnover number is controlled by the heating rate and should be limited by safety concerns. At
446 low turnover numbers, the extraction characteristics will resemble those of reflux and may be
447 limited by equilibrium phenomena. It is recommended that turnover numbers to be at least ten
448 during the course of the extraction.

449
450 Sample amounts should be targeted at 5 g using 200 mL of solvent. Extraction time should be
451 approximately 24 hours and care should be taken to guard against possible degradation of
452 thermally labile or reactive compounds.

453
454 **E. Reflux**

455
456 Reflux extraction is a common and readily implemented approach for the production of
457 extractables. Conditions are easily standardized as the temperature and pressure are at the
458 defined boiling points of the extraction solvents. Unlike Soxhlet extraction, reflux extraction is
459 an equilibrium phenomenon.

460
461 *1. Sample Preparation*

462
463 Transport of extractables out of the complex matrix may be affected by the surface area and
464 thickness of the test article. Test articles may be “processed” (or “sized”) by appropriate
465 methods, cutting, not grinding into appropriately sized pieces) in order to fit into the reflux
466 apparatus.

467
468 *2. Extraction Conditions*

469
470 Sample amounts should be approximately 5 grams in 200 mL of solvent in a round bottom flask.
471 The only adjustable physical parameter for reflux extraction is time. Reflux the sample for a
472 period of time between 1 and 2 hours. The solvent reservoir level must be monitored and
473 periodically recharged to provide the correct amount of solvent. Extractions that produce
474 physical changes in the test materials, especially dissolution, should be terminated.

475
476 In reflux extraction, the sample to solvent ratio may affect the completeness of the technique.
477 Establishing this ratio should be addressed when optimizing the method.

478
479 **F. Sonication**

480
481 Sonication uses ultrasonic energy instead of thermal energy to increase the rate of mass transport
482 of small analytes out of a solid matrix. Similar considerations as reflux extraction (equilibrium
483 conditions) should be evaluated, but these cannot be calculated using thermodynamic
484 parameters. Sonication equipment may be standardized by measuring the temperature rise after a
485 set exposure time and evaluating the energy deposited into the solvent. Standardization of
486 conditions should be accomplished after consultation between participating laboratories.

487
488 *1. Sample Preparation*

489
490 Transport of extractables out of the complex matrix may be affected by surface area and
491 thickness of the test article. Test articles may be “processed” by appropriate methods (e.g.
492 (cutting, not grinding into appropriately sized pieces) in order to fit into the sonication apparatus.

493

494 2. *Extraction Conditions*

495
496 In sonication, the sample to solvent ratio may affect the completeness of the technique. Target
497 sample solvent ratio is 5 grams in 200 mL of solvent. If scaling down it is appropriate to
498 maintain this ratio. The only adjustable physical parameter for sonication is time. Extraction
499 times used in this study shall be approximately 2 hours. The extraction time should be such that
500 the extraction does not produce a noticeable change in the test material (e.g. dissolution). Bath
501 temperatures should be standardized using either ice-water (0 °C), or monitored by a calibrated
502 thermometer. Appropriate safety measures must be implemented to eliminate the potential for
503 unsafe situations to occur.

504
505 **G. Sealed Vessel Extraction**

506
507 Sealed Vessel extraction utilizes thermal energy to facilitate the mass transport of extractables
508 out of a solid matrix. Conditions are easily standardized and sealed vessel extraction is an
509 equilibrium phenomenon.

510
511 1. *Sample Preparation*

512
513 Transport of extractables out of the test articles may be impacted by the physical state of the
514 material to be extracted (e.g. surface area and thickness), the portions of material and extracting
515 solvent in the extraction vessel and the temperature and duration of the extraction. Test articles
516 may be “processed” by appropriate size reduction methods (such as cutting) to fit into and fill the
517 extraction apparatus.

518
519 2. *Extraction Conditions*

520
521 The test material may be rinsed with water and dried prior to testing so as to remove any surface
522 contamination. Approximately 5 grams of material will be contacted with a 200-mL volume of
523 extracting solvent by placing both into the extraction vessel to produce the test unit (the
524 combination of the test material, the extracting solution and the extraction vessel). Add the
525 required quantity of material to a rinsed extraction vessel. Add the required volume of extracting
526 medium to the vessel. Mix and close vessel tightly. Autoclave extraction unit at a nominal
527 temperature of 121 °C for 1 hour. Allow the vessel to cool. Verify that solvent did not leak from
528 container by extraction volume measurement. Separate, by an appropriate means, the extract
529 from the extracted material. Collect the extract in an appropriate vessel with minimal headspace.
530 Retain the extract for analysis. Replicate extractions should be performed. Extracts should be
531 stored prior to and during analysis in such a way as to preserve their compositional integrity
532 (protect from light, heat and evaporation losses).

