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Chemical Analysis for Biocompatibility Assessment of Medical Devices

Draft Guidance for Industry and Food and Drug Administration Staff

DRAFT GUIDANCE

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Preface

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Chemical Analysis for Biocompatibility Assessment of Medical Devices

Draft Guidance for Industry and Food and Drug Administration Staff

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff or Office responsible for this guidance as listed on the title page.

I. Introduction

FDA is issuing this draft guidance to describe recommended methodological approaches for chemical analysis for biocompatibility assessment of medical devices. The recommendations provided in this guidance are intended to improve consistency and reliability of analytical chemistry studies and are based on FDA’s experience evaluating such studies submitted as part of premarket submissions to demonstrate device biocompatibility. However, alternative approaches to conducting chemical characterization may be appropriate. Furthermore, the type of information and/or testing needed in a biocompatibility assessment can vary depending on device characteristics and intended use. Chemical characterization is one approach that manufacturers can consider when developing a strategy for the overall biocompatibility assessment of a device. Manufacturers are encouraged to use an approach that works for their specific purposes, taking into account the considerations discussed in this guidance document, when conducting chemical characterization as part of the biocompatibility assessment for a device.

For the current edition of the FDA-recognized consensus standard(s) referenced in this document, see the [FDA Recognized Consensus Standards Database](#).¹ For more information regarding use of consensus standards in regulatory submissions, please refer to the FDA guidance titled “[Appropriate Use of Voluntary Consensus Standards in Premarket Submissions for Medical Devices](#).”

¹ Available at <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm>

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149 In general, FDA’s guidance documents do not establish legally enforceable responsibilities.
150 Instead, guidances describe the Agency’s current thinking on a topic and should be viewed only
151 as recommendations, unless specific regulatory or statutory requirements are cited. The use of
152 the word *should* in Agency guidances means that something is suggested or recommended, but
153 not required.

154 **II. Background**

155
156 As described in FDA’s biocompatibility guidance “[Use of International Standard ISO 10993-1,](#)
157 [‘Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk](#)
158 [management process.’”² biocompatibility is evaluated through a risk management process.
159 Potential biocompatibility risks are identified through risk assessment of a device and then
160 mitigated using relevant information \(e.g., literature, animal study experience, biocompatibility
161 testing\). Chemical characterization, as also described in the FDA-recognized version of the
162 consensus standard ISO 10993-18 *Biological evaluation of medical devices - Part 18: Chemical*
163 *characterization of medical device materials within a risk management process*, is used to
164 characterize the chemicals that may be released from the medical device to the body, and can be
165 useful to address certain risks during biocompatibility evaluation. For example, chemical
166 characterization can be considered as an alternative to biological testing for evaluating certain
167 biocompatibility endpoints when used in conjunction with toxicological risk assessment \(TRA\)
168 as described in the currently FDA-recognized version of ISO 10993-17 *Biological evaluation of*
169 *medical devices - Part 17: Toxicological risk assessment of medical device constituents*. In
170 addition, chemical characterization studies can be used to support chemical equivalence
171 determinations when evaluating a change in the materials or manufacturing of a device. Use of
172 chemical characterization can reduce the time needed to complete biocompatibility testing by
173 evaluating multiple biocompatibility endpoints at once and can reduce animal testing.³](#)

174
175 ISO 10993-18 describes various chemical characterization approaches, including information
176 gathering, compositional analysis, and extractables studies. Of these approaches, extractables
177 studies are the most frequently employed type of chemical characterization study and are the
178 focus of this guidance. Extractables studies aim to identify and quantify substances that are
179 released from a medical device or material when it is extracted using laboratory extraction
180 conditions and vehicles.

181
182 Chemical analysis of device extracts is intended to result in the identification and semi-
183 quantification of chemical constituents extracted from a device. When the complete chemical
184 composition of a device is not available, analytical chemistry testing is often performed using a
185 non-targeted approach, wherein chemicals present in the extract are identified and semi-
186 quantified. In addition to non-targeted analysis, targeted analysis may be used to fully quantify
187 constituents that are expected to be present in a device.

³ FDA supports the principles of the “[3Rs](#)” to replace, reduce, and/or refine animal use in testing, when feasible. We encourage manufacturers to consult with FDA if they wish to use a non-animal testing method that they believe is suitable, adequate, validated, and feasible. We will consider if a proposed alternative method could be assessed for equivalency to an animal test method.

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188
189 [FDA’s biocompatibility guidance](#) and FDA-recognized consensus standards on biocompatibility
190 do not specify detailed methodology on how to perform extractables studies and chemical
191 analysis of device extracts. FDA and other stakeholders have observed variability in the
192 approaches of individual laboratories performing analytical chemistry that has resulted in
193 inconsistent analytical chemistry reports, and deficiencies in this area are frequently identified in
194 premarket submissions. Therefore, FDA is providing detailed recommendations in this draft
195 guidance to promote consistency and reliability of analytical chemistry studies and to facilitate
196 more efficient review of these studies in premarket submissions, while aligning with ISO 10993-
197 18 and other relevant consensus standards where applicable.
198

199 **III. Scope**

200 This guidance provides recommendations for the collection and reporting of chemical
201 characterization data that could be used to support the following activities:
202

- 203 1. Screening for unspecified extractables (i.e., non-targeted analysis) or testing for specified
204 extractables (i.e., targeted analysis) to evaluate certain biocompatibility endpoints (i.e.,
205 acute, subacute, subchronic, and chronic systemic toxicity, genotoxicity, carcinogenicity,
206 and reproductive/developmental toxicity) in conjunction with TRA.
207
- 208 2. Chemical equivalency comparison to a device with previously demonstrated
209 biocompatibility as part of a biological equivalency evaluation.
210

211 The methods in this guidance are intended to be generally applicable for chemical
212 characterization of devices. However, for some types of devices (e.g., ophthalmic, respiratory,
213 hemodialyzers) different methods may be needed due to the materials used in the device or based
214 on historically established approaches. If there are device-specific FDA guidances or FDA-
215 recognized consensus standards that address chemical characterization for a particular device
216 type (e.g., ISO 11979-5⁴, ISO 15798⁵, and ISO 16672⁶ for ophthalmic implants, device specific
217 guidance on contact lenses,⁷ ISO 18562-3⁸ and ISO 18562-4⁹ for gas pathway devices, and ISO
218 7405¹⁰ for dental materials), those recommendations and methods should be followed.
219

220 Some types of devices commonly raise additional considerations when performing extractables
221 studies, such as absorbable/resorbable/degradable devices, combination products, devices that

⁴ ISO 11979-5 *Ophthalmic implants - Intraocular Lenses - Part 5: Biocompatibility*.

⁵ ISO 15798 *Ophthalmic implants - Ophthalmic viscosurgical devices*

⁶ ISO 16672 *Ophthalmic implants - Ocular endotamponades*

⁷ FDA guidance document “[Class II Daily Wear Contact Lenses - Premarket Notification \[510\(k\)\] Guidance Document](#)”

⁸ ISO 18562-3 *Biocompatibility evaluation of breathing gas pathways in healthcare applications - Part 3: Tests for emissions of volatile organic compounds*.

⁹ ISO 18562-4 *Biocompatibility evaluation of breathing gas pathways in healthcare applications - Part 4: Tests for leachables in condensate*.

¹⁰ ISO 7405 *Dentistry - Evaluation of biocompatibility of medical devices used in dentistry*.

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222 include animal tissues, or devices intended to change phase or other physical state (e.g.,
223 expansion) during use. Note that this is not an exhaustive list of device types where additional
224 considerations may apply. In such cases, the recommendations in this guidance may need to be
225 adapted and it is often helpful to discuss your planned approach prior to study initiation. The [Q-
226 submission process](#)¹¹ can be used to obtain FDA feedback regarding the study design.

227
228 In addition, a supplemental study that simulates clinical use of the device can sometimes be used,
229 when justified, such as to refine the exposure estimate to enable a more accurate TRA. These
230 simulated-use or leachables studies are outside the scope of this guidance. It is often helpful to
231 discuss with FDA the planned approach for such studies prior to study initiation. Q-submissions
232 may be particularly helpful to obtain feedback regarding the study design.
233

234 **IV. Information Gathering**¹²

235

236 **A. Device Components and Materials**

237 As part of the device description, we recommend that sufficient information be provided
238 to understand potential extractables, as well as to support rationales for design of
239 chemical characterization studies. Examples of information that may be useful can be
240 found in [Appendix A](#).

241
242 In certain cases (e.g., products made from animal-derived tissues¹³ or when reporting a
243 change for a clinical study or marketing application), additional information describing
244 the manufacturing process is often needed, such as the manufacturing steps and
245 manufacturing materials. For example, this information may help demonstrate that
246 manufacturing materials are removed or limited to an amount that does not adversely
247 affect the biocompatibility of the device, or to support a justification that a device change
248 is unlikely to adversely impact the biocompatibility of the device.
249

250 When performing chemical equivalence studies, a description of the changes to the
251 device (e.g., materials of construction, manufacturing methods, device geometry) that
252 could affect the equivalence determination should be provided. For example, for changes
253 in device material, formulation, or material supplier/vendor, a list of materials of
254 construction (e.g., base polymer, plasticizers, stabilizers, surfactants, color additive,
255 adhesives) relevant to the change for the previous and proposed material should be
256 provided. Likewise, for changes in device manufacturing, a list of manufacturing
257 materials (e.g., mold releasing agents, detergents) and process changes relevant to the

¹¹ Information regarding the Q-submission process can be found in “[Requests for Feedback and Meetings for Medical Device Submissions: The Q-Submission Program](#)”

¹² See also ISO 10993-18

¹³ FDA guidance document “[Medical Devices Containing Materials Derived from Animal Sources \(Except for In Vitro Diagnostic Devices\)](#)”

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258 manufacturing change (e.g., steps intended for removal of manufacturing materials)
259 should be provided.
260

B. Test Articles, Control Articles, and Blanks

262 Information on the test and control articles can be helpful to support the relevance of the
263 testing and analysis to the device under review. Additionally, a description of any
264 differences between the test articles to the final finished device is helpful to assess the
265 relevance of any subsequent characterization.
266

267 A previous version of the device where biocompatibility has been established should be
268 used as a control if a chemical equivalence study is being performed.
269

270 We recommend the use of a blank (e.g., solvent-only) control to differentiate analytes not
271 contributed by the test article itself. Blank controls also may be useful in chemical
272 equivalence studies.¹⁴
273

C. Test Article Processing Prior to Extraction

275 We recommend test article preparation that mimics the clinical preparation of the device
276 prior to extraction, if applicable, to account for physical transfer of chemicals onto the
277 test article. For example, this may include contact of the test article with all delivery
278 systems, accessories, and packaging materials and any other preparation or processing
279 steps (e.g., rinsing procedure) per the device's instructions for use. In particular, careful
280 test article preparation is recommended for implanted devices.
281

282 Any sample processing that is unrelated to clinical use should be described and
283 explained. For example:

- 284 • Drying/heating test articles before extraction, which may lead to loss of volatile
285 compounds and therefore should not be performed.¹⁵
- 286 • Pre-rinsing test articles prior to the extraction study may remove residuals and
287 should generally not be performed unless the device's instructions for use
288 includes pre-rinsing. Some deviations from the instructions for use may be
289 appropriate with justification, for example, rinsing with water instead of saline
290 prior to Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analysis to
291 reduce measurement interference, if equivalent volumes/times are used.
- 292 • Cutting of devices may lead to generation of particles or exposure of inner device
293 components that would not otherwise be relevant to the biocompatibility
294 evaluation. If cutting is performed:

¹⁴ ISO 10993-12 *Biological evaluation of medical devices - Part 12: Sample preparation and reference materials.*

¹⁵ As evidenced by the use of heating in headspace approaches to analyze volatiles described in Pahl I, Dorey S, Barbaroux M, Lagrange B, Frankl H. Analysis and evaluation of single-use bag extractables for validation in biopharmaceutical applications. *PDA J Pharm Sci Technol.* 2014 Sep-Oct;68(5):456-71.