533
534 Add the required quantity of material to a rinsed extraction vessel. Add the required volume of
535 extracting solution to the vessel. Mix and close vessel tightly. Mark the vessel so that any loss of
536 fluid can be detected and rejected from further analysis. For the IPA/Water mixture the
537 extraction should be performed at a temperature of 55°C (which is 10°C or more below the
538 boiling point of the proposed extraction solvents) for 3 days. Allow the vessel to cool. Separate,

539 by an appropriate means, the extract from the extracted material. Collect the extract in an
540 appropriate vessel with minimal headspace.

541

542

543 **VIII. ANALYTICAL METHODS**

544

545 **A. System Suitability**

546

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

550

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

554

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

561

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

568

569 Table 4 presents a list of system suitability analytes for GC and HPLC based analytical
570 techniques.

571

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

576

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

582

583

584 **Table 4. Composition of the System Suitability Test Mixtures.**

585
 586 *Compounds for HPLC Analysis:*

587
 588 Custom-made test mixture to be prepared by the participating laboratories from standard grade
 589 reference materials:

590

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

591
 592 The test mix should be prepared by appropriate dilution of more concentrated stock solutions,
 593 prepared using solvents appropriate for the individual reagents. The final composition of the test
 594 mixture should be similar to, or compatible with, the mobile phase used in the LC analysis.

595
 596 *Compounds for GC Analysis, Grob Mixture:*

597
 598 Commercial Sources:
 599 e. g.: "Grob-Test-Mix", Cat# 11373, Restek

600
 601 Reference:
 602 K. Grob, Jr., G. Grob and K. Grob, "Testing Capillary Gas Chromatographic Columns", Journal
 603 of Chromatography, 219, p. 13-20, (1981)

604

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

605

606 **Table 4. Composition of the System Suitability Test Mixtures (continued).**

607
608 *Compounds for Headspace GC Analysis:*

609
610 Custom-made test mixture to be prepared by the participating laboratories from standard grade
611 reference materials:

612

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

613

614 ¹ Preparation of SST-Sample:

615 - add 10 µl of the HS-Test-Mixture-I to a 20 ml crimp-cap vial

616 - add 10 µl of internal standard solution (2 mg of 1,4-Dioxane/ml PEG 200)

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

618

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

621

622 *Composition of the ICP Test Mixture:*

623

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

627

628

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

632

633 The evaluation of the system suitability results is as follows:

634

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

641 chromatograms obtained at the beginning and the end of the chromatographic run. See Figure 1
642 for a sample chromatogram of the suitability test mixture.

643
644 *GC Analysis:* The chromatograms for the system suitability test mixture are examined for the
645 presence of peaks corresponding to each analyte in the mix. While all analytes may not produce
646 responses in all sample work-up methods (derivatized and non-derivatized), all analytes should
647 produce peaks in at least one work-up method. All peaks should have a response with a signal to
648 noise ratio (S/N) of 10 or greater. The closest elution peak pair shall exhibit a resolution of
649 greater than 1.5. All peaks should be well-shaped, with a tailing factor less than 2.0. There
650 should be no significant differences in the chromatograms obtained at the beginning and the end
651 of the chromatographic run. See Figure 2 for a sample chromatogram of the suitability test
652 mixture.

653
654 *HSGC Analysis:* The chromatograms for the system suitability test mixture are examined for
655 the presence of peaks corresponding to each analyte in the mix. All analytes should produce
656 peaks that have a response with a signal to noise ratio (S/N) of 10 or greater. The closest elution
657 peak pair shall exhibit a resolution of greater than 1.5. All peaks should be well-shaped, with a
658 tailing factor less than 2.0. There should be no significant differences in the chromatograms
659 obtained at the beginning and the end of the chromatographic run. See Figure 3 for a sample
660 chromatogram of the suitability test mixture.

661
662 *ICP Analysis:* It shall be demonstrated that all elements can be detected at the prepared
663 concentration.

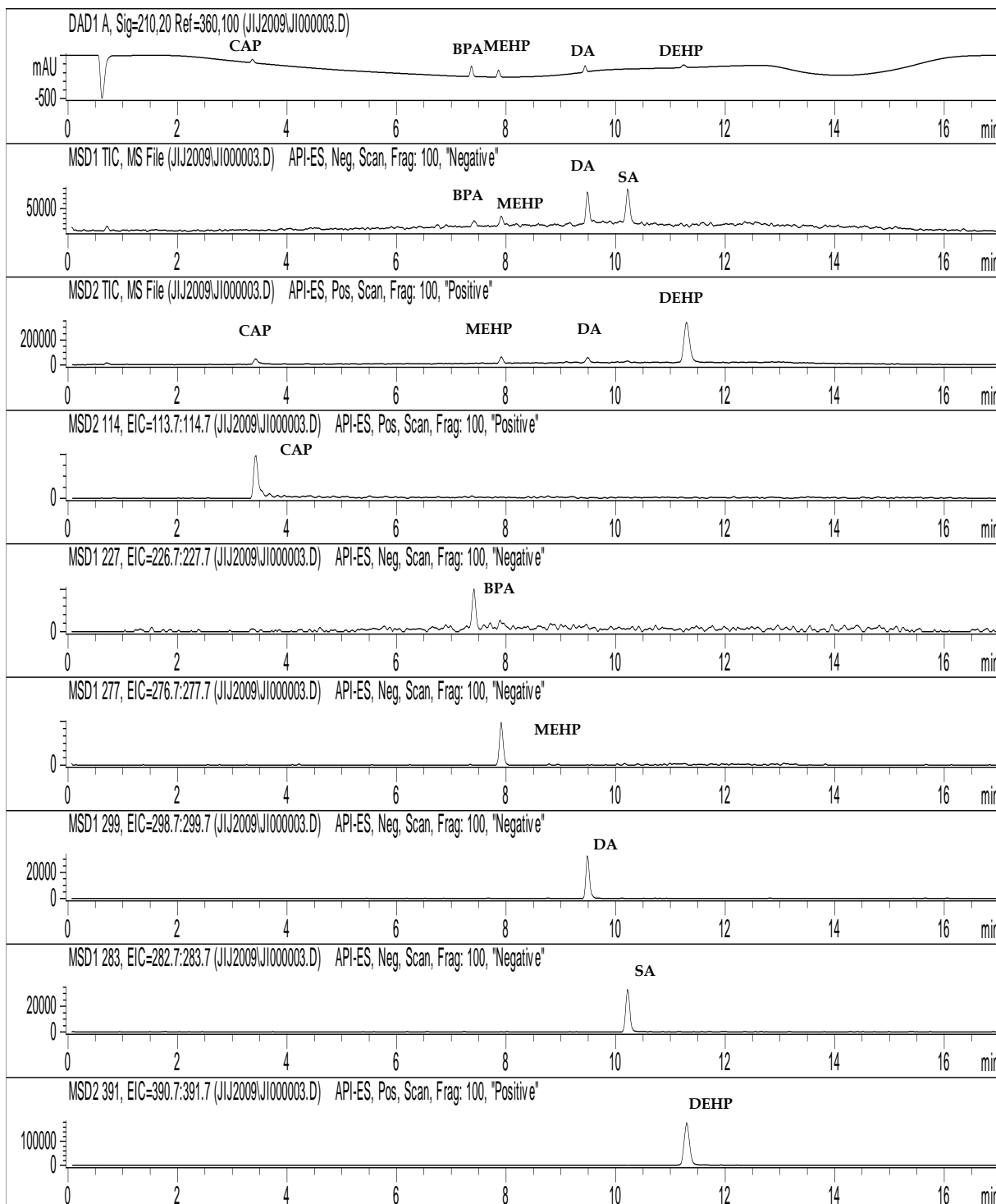
664
665 The performance expectations enumerated previously are general guidelines. All system
666 suitability data shall be reviewed by the Protocol's Study Coordinator and it is the responsibility
667 of the Coordinator to evaluate the system suitability data and establish its acceptability.

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Figure 1. LC/UV/MS Chromatograms of the Suitability Mixture.