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- Additional information may be needed to support that the particles were generated due to cutting and that particles are not shed from the device under normal use. Analysis of particulates is further described in [Section V.F.](#)
 - If applicable, analysis may be needed to confirm that constituents that raise toxicological concern are related to inner device components not exposed during device use.

302 V. Test Article Extraction

303 A. Extraction Conditions

304 Extraction conditions should be chosen to obtain worst-case estimates of amounts of
305 analytes in the device to which the tissue may be exposed. You should provide a rationale
306 for the extraction conditions selected that addresses the device exposure time and type of
307 tissue contact. The recommended extraction approaches are provided in [Table 1](#), which
308 was adapted from ISO 10993-18 Table 2.

309
310

Table 1. Recommended extraction conditions.

	Duration of Contact		
	Limited (< 24 h)	Prolonged (1-30 days)	Long-Term (> 30 days)
Extraction duration/ number of cycles	Exaggerated ^a extractions or clinically relevant worst-case conditions	Exhaustive or exaggerated ^{a,c} extractions	Exhaustive or exaggerated ^{a,c} extractions
Types of solvents	Polar and non-polar ^b	Polar and non-polar ^b	Polar, semi- polar, and non-polar
Non-volatile residue (NVR) analysis recommended to demonstrate exhaustion	N/A	Yes	Yes

311 ^a We recommend that exaggerated conditions exceed both time and temperature of
312 clinical use.¹⁶

313 ^b If a device cannot be evaluated in an analytically expedient polar or non-polar solvent,
314 biological testing may be needed, but in some cases other solvents may be used with
315 justification (Other considerations for extraction solvents can be found in [Appendix B,](#)
316 [Section IX.A.\(3\)](#)).

317 ^c See ISO 10993-18 Table 2 for examples where exhaustive extraction would not
318 typically be warranted.

¹⁶ See also ISO 10993-18.

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319 Performing extraction of test articles in solvents with different polarities (e.g., polar,
320 semi-polar, and non-polar) as described in [Table 1](#) is recommended, unless otherwise
321 justified. ISO 10993-18 summarizes typical extraction solvents of various polarities.
322

323
324 We recommend conducting extractions using a sealed container with minimal dead space
325 (i.e., empty space above the solvent and test article) and temperature control.
326 Additionally, we recommend the use of continuous mechanical agitation during
327 extraction to aid in achieving extraction equilibria.
328

B. Number of Extraction Replicates

330 For each solvent, we recommend that extractions be performed in triplicate¹⁷ and the
331 analyses be conducted on each separate extraction, unless otherwise justified. For
332 example, it may be acceptable to conduct a single replicate for particular device types if
333 this is a historical practice or if a different number of replicates is recommended in
334 device-specific FDA guidance(s) or FDA-recognized consensus standards.
335

336 Triplicates can be particularly important to:

- 337 • Support a statistical comparison to demonstrate chemical equivalence (as part of
338 material equivalency).
- 339 • Evaluate devices that have a higher potential for variability between devices
340 where small changes in chemistry at manufacture, over shelf life and/or while in
341 use could impact safety and effectiveness.¹⁸
- 342 • Evaluate devices where other information (e.g., engineering testing) identifies
343 variability within/across product lots.
344

345 When conducting replicate extractions, we recommend reporting the identity of the
346 extractables and amounts from all replicates separately. Additionally, we recommend
347 using the highest amount for each extractable from any replicate as a worst-case exposure
348 estimate (i.e., not a sum or average of the amounts from all replicates).
349

350 Triplicate extraction may not be necessary if three or more devices need to be pooled for
351 the extraction studies. For example, pooling may be warranted in some cases, such as for
352 very small devices, to generate sufficient extract volume for subsequent chemical
353 analyses. However, if there is other data (e.g., engineering data) that suggests potential
354 unexpected variability across devices, pooling devices instead of conducting triplicate
355 extractions may not be appropriate.
356

C. Extraction Volume

¹⁷ See also ISO 10993-18.

¹⁸ See also ISO 10993-18.

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358 We recommend that extraction volumes be minimized and justified based on relevant
359 published documents and/or the sensitivity needed for the chemical analysis and the
360 subsequent TRA. For example, extraction ratios as described in ISO 10993-12 could be
361 considered if the extraction is not overly dilute (i.e., the limit of quantification (LOQ) is
362 lower than the analytical evaluation threshold (AET)). The test article should be
363 completely covered by solvent.

364
365 While some swelling can be acceptable if there is no device destruction, high levels of
366 swelling/solvent uptake can cause a reduction in the accessible volume of extraction
367 solvent. Compensating for solvent loss by adding more solvent after extraction is
368 complete is not recommended as it could adversely impact the concentration of
369 extractables. If replenishing the solvent, a justification should be provided.
370

371 **D. Extraction Temperature and Time**

372 The temperature and duration of the extraction, including the duration of extraction
373 cycles if conducting an exhaustive extraction, should be provided and justified.
374 Justifications should address how the conditions result in a worst-case exposure estimate.
375 While the recommendations in ISO 10993-12 could be used as a starting point for
376 choosing the temperature and time (e.g., 50 °C with 72-hour cycles), other
377 recommendations in this guidance should also be considered when choosing the
378 temperature and time. For example, for exaggerated extractions we recommend that both
379 the temperature and time exceed clinical use (see [Table 1](#)). As another example, for
380 exhaustive extractions the conditions (including temperature and time) should generate a
381 sufficient quantity of extractables to demonstrate that exhaustion has been achieved,
382 unless justified (see [Section V.G](#)).
383

384 When conducting exhaustive extractions, we recommend that all cycles have the same
385 duration. Using cycles of different durations (e.g., an initial 72-hour cycle followed by
386 repeated 24-hour cycles) may complicate the determination of the exhaustive endpoint.
387 Additionally, we recommend that the same extraction schedule (i.e., duration and number
388 of cycles) be used when preparing extracts for all analyses (e.g., gas chromatography-
389 mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), ICP-
390 MS), unless justified. For example, when analyzing extracts using headspace-gas
391 chromatography-mass spectrometry (HS-GC-MS) it might be appropriate to use a
392 different extraction schedule to avoid the loss of volatile organic compounds (VOCs)).
393

394 When selecting the extraction temperature, we recommend that thermal properties (e.g.,
395 glass transition temperature, melting temperature, degradation temperature) of the bulk
396 materials composing the device be considered. Thermal properties obtained from the
397 literature can be used, if available.
398

399 For extractions *above* the clinical use temperature (e.g., 37 °C) where visible changes in
400 the device are noted, these changes may be due to thermal damage that could also result
401 in changes to the extractables profile. For example, degradation of heat labile substances

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402 (e.g., degradable materials, tissues, biomolecules, and drugs) or material phase change
403 (e.g., from the glassy to the melted state) could occur. Therefore, when visible changes in
404 the device are noted post-extraction, we recommend providing a justification explaining
405 why thermal damage to the test article, or a component, and/or known extractables is not
406 expected based on the thermal properties of the device materials. Please note that visible
407 changes to the device could also be related to solvent incompatibility (see [Section V.E](#)),
408 or device manufacturing issues that may need to be addressed.

409
410 For extractions *at* the clinical use temperature, we recommend that you provide at least
411 one of the following in your submission:

- 412 • A confirmation that the device use duration is limited (< 24 h) and extraction
413 duration is exaggerated compared to the duration of device use.
- 414 • A justification for the temperature used (e.g., the use of higher temperatures
415 would cause thermal damage to a test article or one of its components).
- 416 • Data demonstrating the extraction was exhaustive.

417
418 Extractions *below* clinical use temperature are not recommended because extraction
419 conditions are expected to be at least as aggressive as the conditions of clinical use.
420

421 E. Particulates

422 When particulates are observed in test extracts, we recommend characterization of the
423 particulates to determine the likely source and chemical composition of the particulates,
424 and whether tissue could be exposed to particulates from the final finished device.
425

426 If the particulates are an artifact of sample preparation or extraction, we recommend
427 providing information to support that the particulates do not interfere with subsequent
428 chemical analysis. For example, a justification should be provided to support that any
429 particulate removal steps (e.g., filtration, centrifugation)^{19, 20} do not alter the extractables
430 profile. However, if particulates are thought to be precipitated extractables, re-dissolution
431 is recommended prior to subsequent analysis. Additionally, particulates should be
432 accounted for when determining the exhaustive endpoint (i.e., particulates may raise the
433 apparent NVR mass in the initial extraction cycle, leading to an underestimate of the
434 number of cycles needed to reach exhaustion).
435

436 If particulate release is demonstrated to occur during device use, then we recommend that
437 information be provided to address any concerns related to the clinical safety of the
438 particulates. We recommend that you identify the cause of particulate generation (e.g.,
439 manufacturing process and/or change in stability of the device over the labeled shelf life).
440

¹⁹ Knolhoff AM, Croley TR. Non-targeted screening approaches for contaminants and adulterants in food using liquid chromatography hyphenated to high resolution mass spectrometry. *J Chromatogr A*. 2016 Jan 8;1428:86-96.

²⁰ McDowall RD. Sample preparation for biomedical analysis. *J Chromatogr*. 1989 Aug 11;492:3-58.