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

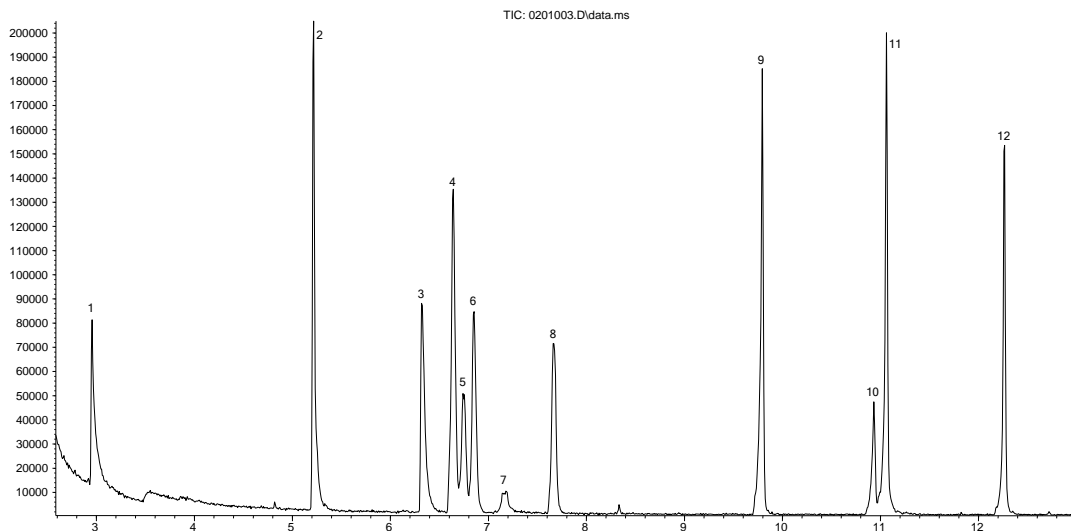


692

693 **Figure 2. GC/FID Chromatograms of the Grob Mixture.**

694
695 A. Underivatized.

Abundance

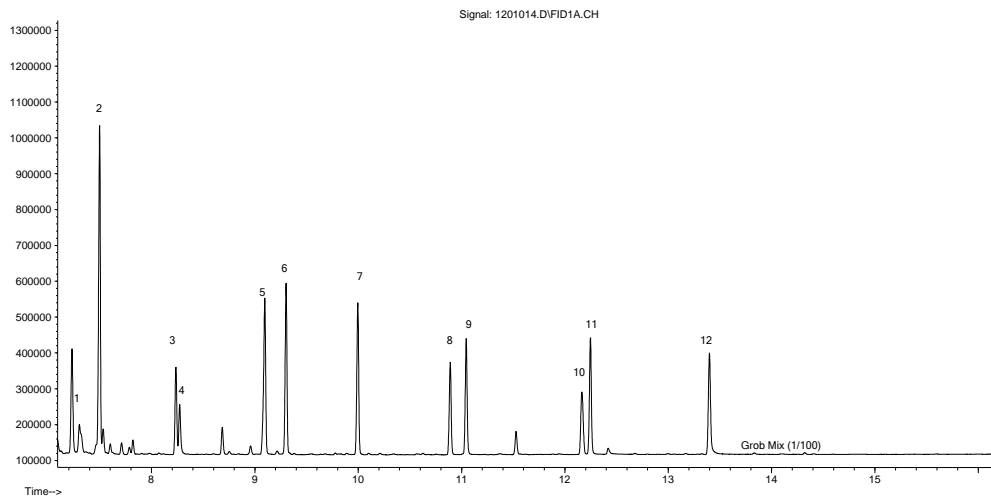


696

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

697 B. Derivatized.

Abundance

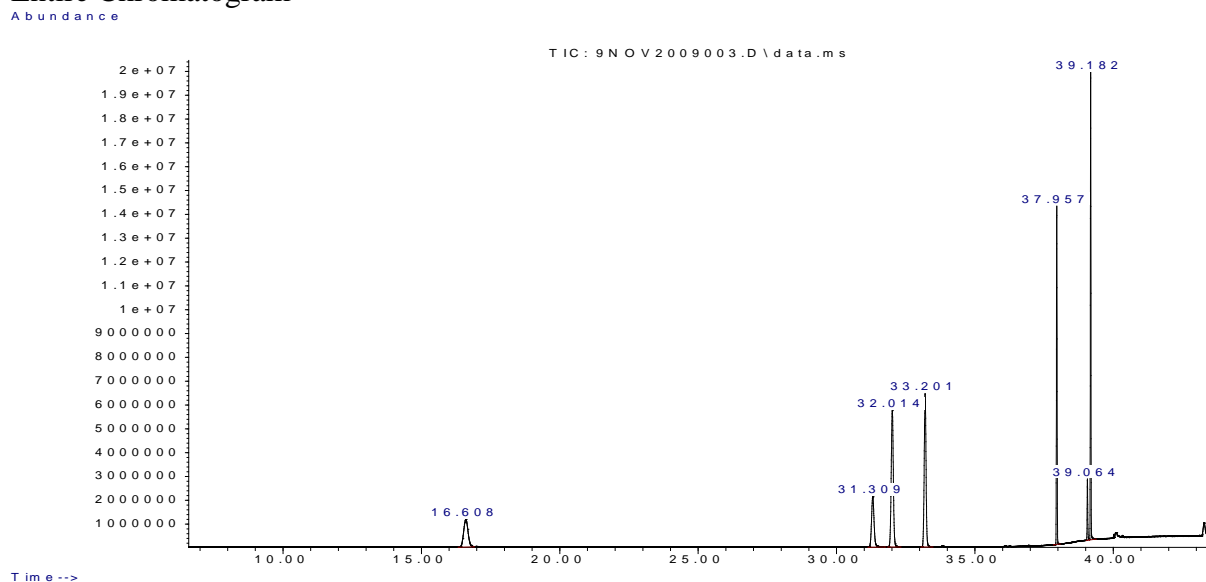


698

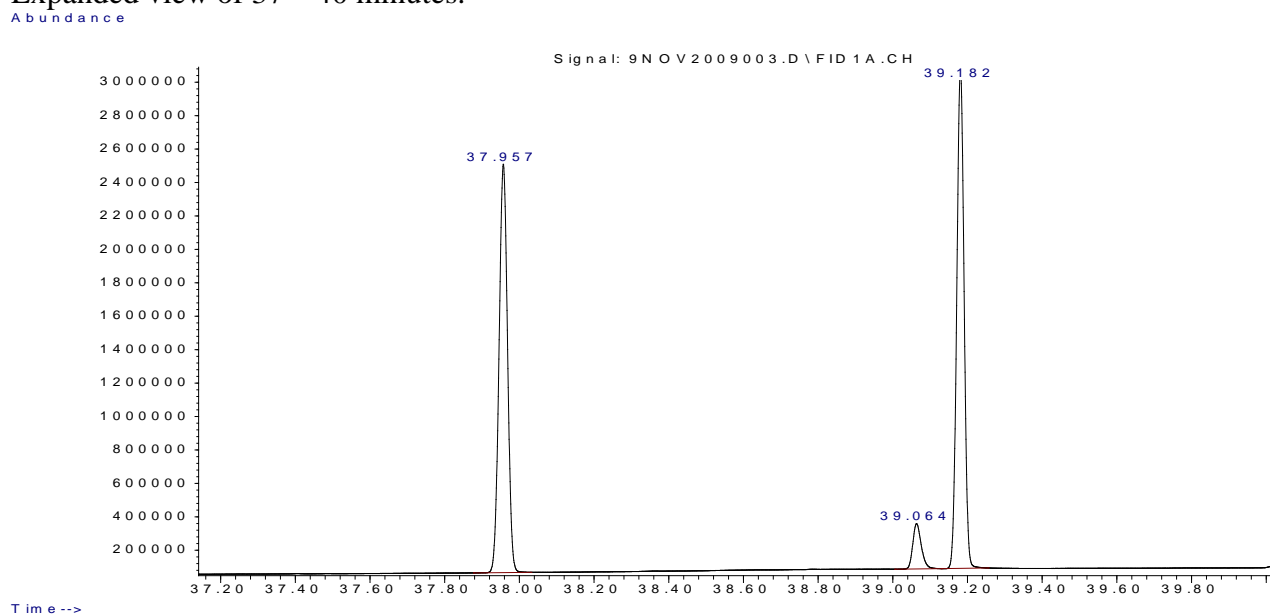
Peak ID	Compound	Peak ID	Compound
1	Decane	7	2,6-Dimethyl phenol [TMS]
2	2,3-Butanediol [2TMS]	8	2,6-Dimethyl aniline [TMS]
3	Undecane	9	Methyl decanoate
4	1-Nonanal	10	Dicyclohexylamine
5	2-Ethyl hexanoic acid [TMS]	11	Methyl undecanoate
6	1-Octanol [TMS]	12	Methyl dodecanoate

699 **Figure 3. GC/MS Chromatograms of the Headspace Suitability Mix.**

700
 701 Entire Chromatogram



702
 703
 704 Expanded view of 37 – 40 minutes.



705
 706

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

707

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

709
710 *1. General*

711
712 Relatively volatile and semi-volatile compounds will be analyzed by Gas Chromatography (GC)
713 using a predominantly non-polar capillary column with wide (40 °C to 300 °C) temperature
714 programming. As noted previously, appropriate detection strategies will be employed (e.g. FID,
715 MS). Each GC analysis will produce an extractables “profile” in the form of a Total Response
716 Chromatogram (e.g. TIC for MS detection). As a first pass, identifications of individual
717 extractables will be accomplished with manual interpretation of the Electron Ionization (EI)
718 spectra assisted by computerized mass spectral library searching. Beyond this, more difficult
719 identifications may require the collection of additional data (such as Chemical Ionization GC/MS
720 for molecular weight confirmation and High Resolution Mass Spectrometry for elemental
721 composition), the purchase of reference compounds, *etc.* The PODP study coordinator shall be
722 consulted before a participating laboratory pursues the more difficult identifications.