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441 Characterization of particulates could include the use of a number of tools, as described
442 in the currently FDA-recognized version of ISO/TS 10993-19 *Biological evaluation of*
443 *medical devices - Part 19: Physico-chemical, morphological and topographical*
444 *characterization of materials.*
445

446 **F. Additional Considerations for Exhaustive Extraction**

447 **(1) Determining the Endpoint of Exhaustive Extraction**

448 We recommend the use of gravimetric NVR analysis to determine the endpoint of
449 an exhaustive extraction. NVR analysis provides an estimate of the amount of
450 non-volatile and some semi-volatile extractables. Other approaches can be used to
451 determine the exhaustive endpoint, if justified (e.g., use of device-specific
452 guidances).
453

454 If NVR analysis is used to demonstrate exhaustive extraction has been achieved,
455 we recommend consideration of the information provided in [Appendix B, Section](#)
456 [IX.B.](#)
457

458 **(2) Combining/Pooling Extracts**

459 It is not necessary to separately perform chemical analysis (e.g., identification and
460 quantification) on the extract from each iteration of an exhaustive extraction.
461 Sequential extractions can be combined (i.e., pooled), and the total combined
462 volume can be used for an AET calculation (i.e., the B parameter) as described in
463 ISO 10993-18, and chemical analysis.
464

465 **VI. Chemical Analysis**

466
467 Justification and explanation of the chemical analysis plan should be provided. In general, we
468 recommend profiling of extractables through a non-targeted analysis with subsequent use of
469 targeted analysis to identify and quantify appropriate extractables, as necessary. Further
470 considerations are provided in [Appendix C.](#)
471

472 **A. Suitability of Detection Methods**

473 We recommend you select methods that ensure a wide range of analytes can be detected,
474 identified, and quantified. For example, we recommend consideration of the following:

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- Similarity in NVR and total amounts determined by analytical methods (e.g., GC, LC, ICP), recognizing that these methods are not sensitive to the same analytes, so achieving 100% mass balance is not expected.²¹
 - Optimization of chromatography conditions to resolve as many compounds as possible.²² FDA has released a preliminary dataset of relative response factors (RRFs) for chemicals with a range of physicochemical properties that could allow analytical chemistry laboratories to assess their ability to detect potential extractables.²³
 - Use of additional detectors (e.g., ultraviolet (UV), charged aerosol detector (CAD), evaporative light scattering detector (ELSD)) to complement MS to assist with detection of non-ionizable analytes.²⁴
 - Chromatographic resolution allows differentiation of co-eluting peaks.^{25, 26,}
 - Mass range allows for identification of low and high molecular weight analytes.²⁷
 - Manual and/or software-based feature finding results in comprehensive discovery of analyte signals (e.g., MS) above the AET.²⁸

491 B. Reference Standards

492 An authentic reference standard is a substance containing a compound of known
493 molecular structure with high purity (e.g., analytical standard grade, > 99.5% purity)
494 suitable for the intended analytical purpose (e.g., targeted analysis, surrogate standard).²⁹
495 An internal reference standard is an authentic reference standard added to (i.e., spiked
496 into) a sample with a known concentration and used to determine the response of the
497 standard in the presence of the sample matrix.³⁰ A surrogate reference standard is an
498 authentic reference standard, which may not match the analyte(s) of interest, used to
499 demonstrate that a non-targeted method can be used to identify and quantify analytes
500 having a wide range of chemical properties and concentrations.³¹

²¹ Gao Y, Allison N. Extractables and leachables issues with the application of single use technology in the biopharmaceutical industry. *J. Chem Technol Biotechnol.* 2016;91(2):289-95.

²² The following articles provide examples of optimization of chromatographic methods: Khan U, Jahangir M. Optimisation and validation of a new gas chromatographic mass spectrometry method for the simultaneous analysis of all regulated flame retardants from consumer articles. *Int J Environ Anal Chem.* 2022;102(2):470-82, and Legrand P, Desdion A, Boccadifuoco G, Dufay Wojcicki A, Worsley A, Boudy V, Dufay SG. Development of an HPLC/UV method for the evaluation of extractables and leachables in plastic: Application to a plastic-packaged calcium gluconate glucoheptonate solution. *J Pharm Biomed Anal.* 2018 Jun 5;155:298-305.

²³ "[Chemicals List for Analytical Performance \(CLAP\)](#)," CDRH Catalog of Regulatory Science Tools to Help Assess New Medical Devices.

²⁴ Jordi MA, et al. *J Pharm Biomed Anal.* 2018 Feb 20;150:368-376.

²⁵ Croley TR, White KD, Callahan JH, Musser SM. The Chromatographic Role in High Resolution Mass Spectrometry for Non-Targeted Analysis. *J Am Soc Mass Spectrom.* 2012;23(9):1569-78.

²⁶ Kind T, Fiehn O. Seven Golden Rules for heuristic filtering of molecular formulas obtained by accurate mass spectrometry. *BMC Bioinf.* 2007 Mar 27;8:105.

²⁷ Jordi MA, et al. *J Pharm Biomed Anal.* 2018 Feb 20;150:368-376.

²⁸ Kind T, et al. *BMC Bioinf.* 2007 Mar 27;8:105.

²⁹ USP <11> USP Reference Standards.

³⁰ Sussman EM, et al. *ACS Biomater Sci Eng.* 2022 Mar 14;8(3):939-963.

³¹ Sussman EM, et al. *ACS Biomater Sci Eng.* 2022 Mar 14;8(3):939-963.

501

502 **C. Calibration, Sensitivity, and Quantification**

503 Various methods are available for determining analyte concentration. When an authentic
504 reference standard is available for quantification of the analyte of interest, the response
505 factor (RF) can be calculated using a calibration curve relating concentration and signal
506 intensity (i.e., fully quantitative or targeted analysis). In semi-quantitative analysis, a
507 calibration curve can be generated using surrogate reference standards, and relative RFs
508 are used to establish the relationship between non-targeted analytes and their
509 concentrations. The sensitivity of the analysis methods (i.e., limit of detection (LOD),
510 and limit of quantification (LOQ)) should be established.

511

512 **D. Chemical Identification**

513 To help understand the potential hazards to which individuals may be exposed, we
514 recommend determining the chemical identity of extractables above the reporting
515 threshold (e.g., AET) and the confidence of each identification (i.e., unknown, tentative,
516 confident, confirmed), unless otherwise justified (e.g., if a device-specific FDA guidance
517 is available). Confident and confirmed identifications are recommended for TRA, unless
518 otherwise justified. [Appendix C, Section X.E](#) includes recommendations for when other
519 identification levels can be used with supporting information, and when additional
520 analytical or biological approaches may be necessary to address the impact of unknown
521 extractables on biocompatibility endpoints of concern. Note that if cohort of concern
522 compounds are known or suspected to be present and the reporting threshold is based on
523 a threshold of toxicological concern (TTC), the presence of these compounds should be
524 investigated even if the amount is below the reporting threshold because they can be toxic
525 at levels below TTC-based reporting thresholds.

526

527 **VII. Data Reporting**

528 Whenever analytical chemistry testing information is included in a submission, we recommend
529 that complete test reports be provided for all tests performed, as described in Attachment E of
530 [FDA's biocompatibility guidance](#). We recommend the test report provide a summary of the
531 method used, such as described in ISO 10993-18. The test report should identify any protocol
532 deviations and their impact on the conclusions drawn from the test. The test report should
533 provide a summary of the test methods and results as described in [Appendix D](#).

534

535

536

537 **VIII. Appendix A: Information Gathering Steps, Further**
538 **Considerations**

539 **A. Information for Device Components and Materials**

540 The intended use of the device should be described to include the following information
541 and should consider clinically relevant worst-case exposure.

- 542 • number of devices per procedure
- 543 • number of procedures
- 544 • duration of contact per procedure
- 545 • description of maximum exposure time and exposure duration category, i.e.,
546 limited (< 24 hours), prolonged (1-30 days) and long-term (> 30 days)
- 547 • devices with short duration but repeated use (e.g., ten minutes daily for two
548 months) are categorized according to the total number of days (e.g., long-term
549 >30 days for this example).

550
551 A detailed list of device components and device materials should be provided, including:

- 552 • name of device components
- 553 • duration and type of tissue contact (e.g., direct, indirect, none) of each component
- 554 • materials comprising each component
- 555 • material-specific information
 - 556 ○ chemical name
 - 557 ○ trade name (if relevant)
 - 558 ○ supplier
 - 559 ○ component contribution to the total device by surface area (cm²) or, if surface
560 area is not relevant, the amounts (e.g., by mass for textiles)
 - 561 ○ material standards
 - 562 ○ technical data sheets (e.g., from a supplier of a component or material) with
563 information on surface finish and manufacturing processes, if available
 - 564 ○ other material-specific information, such as formulation information including
565 known impurities, safety data sheets, and certificates of analysis

566

567 **B. Information for Test Articles, Controls and Blanks**

568 We recommend the following information be provided:

- 569 • A statement that the test article is the device/component in its final, finished form
570 (including sterilization and packaging, if applicable). Alternatively, an
571 explanation for why the test article accurately represents the device/component in
572 its final finished form.
- 573 • Device configuration/size
- 574 • Any specific manufacturing information relevant to test article used (e.g.,
575 sterilization method and number of cycles)
- 576 • Additional information describing that the test article represents the worst-case
577 tissue exposure scenario:

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- Complete whole (not partial) device (or multiple complete devices)
 - Typically, the device with the largest surface area should be used because larger devices tend to contain greater quantities and/or numbers of extractables. In most cases, it is not considered worst-case to extract smaller devices and extrapolate based on the direct proportion of extraction surface area.
 - When applicable, the worst-case manufacturing process (e.g., the device that undergoes the greatest number of sterilization and/or reprocessing cycles)
 - Other information that could be helpful in a biocompatibility evaluation (e.g., the lot number/other identification number). For example, the time from manufacture and storage conditions could be helpful if chemical analysis is being used to support shelf-life studies or determination of root cause for biological test failure.

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593 IX. Appendix B: Extraction Conditions, Further 594 Considerations

595 A. Extraction Solvents

596 (1) Considerations Regarding Alcohol/Water Mixtures

597 While both neat alcohol and alcohol-water mixtures (e.g., 50% isopropyl alcohol)
598 could be considered “semi-polar” by definition, if using an alcohol as a semi-
599 polar solvent for extractables studies, we recommend neat alcohol be used unless
600 otherwise justified, for the following reasons:

- 601 • Extractions using alcohol/water mixtures may result in fewer numbers
602 and/or lower amounts of extractables compared to pure alcohol alone.
- 603 • Water mixed with alcohol may introduce complications in terms of
604 instrument compatibility, such as in gas chromatography systems (i.e., if
605 additional processing needed, some extractables may be lost).
- 606 • Polymers may selectively absorb one component of the mixture, resulting
607 in a change in the composition of the extraction solvent mixture and
608 altered polarity.

609 (2) Other Considerations

610 When extraction solvents not included in ISO 10993-18 are used, a justification
611 should be provided to support the suitability of the solvent and the rationale for its
612 selection. Examples include:

- 613 • In addition to polarity, solvent properties such as pH, dielectric constant,
614 and the solubility parameter make the solvent an appropriate extraction
615 vehicle.
- 616 • Hildebrand or Hansen solubility parameters (δ) can be used to provide an
617 estimation of the degree of interaction between materials and solvents. A
618 small difference in a solubility coefficient between the selected solvent
619 and the polymer generally indicates good solubility of that polymer in the
620 selected solvent. The Hildebrand solubility coefficient is useful for non-
621 polar materials and solvents, whereas the Hansen solubility coefficient is
622 more suitable for polar systems.

623
624 Solvents with high boiling points (e.g., dimethyl sulfoxide (DMSO)) may
625 confound subsequent analytical steps, such as sample concentration (see
626 [Appendix C, Section X.A.\(2\)](#)) due to loss of analytes during the concentration
627 process.³² Similarly, solvents with low boiling points (e.g., dichloromethane) may

³² Solvents with higher boiling points have lower vapor pressures at a given temperature (e.g., see the Clausius-Clapeyron equation in Atkins PW, De Paula J, Keeler J. *Atkins' Physical Chemistry*, 11th ed.; Oxford University Press: 2018). Therefore, solvents with higher boiling points may necessitate harsher conditions for evaporation (e.g., higher temperatures, lower pressures, longer times), which increases the potential for losses of volatile compounds.

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628 increase the difficulty of extraction studies because these solvents may evaporate
629 under typical extraction conditions.

(3) Considerations for Solvents that Cause Destructive Swelling of the Test Article

632 Extraction should not cause destructive swelling of the test article (e.g., severe
633 swelling, particulate generation, degradation, and/or dissolution). Some swelling
634 of test articles is expected and is acceptable if the test article remains intact,
635 particularly for some devices (e.g., hydrogels) that are designed to be swollen
636 during use. Destructive swelling may induce material/device disintegration and
637 result in particulate debris and extractables that may not be clinically relevant but
638 that can interfere with analysis. However, if unintended destructive swelling
639 occurs in an aqueous extraction solvent, the clinical implications should be
640 considered.

642 If solvent incompatibility is expected or when investigating a novel medical
643 device material, pilot studies are recommended to identify compatible (i.e., non-
644 destructive) solvents. If destructive swelling occurs due to solvent effects, we
645 recommend evaluating at least two additional alternative solvents that are
646 representative of similar polarity (e.g., semi-polar) and varying chemical
647 functionality (e.g., alcohols, esters) and providing photographic evidence of the
648 solvent effects on the test article. For example, if alcohols are incompatible with
649 your device, we recommend that solvents with different functional groups (e.g.,
650 butyl acetate, acetonitrile) be evaluated. Similarly, if hexane is incompatible with
651 your device, we recommend longer-chain solvents (e.g., heptane, iso-octane) and
652 cyclic solvents (e.g., cyclohexane) be evaluated. Non-destructive swelling does
653 not indicate solvent incompatibility, as long as the extraction vehicle can be
654 recovered for analysis. If non-destructive swelling occurs in multiple solvents of a
655 particular type (e.g., semi-polar), we recommend selecting the tested solvent
656 exhibiting the lowest swelling.

658
659 If a compatible pure semi-polar solvent cannot be identified, then a polar/semi-
660 polar mixture may be appropriate, with justification. Likewise, if a compatible
661 pure non-polar solvent cannot be identified, then a semi-polar/non-polar mixture
662 may be appropriate, with justification. However, using solvent mixtures can
663 present analytical challenges (e.g., see [Appendix B, Section IX.A.\(1\)](#)). We
664 recommend seeking feedback from FDA on your study design if you intend to use
665 a solvent mixture.

666
667 For devices with limited or prolonged contact, where use of two solvents (i.e.,
668 polar and non-polar) are recommended for extraction studies, occurrence of
669 destructive swelling may be an appropriate justification for the use of a semi-polar
670 solvent instead of the solvent (e.g., non-polar) that resulted in destructive
671 swelling.

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672
673 If compatible solvents cannot be identified, your chemical characterization data
674 would generally not be appropriate to support a TRA. In these cases, we
675 recommend providing additional information (e.g., biological test data, materials
676 information, manufacturing information) to address the relevant biocompatibility
677 endpoints.

(4) Extraction Media for Elemental Analysis

678
679 Elemental analysis is commonly performed on polar extracts (e.g., deionized
680 water). However, it also can be performed using saline or an acidic solution (e.g.,
681 diluted hydrochloric acid (HCl) or nitric acid (HNO₃) solutions), if justified.^{33, 34,}
682 ³⁵ For example, when certain elemental analytes are expected based on the device
683 materials or manufacturing processes, there may be a preferred extraction media
684 to maximize analyte release. Likewise, if corrosion of metallic device components
685 is a concern, then testing conditions specific to the metal should be considered.
686 For example, phosphate-buffered saline (PBS) has been recommended for studies
687 on nitinol, where corrosion and release of nickel from the bulk is a concern.³⁶
688

689 We recommend acidification of extracts prior to ICP-MS analysis to promote
690 dissolution and detection.^{37, 38, 39, 40} It is acceptable to transfer a polar extract
691 aliquot (i.e., control, test sample) to an inert container (e.g., polypropylene) prior
692 to acidification to minimize the borosilicate glass leachate.
693

B. Considerations for Determining the Endpoint of Exhaustive Extraction

694
695
696 If NVR analysis is used to demonstrate that exhaustion has been achieved, we
697 recommend the following:
698

³³ Jordi MA, Khera S, Roland K, Jiang L, Solomon P, Nelson J, Lateef SS, Woods J, Martin L, Martin S, Aiello F, Chen N. Qualitative assessment of extractables from single-use components and the impact of reference standard selection. *J Pharm Biomed Anal.* 2018 Feb 20;150:368-376.

³⁴ Solomon P, Nelson J. Profiling extractable and leachable inorganic impurities in ophthalmic drug containers by ICP-MS. *Pharm Dev Technol.* 2018 Mar;23(3):247-254.

³⁵ Dorival-García N, Carillo S, Ta C, Roberts D, Comstock K, Lofthouse S, Ciceri E, D'Silva K, Kierans G, Kaisermayer C, Lindeberg A, Bones J. Large-Scale Assessment of Extractables and Leachables in Single-Use Bags for Biomanufacturing. *Anal Chem.* 2018 Aug 7;90(15):9006-9015.

³⁶ FDA guidance document "[Technical Considerations for Non-Clinical Assessment of Medical Devices Containing Nitinol](#)," Section IV.C.2, first paragraph, page 16.

³⁷ Jenke D, et al. *PDA J Pharm Sci Technol.* 2013 Sep-Oct;67(5):448-511.

³⁸ Houk RS, Fassel VA, Flesch GD, Svec HJ, Gray AL, Taylor CE. Inductively coupled argon plasma as an ion source for mass spectrometric determination of trace elements. *Anal Chem.* 1980;52(14):2283-9.

³⁹ Grotti M, Todolí J-L. Nitric acid effect in inductively coupled plasma mass spectrometry: new insights on possible causes and correction. *J Anal At Spectrom.* 2020;35(9):1959-68.

⁴⁰ Wollenweber D, Straßburg S, Wunsch G. Determination of Li, Na, Mg, K, Ca and Fe with ICP-MS using cold plasma conditions. *Fresenius' J Anal Chem.* 1999;364(5):433-7.

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- Use of replicate extractions (e.g., triplicate), unless otherwise justified. Additionally, see [Section V.B](#) for information about the number of replicates.
 - Drying the entire volume of an extraction for NVR analysis. Because total NVR quantities can be small, it can be challenging to use an NVR measurement to determine that exhaustive extraction has been achieved (i.e., less than 10% of the initial extracted quantity remains) if only an aliquot is dried. Consequently, we recommend conducting a specific extraction for exhaustive endpoint determination separate from the extractions used for other analytical testing (e.g., GC-MS, LC-MS, ICP-MS).
 - Use of extraction conditions (including temperature, time, solvent volume, and number of test articles) that produce a measurable NVR amount during at least the first extraction cycle. If the NVR amount from the first extraction cycle is not measurable, it may be challenging to demonstrate that exhaustion has been achieved, unless justified. For example, justifications could include that the materials of construction are expected to yield very low extractable amounts under the extraction conditions (e.g., some polymers in water).
 - Use of a balance with the capability to precisely measure NVR in the 10-100 µg range. As noted above, we recommend that the entire extract volume be dried for NVR analysis. However, if only a portion or an aliquot is used for NVR analysis, we recommend providing information on the aliquot volume and percentage of the whole extract, accompanied with a justification that indicates that the sensitivity of the approach in units of mass/device is appropriate.
 - Provide a tabular comparison of the total NVR amount to the total mass from other chemical analyses conducted for identification and quantification (e.g., GC-MS for semi-volatile organic compounds (SVOCs), LC-MS for non-volatile organic compounds (NVOCs), ICP-MS for elemental constituents) to support that significant loss of extractables has not occurred during processing and analysis.

727 NOTE: NVR does not provide the identities or concentrations of individual extractables.

728

729 We also recommend the following additional points when determining the exhaustive

730 endpoint:

- 731
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- 738
- Exhaustion should be demonstrated separately for each solvent (i.e., the duration needed to reach exhaustion in one solvent should not be used to define the exhaustive endpoint in other solvents).
 - Extractions should be conducted for exhaustive endpoint determination and for analytical testing in an identical manner (i.e., use the same temperature, cycle duration and number, extraction solvent, extraction ratio, and number of test articles). Serial extraction may result in different identities and amounts of extractables compared to a single extraction of the same total duration because

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739 differences in extraction conditions may alter equilibria due to changes in the
740 partitioning of analytes.^{41, 42}
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⁴¹ Turner P, Elder RM, Nahan K, Talley A, Shah S, Duncan TV, Sussman EM, Saylor DM. Leveraging Extraction Testing to Predict Patient Exposure to Polymeric Medical Device Leachables Using Physics-based Models. *Toxicol Sci.* 2020 Nov 1;178(1):201-211.

⁴² Crank J. *The Mathematics of Diffusion*. Clarendon Press: UK, 1975.

744 **X. Appendix C: Chemical Analysis and Extractables**
745 **Profiling, Further Considerations**

746 **A. Extract Processing**

747 There are various situations where sample processing (e.g., solvent exchange, dilution, or
748 concentration) may be needed. This section describes types of sample processing and
749 common scenarios where sample processing may be useful.