723
724 *2. Sample Preparation*

725
726 The resulting extracts will usually contain low-level amounts of extractables. Sample
727 concentration and/or solvent switching may be necessary to provide compatible samples for the
728 analytical instrumentation. While it is possible to manipulate extracts to provide very large
729 concentration ratios, this has the undesirable effect of concentrating normal solvent impurities.
730 Therefore, extracts will be concentrated no more than 100X, which is reasonable given normal
731 ACS reagent purities of 99+%. The process for preparing (working-up) the aqueous extracts for
732 GC analyses is shown in Table 5. Similar evaporative sample concentration strategies may be
733 utilized with the organic extracts.

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Table 5. Sample Work-up for Aqueous Extracts, GC Analysis	
Sample Preparation, Liquid-liquid Extraction; pH 2.5 and pH 9.5 Solutions.	<ol style="list-style-type: none"> 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	<p>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 \approx pH 2 and extracted twice. In the second extraction step, the samples will be adjusted to \approx pH 10 and extracted twice. The resultant DCM extracts will be combined, dried and concentrated per steps 9 through 11 above.</p>
TMS Derivatization of Residues	<ol style="list-style-type: none"> 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.

753
 754 The procedure contained in this Table is an example only and it is not required that participating
 755 laboratories adopt this procedure in either whole or in parts. However, any and all sample
 756 preparation procedures that will be used by a participating laboratory must be discussed with the
 757 PODP study coordinator prior to their utilization so that appropriate testing methodologies are
 758 utilized and harmonization between laboratories working on the same test articles can be
 759 achieved.

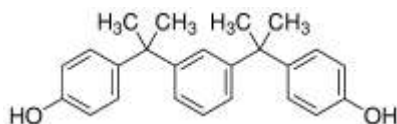
760
 761 The procedure calls for the addition of a surrogate and injection internal standard, consistent with
 762 the system suitability assessment strategy enumerated previously. A surrogate internal standard
 763 is used to monitor the performance of the total procedure and is added to each extract in the initial
 764 stage of its work-up. Requirements for such an internal standard are:

- 765
766 - sufficiently stable
767 - sufficiently soluble in all extraction solvents
768 - amenable to back-extraction from aqueous extracts by organic solvents
769 - semi-volatile
770 - amenable to all detection principles
771 - selectively detectable
772 - amenable to TMS-derivatization

773
774 The surrogate internal standard compound that meets these criteria has been identified as 4,4'-(m-
775 Phenylenediisopropylidene)diphenol (Bisphenol M):
776

CAS-no.: 13595-25-0
Molecular weight: 346.46
Molecular formula: $C_{26}H_{30}O_2$

Structure:



Source: e. g. Aldrich #450464

777
778 The Surrogate Standard Solution is prepared by dissolving 100 mg of Bisphenol M in 100 ml of
779 methanol, resulting in a concentration of 1000 µg/ml. This stock is further diluted 1 to 20 with
780 methanol to produce the surrogate internal standard solution containing 50 µg/mL Bisphenol M.
781

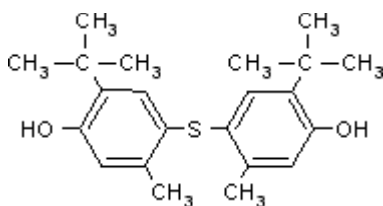
782 An injection internal standard is used to monitor the performance of the instrumental process
783 only and is added to each sample at the last stage of its work-up. Such an internal standard must
784 be:

- 785
786 - sufficiently stable
787 - sufficiently soluble in final extract
788 - semi-volatile
789 - amenable to all detection principles
790 - selectively detectable
791

792 The injection internal standard compound that meets these criteria has been identified as 4,4'-(m-
793 4,4'-Thiobis(3-methyl-6-t-butylphenol), Irganox 415:

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

Structure:



Source: e. g. Aldrich #366285

794
 795 The Injection Standard Solution is prepared as follows: 100 mg of Irganox 415 are dissolved in
 796 20 ml of methanol, concentration = 5000 µg/ml.. This stock is further diluted 1 to 100 with
 797 methanol to produce the surrogate internal standard solution containing 50 µg/mL Irganox 415.
 798

799 The surrogate and injection internal standards are added to all samples to ensure that they are
 800 properly worked-up and injected. Two internal standards are used to isolate the analytical
 801 processes of sample work-up and instrumental analysis. The minimum performance expectation
 802 for the internal standards is that they be present in the sample chromatograms with a response
 803 whose signal to noise ratio is 10 or greater.
 804

805 3. Operating Conditions

806
 807 The following GC conditions (Table 6) serve as an illustration of a methodology which is
 808 suitable for testing the prepared samples. The procedure contained in this Table is an example
 809 only and it is not required that participating laboratories adopt this procedure in either whole or
 810 in parts. However, any and all sample analysis procedures that will be used by a participating
 811 laboratory must be discussed with the PODP study coordinator prior to their utilization so that
 812 appropriate testing methodologies are utilized and harmonization between laboratories working
 813 on the same test articles can be achieved.
 814