750
751 Solvent exchange may lead to a loss of extractables that do not fully partition into the
752 new solvent. Likewise, extract concentration may result in the loss of higher-volatility
753 analytes, particularly if concentrating solvents with high boiling points (e.g., DMSO). We
754 recommend that any extract processing methodologies are accompanied with method
755 qualification and verification information, which generally involves a spike and recovery
756 report. We recommend assessing the method recovery rates using internal reference
757 standards representative of a wide range of chemical properties (e.g., charge state,
758 polarity, and volatility).⁴³ We also recommend at least 5 reference standards be used to
759 assess recovery. Additionally, we recommend including the reference standards used for
760 semi-quantification in the set of reference standards used to assess recovery. Moreover,
761 the concentration of the reference standards used to assess recovery should be justified.
762 We recommend using concentrations near the middle of the linear range of the calibration
763 curve. If adequate recovery (e.g., 80-120%)^{44, 45, 46} is not achieved, you should take steps
764 to improve recovery.

765 **(1) Solvent Exchange**

766 Solvent exchange (also known as liquid/liquid extraction or vehicle exchange)
767 may be performed when there is extract solvent incompatibility with an analytical
768 approach.⁴⁷

769
770 If adequate recovery (e.g., 80-120%) is not achieved, we recommend the
771 following be considered to improve the performance of the solvent exchange
772 method:

⁴³ Ramos L. Critical overview of selected contemporary sample preparation techniques. *J Chromatogr A*. 2012 Jan 20;1221:84-98.

⁴⁴ CLSI C62-A, *Liquid Chromatography-Mass Spectrometry Methods*.

⁴⁵ Li J, Cai Y, Shi Y, Mou S, Jiang G. Analysis of phthalates via HPLC-UV in environmental water samples after concentration by solid-phase extraction using ionic liquid mixed hemimicelles. *Talanta*. 2008 Jan 15;74(4):498-504.

⁴⁶ Zdravkovic SA. Solid phase extraction in tandem with GC/MS for the determination of semi-volatile organic substances extracted from pharmaceutical packaging/delivery systems via aqueous solvent systems. *J Pharm Biomed Anal*. 2015 Aug 10;112:126-38.

⁴⁷ For example, see Product Quality Research Institute (PQRI), 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," 2009, Table 5, page 24.

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- Perform solvent exchange multiple times. We recommend performing three exchanges, although more exchanges may be needed for adequate recovery depending on the solvents and solutes.⁴⁸
 - Perform solvent exchange at three pH levels (i.e., acidified, neutral, and basified aqueous phase).⁴⁹

779

780

Generally, we do not recommend solvent exchange by evaporation to dryness and redissolution, as it could result in the loss of VOC and SVOC analytes.

(2) Dilution and Concentration

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Sample dilution may be performed to assist in accurately quantifying high-abundance analytes or to assist in quantifying analytes in the presence of other co-eluting analytes that may interfere with the analysis. Sample concentration may be performed to achieve method sensitivity to the appropriate level (e.g., below the AET).^{50, 51}

788

789

If sample dilution or concentration are used, we recommend the following be addressed:

- 790
- 791
- 792
- 793
- 794
- Report all sample dilution and concentration steps.
 - In the quantification of analytes and/or the AET determination, incorporate calculations that account for sample dilution/concentration (i.e., adjust the dilution/concentration factor D).

795

796

797

If adequate recovery (e.g., 80-120%) is not achieved, we recommend the following be considered to improve the detection and quantification of various analytes in the sample:

- 798
- 799
- 800
- 801
- 802
- Perform a separate analysis on non-concentrated samples to determine if better quantification for some of the analytes is possible.
 - Employ other qualified methods for analyte concentration (e.g., solid phase extraction).

(3) Extract Processing Scenarios

803

804

The following are common scenarios in which sample processing may be useful.

⁴⁸ Performing three solvent exchanges results in ~90% recovery, assuming equal solvent volumes in each exchange and a solute distribution constant of 1 between the two solvents. (Harris DC, Lucy, CA. *Quantitative Chemical Analysis*, 10th ed.; W.H. Freeman: New York, NY, 2020.)

⁴⁹ Ramos L. *J Chromatogr A*. 2012 Jan 20;1221:84-98.

⁵⁰ USP <1664> Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging Delivery Systems.

⁵¹ Norwood, D. Brief Overview of the PQRI Recommendations: Challenges and Successes. International Pharmaceutical Aerosol Consortium on Regulation & Science (IPAC-RS) Conference; Rockville, MD; March 29-31, 2011.

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805 **Scenario 1: Water-containing solvents to be analyzed by GC-MS**

806 Issue: Water can interfere with direct-injection GC-MS analysis (i.e., it expands in
807 the inlet, causing pressure/flow problems).^{52, 53}

808
809 We recommend the following alternative approaches be considered:

- 810 • Perform solvent exchange.⁵⁴
- 811 • Use sample introduction instrumentation, including static or dynamic HS-
812 GC-MS and/or solid-phase microextraction (SPME).⁵⁵
- 813 • Inject a smaller volume.⁵⁶
- 814 • Dilute the extract in an organic solvent.⁵⁷

815
816 NOTE: Evaporation and reconstitution steps are generally not recommended for
817 GC analysis due to the likelihood of VOC/SVOC analyte loss during evaporation.
818 However, evaporation and reconstitution may be used if another method (e.g.,
819 HS-GC-MS) is used on the unconcentrated extract to improve the range of
820 volatilities collected and analyzed.⁵⁸

821
822 NOTE: For water-containing extracts, we do not generally recommend directly
823 injecting a smaller volume or diluting with an organic solvent because these
824 approaches may reduce sensitivity and may prevent detection of some compounds
825 due to interactions with the column (e.g., peak broadening, retention time (RT)
826 change, loss of resolution).^{59, 60, 61, 62} However, if you need to use these
827 approaches (e.g., to evaluate polar extractables that may be lost during solvent
828 exchange), we recommend providing a justification that you have addressed these
829 issues (e.g., ensure the LOQ is less than the reporting threshold, provide
830 chromatograms for surrogate reference standards to demonstrate that peak

⁵² Kuhn ER. Water injections in GC-How wet can you get? *LC-GC North America*. 2002;20:474-8.

⁵³ Mazzucotelli M, Minteguiaga MA, Sgorbini B, Sidisky L, Marengo A, Rubiolo P, Bicchi C, Cagliero C. Ionic liquids as water-compatible GC stationary phases for the analysis of fragrances and essential oils: Quantitative GC-MS analysis of officially-regulated allergens in perfumes. *J Chromatogr A*. 2020 Jan 11;1610:460567.

⁵⁴ Ramos L. *J Chromatogr A*. 2012 Jan 20;1221:84-98.

⁵⁵ Yan X, Zhan Y, Zhong D, Li Y, Wu D. Inhibition of water adsorption into polar solid-phase microextraction materials with ultrathin polydimethylsiloxane coating for thermal desorption-gas chromatography analysis. *J Chromatogr A*. 2018 Nov 30;1578:1-7.

⁵⁶ Mazzucotelli M, et al. *J Chromatogr A*. 2020 Jan 11;1610:460567.

⁵⁷ Mazzucotelli M, et al. *J Chromatogr A*. 2020 Jan 11;1610:460567.

⁵⁸ Teasdale A, Jahn M, Bailey S, Feilden A, Taylor G, Corcoran ML, Malick R, Jenke D, Stults CL, Nagao LM. Controlled Extraction Studies Applied to Polyvinyl Chloride and Polyethylene Materials: Conclusions from the ELSIE Controlled Extraction Pilot Study. *AAPS PharmSciTech*. 2015 Jun;16(3):664-74.

⁵⁹ Grob K. Solvent effects in capillary gas chromatography. *J Chromatogr A*. 1983;279:225-32.

⁶⁰ Grob K, Li Z. Introduction of water and water-containing solvent mixtures in capillary gas chromatography: I. Failure to produce water-wettable precolumns (retention gaps). *J Chromatogr A*. 1989;473:381-90.

⁶¹ Grob K, Li Z. Introduction of water and water-containing solvent mixtures in capillary gas chromatography: II. Wettability of precolumns by mixtures of organic solvents and water; retention gap techniques. *J Chromatogr A*. 1989;473:391-400.

⁶² Norwood D, Michelson A, Dunn N, Duett J, Fleck L, Vas G. Impact of the GC-MS Injection Solvent and the Analyte Concentration on Relative Responses for common Extractables. *Rev Sep Sci*. 2022;4(1):e22002.

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831 separation and shape are unaffected by the presence of water, confirm that RF
832 determinations are based on measurements performed with the applicable
833 solvent).⁶³

834 **Scenario 2: Non-polar solvents (e.g., hexane) to be analyzed by LC-MS**

835 Issue: The non-polar solvent is immiscible with the chromatographic mobile
836 phase or extractables precipitate during sample preparation.⁶⁴
837

838 We recommend the following alternative approaches be considered:

- 839 • Evaporate and reconstitute in a suitable solvent; ensure that SVOCs that
840 might be lost during evaporation and reconstitution are captured with
841 additional techniques (e.g., GC-MS).⁶⁵
- 842 • Perform solvent exchange.
- 843 • Dilute the extract in an analytically expedient solvent. Separate any
844 precipitate to avoid instrument damage with injection of particulates. In
845 addition, we recommend characterizing the precipitates (e.g., after
846 dissolving the precipitates in an appropriate solvent) to ensure that all
847 extracted constituents are analyzed.

848 **Scenario 3: Proposed extraction solvent is incompatible with a laboratory-** 849 **qualified method**

850 Issue: Laboratory has not qualified a method for analysis of a solvent that is
851 proposed.⁶⁶
852

853 We recommend the following alternative approach be considered:

- 854 • Qualify the method using the proposed solvent (e.g., typical solvents used
855 for extraction studies can include water, isopropanol, and hexane).

856
857 If method qualification with the proposed solvent is not feasible, the following
858 approaches may be considered, with justification:

- 859 • Perform solvent exchange.
 - 860 • Dilute the extract in an analytically expedient solvent.
- 861

862 **B. Extractables Profiling**

⁶³ Norwood D, *et al.* *Rev Sep Sci.* 2022;4(1):e22002.

⁶⁴ Nahan K, Sussman EM, Oktem B, Schultheis L, Wickramasekara S. Screening for extractables in additive-manufactured acrylonitrile butadiene styrene orthopedic cast. *Talanta.* 2020 May 15;212:120464.

⁶⁵ Norwood DL, Stults CLM, Nagao LM, Ball DJ, *Leachables and Extractables Handbook: Safety Evaluation, Qualification, and Best Practices Applied to Inhalation Drug Products.* Wiley: 2012.

⁶⁶ ISO 10993-18 Second Edition 2020-01, Annex F, pages 58-60.

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(1) Primary Tools used in Extractables Profiling

[Table 2](#) identifies the primary tools we recommend be used in extractables profiling to understand the identity and quantity of VOCs, SVOCs, NVOCs and elemental constituents that can be extracted from the device. NVR analysis is also recommended to support that analyte discovery during identification is adequate (i.e., that a significant proportion of extracted non-volatile substances above the AET are identified).

In all cases, we recommend a justification for the selected analytical methods be provided. Additionally, we recommend initiating the analysis as soon as is practically possible after performing the extraction to avoid deterioration of the extracts (e.g., within 24 hours).

Table 2. Recommended analytical techniques.⁶⁷

Solvent / Technique ^a	VOC & SVOC ^b	SVOC & NVOC ^b	Elemental Constituents	NVR
Polar	GC-MS (Complementary: HS-GC-MS ^c)	LC-MS	ICP-MS or ICP-OES	Gravimetric analysis
Semi-polar	GC-MS	LC-MS	n/a	Gravimetric analysis
Non-Polar	GC-MS	LC-MS	n/a	Gravimetric analysis
Solvent-free	Complementary: HS-GC-MS ^d	n/a	n/a	n/a

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^a If a solvent is not compatible with an analytical method, then use a complementary analytical technique or appropriate sample preparation prior to analysis (e.g., solvent exchange, see [Appendix C, Section X.A.\(1\)](#)).

^b High-resolution MS is recommended to detect, identify, and/or quantify extractables.

^c In addition to direct injection GC-MS, HS-GC-MS may be performed directly on polar solvents to characterize substances not easily separated or detected by direct injection techniques (e.g., silicone cyclic oligomers such as D3).

^d In addition to direct injection GC-MS, to analyze substances not easily detected by direct injection techniques (e.g., liquid adhesives containing volatile solvents), HS-GC-MS may be performed directly on the test article (e.g., solvent-free HS-GC-MS).

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(2) Ionization Methods for LC-MS

For detection of a wide range of SVOC/NVOC compounds, we recommend the use of electrospray ionization (ESI) in both positive and negative modes as the primary LC-MS analysis technique. Additional analysis using atmospheric

⁶⁷ See also ISO 10993-18.

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893 pressure chemical ionization (APCI) can further improve compound detection and
894 identification for some types of matrices and analytes.

(3) Primary Detection Methods

895 Various types of MS and non-MS detectors can be used together in non-targeted
896 analysis for detection of a wide range of compounds. For example, it may be
897 helpful to use optical detection methods (e.g., UV, diode array detector (DAD))
898 with LC-MS because these orthogonal techniques do not rely on ionization of a
899 compound for detection, they provide some supporting structural information, and
900 in the absence of co-eluting compounds can be used to support identification.
901

902 Additional analysis using an ELSD or a CAD can be useful as complementary,
903 orthogonal techniques to liquid chromatography-ultraviolet-mass spectrometry
904 (LC-UV-MS) to further improve compound detection and quantification.
905 Similarly, a flame ionization detector (FID) can be used with HS-GC-MS or GC-
906 MS to further improve compound detection and quantification.
907

(4) Secondary Detection Methods

908 The following secondary detection methods, while generally not needed, may be
909 helpful in some cases:
910

- 911 • Gel permeation chromatography (GPC) is useful for characterization of
912 higher molecular weight compounds (e.g., polymers).
- 913 • Ion chromatography (IC) can be useful for analyzing counterions in
914 aqueous extracts, such as when high levels of salts are present.
915

916 The following secondary detection methods are less relevant for extractables
917 profiling:

- 918 • Fourier-transform infrared (FTIR) spectroscopy is useful for
919 characterizing substances based on functional groups and covalent bonds.
920 However, FTIR has limited use for mixtures, and does not provide
921 sufficient information to determine the complete molecular structure of a
922 compound. FTIR is also less sensitive for quantification compared to other
923 instruments. Therefore, FTIR data alone is generally not sufficient to
924 quantitatively identify individual compounds present or extracted from
925 medical devices. However, FTIR analysis can provide useful qualitative
926 information about, for example, the chemical composition of particulates
927 or the NVR.
- 928 • Total organic carbon (TOC) can be useful when used with NVR to help
929 discriminate inorganic and organic compounds. However, TOC analysis
930 excludes various species including but not limited to inorganic carbon,
931 inorganic salts, metals, and inorganic ions.

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(5) Targeted Analysis

If analytical chemistry data is needed to support overall biocompatibility, we recommend a non-targeted study be conducted. However, targeted analysis may be performed for one or more constituents, in parallel or in addition to non-targeted analysis, as needed. For example, targeted analysis can be used to confirm the identity and refine the quantity of a constituent (e.g., a cohort of concern compound)⁶⁸ whose presence is suspected based on either (a) *a priori* knowledge or (b) analytical data. As another example, targeted analysis can be used to analyze extractables with high concentrations that may be over- or underestimated by semi-quantification. We recommend that targeted analysis be performed using relevant analytical techniques that have been qualified for the device matrix material and the analyte(s) of interest.

Generally, non-targeted data is insufficient to conclude that a particular substance is absent from an extract.⁶⁹ Targeted analysis that is calibrated using an authentic reference standard or appropriate surrogate is the most robust approach to address suspected analytes. Further, some substances cannot be detected using routine non-targeted approaches (e.g., formaldehyde), and targeted analysis is recommended for quantification of these analytes.⁷⁰

⁶⁸ ISO/TS 21726 *Biological evaluation of medical devices — Application of the threshold of toxicological concern (TTC) for assessing biocompatibility of medical device constituents*

⁶⁹ Collaborative trials have demonstrated low reliability in identification of spiked compounds analyzed using non-targeted approaches as presented in Sobus JR, Grossman JN, Chao A, Singh R, Williams AJ, Grulke CM, Richard AM, Newton SR, McEachran AD, Ulrich EM. Using prepared mixtures of ToxCast chemicals to evaluate non-targeted analysis (NTA) method performance. *Anal Bioanal Chem.* 2019 Feb;411(4):835-851.

⁷⁰ Dugheri S, Massi D, Mucci N, Marrubini G, Cappelli G, Speltini A, *et al.* Exposure to airborne formaldehyde: Sampling and analytical methods—A review. *Trends Environ Anal Chem.* 2021;29:e00116.

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C. Reference Standard Selection

958 Use of more than a single surrogate reference standard for semi-quantification of
959 mixtures is recommended because approaches using a single surrogate reference standard
960 have been shown to result in underestimation of chemicals with low RFs. Use of more
961 than a single reference standard, so that the range of chemicals potentially present in the
962 extract is covered, can improve accuracy of quantification and TRA.⁷¹ We recommend at
963 least 3 surrogate reference standards for direct injection GC-MS and at least 5 for LC-
964 MS.⁷² If the 5 surrogate reference standards used for LC-MS do not ionize in both
965 positive and negative modes, additional surrogate reference standards are recommended
966 so that there are at least 5 that ionize in each polarity mode to address possible
967 differences in RFs.

968

969 We recommend the method-specific⁷³ surrogate reference standards chosen be described
970 and justified.

971

972 The following can be considered in developing a justification for the use of surrogate
973 reference standards for detecting a wide range of compounds:

- 974 • Selected reference standards represent a range of chemical properties including
975 volatility, molecular weight, polarity, solubility.
- 976 • Selected reference standards bracket the RT range of analytes during
977 chromatographic separation.

978

979 We also recommend justifying that the selected surrogate reference standards provide a
980 reasonable estimation of extractable concentrations by considering the following:

- 981 • Selected reference standards have the same or similar chemistry and
982 characteristics as the expected analytes for each particular method.⁷⁴
- 983 • Selected surrogate reference standards used for semi-quantification exhibit
984 conservative (e.g., lower) RFs.

985

986 The following can be considered in developing a justification for the use of reference
987 standards (either surrogate reference standards or authentic reference standards) for ICP
988 analysis:

⁷¹ For example, see Jenke D, *et al. J Chromatogr Sci.* 2012 Mar;50(3):206-12.

⁷² More reference standards are needed for LC to account for greater variability in instrument sensitivity to different chemicals compared to GC. Studies of response factor variability/optimization have reported that 3 to 30 reference standards can be used.

⁷³ Pieke EN, Granby K, Trier X, Smedsgaard J. A framework to estimate concentrations of potentially unknown substances by semi-quantification in liquid chromatography electrospray ionization mass spectrometry. *Anal Chim Acta.* 2017 Jul 4;975:30-41.

⁷⁴ Norwood DL, Mullis JO, Pennino, SJ. "The Analytical Evaluation Threshold (AET) and its Relationship to Safety Thresholds." in *Leachables and Extractables Handbook*, Ball DJ, Norwood DL, Stults CLM, Nagao LM eds.; Section 5.3 Determination of the AET, page 66.

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- Multiple elemental reference standards are used to evaluate the sensitivity and accuracy of the method, including sample preparation.
 - Elemental reference standards are also selected based on the need for more accurate quantification data (e.g., full versus semi-quantification). For example, specific reference standards can be selected for full quantification to confirm the amount of potentially toxic elements. The following elements can be considered for this type of analysis: Class 1, 2A, 2B and 3 elements per the International Council for Harmonisation (ICH) guideline for elemental impurities.⁷⁵

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NOTE: When a constituent is expected to be present in quantities that could be toxic, we recommend that the constituent be selected as a reference standard to allow accurate quantification (e.g., targeted analysis). For example, if flexible polyvinyl chloride tubing is plasticized with diethylhexyl phthalate (DEHP), this plasticizer can be used as an authentic reference standard for LC and/or GC analysis. Similarly, if a metal catalyst is used to manufacture a polymer, this element can be used as an authentic reference standard for ICP analysis.

1006 D. Calibration, Sensitivity, and Quantification

1007 (6) Calibration

1008 We recommend a description of the calibration method be provided in your test

1009 report, which includes:

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- Solvent in which each reference standard is diluted.
 - Analytical measure used for quantification (e.g., MS or UV signal).
 - For example, if MS signal is used, specify whether the precursor (i.e., molecular) ion or product ion is used, and a description of the adduct.⁷⁶
 - For example, if UV signal is used, provide the wavelength.
 - Concentration levels for each reference standard, resulting calibration curve, linearity of the curve, and the RFs.
 - Justification for the number of calibration concentration levels. For example, when fewer than 5 non-zero concentration levels are used,^{77, 78} we recommend data be provided to confirm reasonable linearity (e.g., a linear fit with $r^2 \geq 0.95$) over the calibration range.
 - Confirmation that the calibration levels of the reference standards produce signals that bracket the signal levels of the analytes (e.g., from at or below the AET to above the highest analyte concentration).