815 Data cannot be collected while the injection solvent is in the ion source.
 816

Table 6. 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)

817

818 4. *General Comments.*

819
820 Note that the presented GC sample preparation and/or instrumental conditions are target
821 conditions for all participating laboratories and investigators. The actual conditions employed by
822 any participating laboratory should be reviewed by the PODP study coordinator prior to their
823 utilization so that appropriate testing methodologies are utilized and harmonization between
824 laboratories working on the same test articles can be achieved. In any event, the analyses
825 performed by the participating laboratory must meet system suitability criteria, as established in
826 Section VIII.A.

827
828 Any additional identification work beyond the first pass analysis will be performed only after
829 consultation with the PODP study coordinator.

830
831 Chromatograms of the extracts should be compared to chromatograms of the extraction blanks so
832 that peaks due to extractables can be delineated from peaks that reflect analytical artifacts.

833

834 **C. High Performance Liquid Chromatography (HPLC)**

835

836 1. *General*

837

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

847

848 2. *Sample Preparation*

849

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

855

856 3. *Operating Conditions*

857

858 The LC conditions in Table 7 serve as an illustration of a methodology which is suitable for
859 testing the prepared samples. The procedure contained in this Table is an example only and it is
860 not required that participating laboratories adopt this procedure in either whole or in parts.
861 However, any and all sample analysis procedures that will be used by a participating laboratory
862 must be discussed with the PODP study coordinator prior to their utilization so that appropriate

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

865
 866

Table 7. 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	

867

868 4. *General Comments*

869

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

872

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

875

876

877 **D. Inductively Coupled Plasma Atomic Spectroscopy (ICPAS)**

878

879 1. *General*

880

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

885

886

887

⁴ USP 30, <730> Plasma Spectroscopy.

888 2. *Sample Preparation*

889
890 The resulting extracts will usually contain low-level amounts of extractables as well as the ionic
891 constituents of the extracting media. The pH 2.5 and pH 9.5 extracts will contain large quantities
892 of sodium and the pH 9.5 extracts will have large quantities of phosphorous. Thus these analytes
893 cannot be determinable in these extracts.

894
895 The material extracts will need to be processed to some extent prior to testing. Aqueous samples
896 will be acidified directly via addition of nitric acid. Place 10 mL of aqueous extract in a trace
897 metal-free plastic vessel. Add 0.5 mL of concentrated nitric acid and mix well.

898
899 3. *Operating Conditions*

900
901 The ICP spectrometers shall be operated consistent with good laboratory practices and standard
902 procedures in place in the participating testing facilities. The following is a list of elements that
903 must be included in the ICP analysis: Al, As, Be, B, Cd, Cr, Co, Cu, Fe, Li, Mg, Mn, Mo, Ni, Pd,
904 Pt, S, Sb, Se, Si, Sn, Sr, Ti, V, W, Zn, and Zr. Additional elements may be reported depending
905 on the capabilities of the lab. The analysis conditions should be such that these elements can be
906 measured at the appropriately low levels, typically 0.25 µg/mL or less in the material extracts.

907
908 4. *General Comments*

909
910 The analyses performed by the participating laboratory must meet system suitability criteria, as
911 established in Section VIII.A

912
913 Results for the extracts should be compared the results for the extraction blanks so that
914 extractables can be delineated from analytical artifacts and solvent impurities/components.

915
916 **E. Headspace GC/MS**

917
918 1. *General*

919
920 Direct headspace analysis of materials allows for an assessment of their volatile components,
921 which may (or may not) be extractables or leachables. Headspace analysis augments the solvent
922 extraction of materials (and the subsequent analysis of the extracts) because (a) the volatile
923 entities may not be captured in the solvent extract and/or (b) the volatile entities may not persist
924 in the analytical methods used to test the solvent extracts.

925
926 Headspace analysis couples thermal “extraction” of a material with the transfer of the “extract”
927 to an appropriate analytical methodology. In headspace the analysis, the thermal “extraction” is
928 accomplished by heating the material in a closed vessel. The evolved volatile entities are
929 “captured” in the headspace gas, which is transferred, in whole or in part, to an appropriate
930 analytical technique. Since the headspace sample is a gas, gas chromatography is the analytical
931 method of choice. Mass spectrometry is the detection method of choice because it facilitates the
932 identification of evolved entities.

933 The headspace methodology is intended to uncover volatile entities that are present in the test
 934 material; it is not intended to produce “volatiles” by causing the test material to thermally
 935 decompose. Thus the headspace “extraction” is accomplished at relatively low temperatures
 936 (e.g. 120°C or less).

937
 938 2. *Sample Preparation*

939 Weigh approximately 1.0 g of sample into a 20 mL headspace autosampler vial. If necessary
 940 reduce the size of the sample (for example, by cutting) so that it fits into the vial. Seal the vial by
 941 crimping a cap onto it.
 942

943 For semiquantitative evaluation and also to check for proper performance of the measurement, an
 944 10 µL aliquot of a solution of 1,4-Dioxane in polyethylene glycol 200 (concentration 2 mg/mL)
 945 is added to each vial. This solution is prepared as follows: 20 mg of 1,4-Dioxane are dissolved in
 946 10 mL of polyethylene glycol 200, resulting in a concentration of 2 mg/mL.
 947

948
 949 Note: A positive displacement pipetting system (e. g. Gilson Microman[®]) should be used for
 950 dosing this solution due to its high viscosity.

951
 952 3. *Operating Conditions*

953 The operating conditions for the Headspace GC/MS are contained in Table 8.
 954
 955

Table 8. 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

956
 957
 958
 959

960 4. *General Comments*

961
962 The analyses performed by the participating laboratory must meet system suitability criteria, as
963 established in Section VIII.A.

964
965 The Headspace GC/MS analysis will produce an extractables “profile” in the form of a Total
966 Response Chromatogram (e.g. TIC for MS detection). As a first pass, identifications of
967 individual extractables will be accomplished with manual interpretation of the Electron
968 Ionization (EI) spectra assisted by computerized mass spectral library searching. More difficult
969 identifications may require the collection of additional data (such as Chemical Ionization GC/MS
970 for molecular weight confirmation and High Resolution Mass Spectrometry for elemental
971 composition), should be discussed with the PODP study coordinator before a participating
972 laboratory pursues these more difficult identifications.

973
974 Chromatograms of the extracts should be compared to chromatograms of the extraction blanks
975 (Headspace vials containing no test material) so that peaks due to extractables can be delineated
976 from peaks that reflect analytical artifacts.

977
978 The concentration of any extractables can be estimated via the use of the internal standard.
979

980 **IX. DATA EVALUATION AND REPORTING**

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982 **A. Qualitative Analysis**

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- 984 • A list of all identified entities (compounds, elements) that were not detected in the
985 corresponding blank. This list should include the recognized compound name,
986 CAS Registry number, chemical formula, and chemical structure.
 - 987 • A list of all unidentified chromatographic peaks that were not detected in the
988 corresponding blank at signal to noise ratios greater than 10. The participating
989 laboratory should determine and report the analyte concentration that corresponds
990 to this signal to noise ratio (typically defined as the limit of quantitation, LOQ).
 - 991 • Copies of chromatograms, spectra, etc.
 - 992 • Complete methodological information for both the extraction and analysis
993 processes.
 - 994 • The required system suitability results, which should include an assessment of
995 detectability.
 - 996 • The identification status for all compounds shall be established and reported as
997 follows:
998
 - 999 • A *Confirmed* identification means that collaborating information has been
1000 obtained including mass spectrometric fragmentation pattern, confirmation of
1001 molecular weight (or elemental composition), match in retention time and
1002 spectrum with authentic standard.
 - 1003 • A *Confident* identification means that sufficient data to preclude all but the most
1004 closely related structures have been obtained

1005 • A *Tentative* identification means that data have been obtained that are consistent
1006 with a class of molecule only.

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1008 • A report format will be distributed to the participating laboratories.

1009

1010 **B. Semi-Quantitative Analysis**

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1012 While it is not the primary intent of this Stage 1 Protocol to produce quantitative data,
1013 some of the test methods employed may be amenable to concentration estimation (e.g.
1014 ICP, GC with internal standards). In the case that a participating laboratory reports
1015 concentration estimates, the means by which such estimates were obtained must be
1016 indicated. Additionally, all such estimates shall be reported with a convention (e.g.
1017 significant figures) which effectively reflects the uncertainty in the determination. As
1018 was noted previously, the threshold for reporting semi-quantitative results is 10 µg/g.

1019

1020 **X. GLOSSARY**

1021

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
PODP	Parenteral and Ophthalmic Drug Products
TIC	Total Ion Chromatogram
API-ES	Atmospheric Pressure Ionization - Electrospray
HS	Headspace
PQRI	Product Quality Research Institute
OINDP	Orally Inhaled and Nasal Drug Products

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1025 **XI. REFERENCES**

1026

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1032 *Biologics-Chemistry Manufacturing, and Controls Documentation*, (May 1999).

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1035 (May, 2005).