⁷⁵ [ICH guideline Q3D for elemental impurities](#)

⁷⁶ Jeon SH, Kim YP, Kho Y, Shin JH, Ji WH, Ahn YG. Development and Validation of Gas Chromatography-Triple Quadrupole Mass Spectrometric Method for Quantitative Determination of Regulated Plasticizers in Medical Infusion Sets. *J Anal Methods Chem.* 2018 Feb 5;2018:9470254.

⁷⁷ Raposo F. Evaluation of analytical calibration based on least-squares linear regression for instrumental techniques: A tutorial review. *TrAC, Trends Anal Chem.* 2016;77:167-85.

⁷⁸ USP <1225> Validation of Compendial Procedures.

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(7) Sensitivity

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We recommend a rationale be provided to support that the sensitivity for each reference standard is adequate for the study. For example, if LOQs⁷⁹ are used for sensitivity, the LOQ for each reference standard should be lower than the reporting threshold (e.g., AET).

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If an LOQ is used, we recommend that you support the LOQ determination by providing experimental evidence, including the calibration curves and calibration chromatograms of the reference standards used and the blank control, and the analysis of a suitable number of samples prepared near the LOQ for each analytical method. We do not recommend using statistical approaches (e.g., based on the signal-to-noise ratio or S/N ratio) alone to establish the LOQ, although these approaches may be useful in establishing an LOQ that can be verified using experimental evidence as described above.

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We do not generally recommend the use of LODs to support the sensitivity for each reference standard as LODs are typically closer to background noise levels, and therefore they are not considered as reliable as LOQ for quantification.⁸⁰

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(8) Semi-Quantification Method

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We recommend including in your submission, a description of the semi-quantification method used and information to demonstrate that the method does not result in underestimation of the concentration of analytes. We also recommend describing how specific reference standards and their RFs were used for semi-quantification of specific analytes. For example, information to support the semi-quantification method used may be based on one or more of the following:

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- nearest RT⁸¹
- similarity in chemistry between the analyte and the surrogate reference standard
- worst-case (i.e., minimum) RF
- an RF database based on prior analysis of a reference standard (i.e., RRF approach)^{82, 83, 84}

⁷⁹ Skoog DA, West DM, Holler FJ, Crouch SR. *Fundamentals of analytical chemistry*, 9th ed.; Brooks/Cole, Cengage Learning: Belmont, CA, 2014.

⁸⁰ Skoog DA, West DM, Holler FJ, Crouch SR. *Fundamentals of analytical chemistry*, 9th ed.; Brooks/Cole, Cengage Learning: Belmont, CA, 2014.

⁸¹ Nahan K, et al. *Talanta*. 2020 May 15;212:120464.

⁸² Paskiet D, Jenke D, Ball D, Houston C, Norwood DL, Markovic I. The Product Quality Research Institute (PQRI) Leachables and Extractables Working Group Initiatives for Parenteral and Ophthalmic Drug Product (PODP). *PDA J Pharm Sci Technol*. 2013 Sep-Oct;67(5):430-47.

⁸³ Jenke D, et al. *J Chromatogr Sci*. 2012 Mar;50(3):206-12.

⁸⁴ Norwood DL, Paskiet D, Ruberto M, Feinberg T, Schroeder A, Poochikian G, Wang Q, Deng TJ, DeGrazio F, Munos MK, Nagao LM. Best practices for extractables and leachables in orally inhaled and nasal drug products: an overview of the PQRI recommendations. *Pharm Res*. 2008 Apr;25(4):727-39.

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E. Chemical Identification

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We recommend that the following general principles be applied when analytical chemistry testing is conducted per ISO 10993-18, as shown in the flowchart below ([Figure 1](#)).

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- Analytical instrumentation, methods, libraries and standards should be adequate to identify and semi-quantify extractables, and generate chemistry data for TRA.

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- Chemical identification of all analytes above the reporting threshold (e.g., AET). In addition, if cohort of concern compounds are known or suspected to be present, the presence of these compounds should be investigated because they can be toxic at levels below reporting thresholds that are based on TTCs.

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- Additional structural elucidation of extractables (e.g., for TRA) based on identification level and supporting information (e.g., orthogonal data).^{85, 86}

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- Knowledge of the materials of construction and the manufacturing process (e.g., *a priori* information) to support the chemical identifications (e.g., tentative, confident, confirmed).⁸⁷

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- Reporting of all plausible candidate identifications when multiple candidate identifications are found, unless otherwise justified.^{88, 89}

⁸⁵ Sobus JR, Wambaugh JF, Isaacs KK, Williams AJ, McEachran AD, Richard AM, *et al.* Integrating tools for non-targeted analysis research and chemical safety evaluations at the US EPA. *J Exposure Sci Environ Epidemiol.* 2018;28(5):411-26.

⁸⁶ Ulrich E, Sobus J, Richard A, Grulke C, Wambaugh J, Newton S, *et al.* EPA's Non-Targeted Analysis Research Program: Expanding public data resources in support of exposure science. Society of Toxicology; San Antonio, TX; March 11-15, 2018.

⁸⁷ Milman BL. "Prior Data for Non-target Identification." in *Chemical Identification and its Quality Assurance*, Milman BL ed.; Springer Berlin Heidelberg: 2011, Chapter 6, pages 141-164.

⁸⁸ Milman BL. "Prior Data for Non-target Identification." in *Chemical Identification and its Quality Assurance*, Milman BL ed.; Springer Berlin Heidelberg: 2011, Chapter 6, pages 141-164.

⁸⁹ Milman BL. "Non-target Identification. Chromatography and Spectrometry." in *Chemical Identification and its Quality Assurance*, Milman BL ed.; Springer Berlin Heidelberg: 2011, Chapter 7, pages 165-234.

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1078 We recommend the following guidelines for the identification levels (i.e.,
1079 unknown, tentative, confident, confirmed)^{90,91, 92} for compounds above the
1080 reporting threshold (e.g., AET).

1081
1082 We recommend that the following extractables be reported as Unknown:

- 1083 • Compounds that cannot be at least tentatively identified.
- 1084 • Analytes identified as a member of a class of compounds where only
1085 partial structural identification is available. This information is helpful in
1086 understanding whether additional structural elucidation may be needed for
1087 the TRA. In general, grouping of extractables into compound classes
1088 based only on structural groups (e.g., branched and linear alkane
1089 hydrocarbons) may be inadequate chemical information for TRA.

1090
1091 Tentative identification means that one or more candidate molecular structure can
1092 be assigned to an analyte. We recommend the following minimum information be
1093 provided to support a tentative identification:

- 1094 • A library match or expert interpretation of mass spectral data;
1095 AND
- 1096 • Review of tentative identifications by an experienced analytical chemist to
1097 support that the proposed chemical identities are plausible.⁹³ For example,
1098 expert judgement can be supplemented by qualified approaches reported
1099 in peer-reviewed literature to eliminate false candidate molecular
1100 formulas;^{94, 95}
1101 AND
- 1102 • Justification for why additional analysis to increase the identification level
1103 is not needed, because confident or confirmed identification are
1104 recommended for TRA.

1105
1106 Confident identification means that a single candidate structure can be assigned to
1107 an analyte. We recommend the following minimum information be provided to
1108 support a confident identification:

- 1109 • A library match or expert interpretation of mass spectral data;
1110 AND

⁹⁰ Cuadros-Rodríguez L, Lazúen-Muros M, Ruiz-Samblás C, Navas-Iglesias N. Leachables from plastic materials in contact with drugs. State of the art and review of current analytical approaches. *Int J Pharm.* 2020 Jun 15;583:119332.

⁹¹ Schymanski EL, Jeon J, Gulde R, Fenner K, Ruff M, Singer HP, *et al.* Identifying Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence. *Environ Sci Technol.* 2014;48(4):2097-8.

⁹² De Vijlder T, Valkenburg D, Lemièrre F, Romijn EP, Laukens K, Cuyckens F. A tutorial in small molecule identification via electrospray ionization-mass spectrometry: The practical art of structural elucidation. *Mass Spectrom Rev.* 2018 Sep;37(5):607-629.

⁹³ Milman BL. "Non-target Identification. Chromatography and Spectrometry." in *Chemical Identification and its Quality Assurance*, Milman BL ed.; Springer Berlin Heidelberg: 2011, Chapter 7, pages 165-234.

⁹⁴ Kind T, *et al.* *BMC Bioinf.* 2007 Mar 27;8:105.

⁹⁵ De Vijlder T, *et al.* *Mass Spectrom Rev.* 2018 Sep;37(5):607-629.

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- Supporting information (e.g., molecular formula/molecular weight, elemental composition, spectral data, RT) from one or more orthogonal methods (e.g., chromatography, spectroscopy).

1115 Confirmed identification means that the molecular structure has been verified
1116 using an authentic reference standard. We recommend the following minimum
1117 information be provided to support a confirmed identification:

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- A library match or expert interpretation of mass spectral data;
AND
 - Supporting information (e.g., molecular formula/molecular weight, elemental composition, spectral data, RT) from one or more orthogonal methods (e.g., chromatography, spectroscopy);
AND
 - Identity verification using an authentic reference standard.

1125

1126 Supporting information^{96, 97} for identification can include one or more of the
1127 following:

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- 1136
- molecular formula generation (based on accurate mass) and/or confirmation (with an authentic reference standard of the candidate structure or a close structural analog)
 - RT or retention index matching
 - isomer assignment based on interpretation of data
 - spectral interpretation (e.g., for MS spectra)
 - fragmentation spectra interpretation based on data (e.g., for EI-based MS spectra)
 - MSⁿ elucidation of fragments

1137

1138 NOTE: For extractables reported as unknown, we recommend including
1139 supporting identification information in your submission. If only an RT and semi-
1140 quantified amount are reported for an unknown extractable above the AET, then
1141 additional analytical or biological approaches may be necessary to address the
1142 impact of unknown extractables on biocompatibility endpoints of concern.

1143

1144 We recommend the LC-MS mass accuracy and mass resolution meet the
1145 following criteria to support identification:

- 1146
- 1147
- The mass accuracy of the parent ion and product ion should be < 10 ppm and < 20 ppm, respectively, and these values should be supported by the

⁹⁶ Milman BL. "Prior Data for Non-target Identification." in *Chemical Identification and its Quality Assurance*, Milman BL ed.; Springer Berlin Heidelberg: 2011, Chapter 6, pages 141-164.

⁹⁷ Sussman EM, Oktem B, Isayeva IS, Liu J, Wickramasekara S, Chandrasekar V, Nahan K, Shin HY, Zheng J. Chemical Characterization and Non-targeted Analysis of Medical Device Extracts: A Review of Current Approaches, Gaps, and Emerging Practices. *ACS Biomater Sci Eng.* 2022 Mar 14;8(3):939-963.

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- 1148 reference standards.⁹⁸ This is considered achievable on most quadrupole
1149 time-of-flight (qTOF), triple TOF, and Orbitrap instruments.⁹⁹
1150 • Database matches should meet the above mass accuracy criteria at
1151 minimum.
1152 • A minimum instrument mass resolution of 10,000 is recommended to
1153 achieve identification of co-eluting substances.¹⁰⁰
1154

1155 NOTE: Improved mass accuracy and mass resolution may be needed to select
1156 between multiple possible matches (e.g., if all the potential matches are not
1157 included in the TRA).
1158

1159 We recommend the following information be provided to describe the
1160 identification approach, as applicable:

- 1161 • Method for calculating match score(s) (e.g., algorithm, range of possible
1162 scores, score thresholds used to support selected identification(s))
1163 • Spectral reference library/software information, including:
1164 ○ Library/software name(s) (e.g., NIST, ChemSpider, MassBank)
1165 ○ Library/software type(s) (e.g., commercial, built in-house, publicly
1166 available)
1167 ○ Library/software version number(s) and date(s) of last update
1168 ○ Rationale for the spectral library's applicability to medical devices. As
1169 discussed in literature, a library based on an incomplete library is
1170 unable to provide any level of identification.¹⁰¹ To address this
1171 concern, for commonly used libraries, the following rationale may be
1172 sufficient: "The library contains [number of compounds] compounds
1173 across a wide variety of chemical classes relevant to medical devices."
1174 It should also include a description of the relevance of spectral library
1175 constituents specifically to the device under consideration. For
1176 example, "Expected extractables from the [device material] such as
1177 [extractables] and structural analogs or representative compounds are
1178 included in the library represented by [representative compounds
1179 included in the library] which supports the applicability of the selected
1180 spectral library to the medical device under consideration." This is
1181 particularly important when novel materials of construction and/or
1182 manufacturing and processing steps are involved. Alternatively, test
1183 labs can submit a Masterfile containing the details of the spectral
1184 library and reference that with their submissions.
1185 • Instrument mass accuracy and mass resolution for every mode of
1186 operation (e.g., MS, MS/MS)

⁹⁸ Gross ML. Accurate masses for structure confirmation. *J Am Soc Mass Spectrom.* 1994 Feb;5(2):57.

⁹⁹ Marshall AG, Hendrickson CL. High-resolution mass spectrometers. *Annu Rev Anal Chem.* 2008;1:579-99.

¹⁰⁰ Marshall AG, et al. *Annu Rev Anal Chem.* 2008;1:579-99.

¹⁰¹ Stein S. Mass spectral reference libraries: an ever-expanding resource for chemical identification. *Anal Chem.* 2012 Sep 4;84(17):7274-82.

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- How supporting information may be used for identification (e.g., molecular formula generation, RT matching)
 - Method(s) to determine that proposed identifications are plausible (i.e., to avoid incorrect identifications) and that identifications are not missed (i.e., all extractables above the reporting threshold have been quantified and reported)¹⁰²

1194 For individual identifications, other types of information may be used to support
1195 identification (e.g., to show that an identification is plausible/expected for the
1196 device, to distinguish between multiple candidate structures, or when a chemical
1197 is suspected to be a toxicological risk), including:¹⁰³

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- formulation and manufacturing information
 - literature and reference information (e.g., constituent is expected in a class of polymers when synthesized by a certain method)
 - other chemistry data (e.g., analytical chemistry data from the material supplier)
 - comparison to a reference material or comparator device
 - functional group(s) and/or other chemical/physical properties

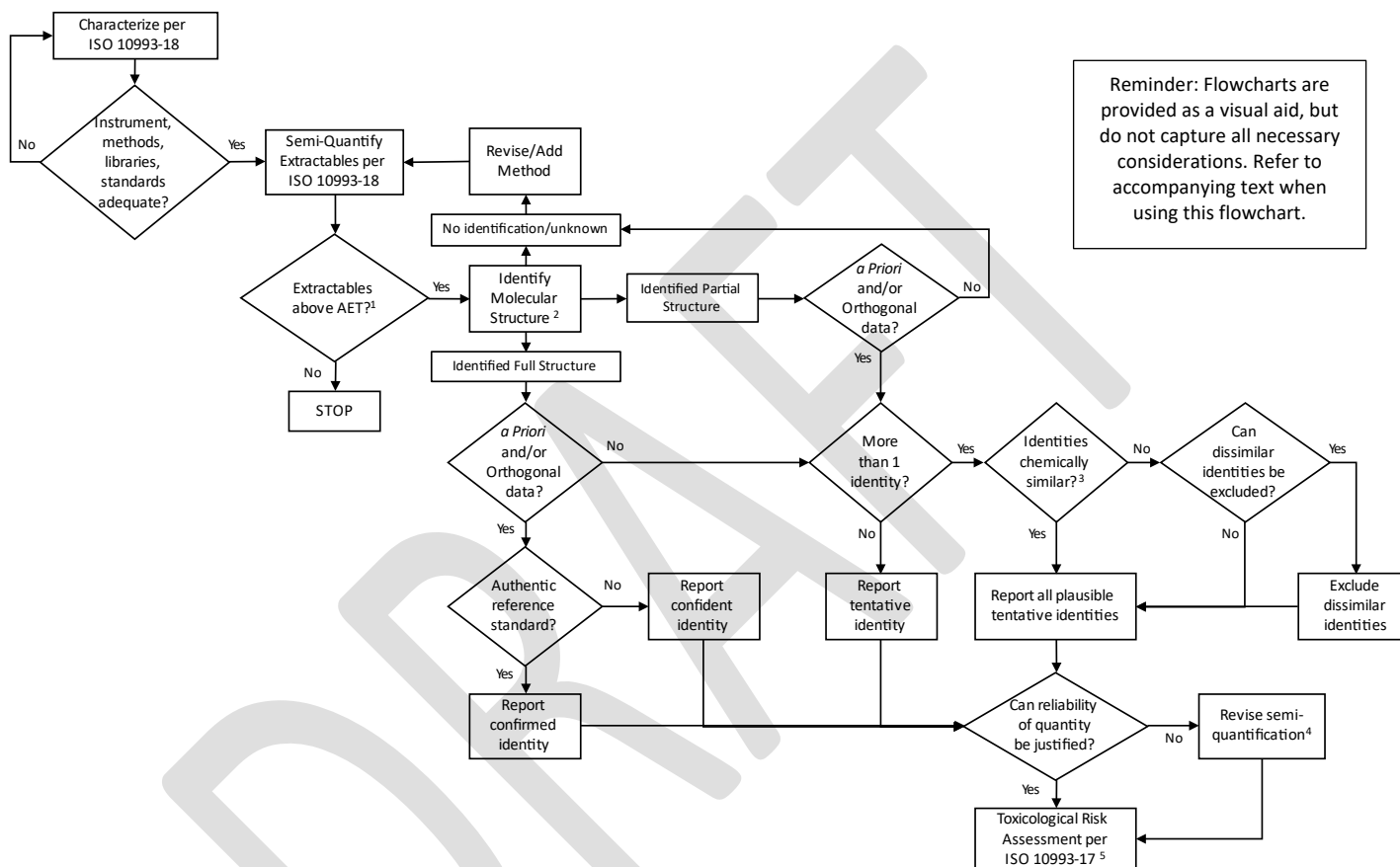
¹⁰² Sussman EM, *et al.* *ACS Biomater Sci Eng.* 2022 Mar 14;8(3):939-963.

¹⁰³ Milman BL. "Prior Data for Non-target Identification." in *Chemical Identification and its Quality Assurance*, Milman BL ed.; Springer Berlin Heidelberg: 2011, Chapter 6, pages 141-164.

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Figure 1. Diagram of process for chemical identification.

1208 ¹ When the AET is based on a TTC value, a justification that cohort of concern compounds are not present should be provided.

1209 ² See recommendations about chemical identification in this section ([Section VI.D](#)).

1210 ³ “Chemically similar” means similarity between two or more chemical structures as assessed by experts.

1211 ⁴ For example, by using an alternative surrogate reference standard.

1212 ⁵ If the TRA raises a toxicological concern, additional chemical analysis or information may be requested (e.g., targeted analysis, release kinetics study).

1213 **XI. Appendix D: Reporting Considerations**

1214 **A. Reporting Threshold/Analytical Evaluation Threshold** 1215 **Calculation**

1216 We recommend that the reporting threshold for the analytical methods be described and
1217 justified (e.g., by reference to a device-specific guidance).

1218
1219 If an AET is used to determine the reporting threshold,¹⁰⁴ we recommend calculating the
1220 AET using the following equation, which is based on the currently FDA-recognized
1221 version of ISO 10993-18. The only difference is that the equation below includes a factor
1222 (*D*) to account for extract processing (i.e., concentration or dilution), as noted in the
1223 consensus standard:

$$1224 \quad \quad \quad AET = DBT \times \frac{A}{BCD} \times \frac{1}{UF} \quad \quad \quad \text{Eq. 1}$$

1226
1227 In this equation, *A* is the number of devices or test articles that were used to generate the
1228 extract, *B* is the volume of the extract (in mL), *C* is the clinical exposure to the medical
1229 device (i.e., the number of devices a user would be exposed to in a day under expected
1230 clinical practice), DBT is the dose-based threshold (in µg/day), and UF is an uncertainty
1231 factor applied to account for the analytical uncertainty of the screening methods used to
1232 semi-quantify extractable concentrations. The parameter *D* is the dilution or
1233 concentration factor (i.e., $D = V_{\text{final}}/V_{\text{initial}}$). If the extract is diluted, $D > 1$. If the extract is
1234 concentrated, $D < 1$. If the extract is not processed, $D = 1$ and inclusion of the parameter
1235 *D* is optional for AET calculations. Thus, dilution of an extract results in a lower AET
1236 value and concentration of an extract results in a higher AET value in comparison to an
1237 unprocessed ($D = 1$) extract.

1238
1239 If an AET is used to determine the reporting threshold, we also recommend that the
1240 following be addressed:

- 1241 • Clearly describe the AET calculation, including the value used for each variable
1242 and the calculation result in units of concentration (i.e., mass/volume).
- 1243 • Justify the value used for *C* and the selected DBT based on the device intended
1244 use (e.g., Instructions For Use and duration of use including repeat or cumulative
1245 use). Typical DBTs are selected on the basis of an appropriate TTC, such as those
1246 described in ISO/TS 21726. For example, for a device that contacts the tissue for
1247 30 days or more, a DBT based on a 1.5 µg/day TTC would be conservatively
1248 protective.
- 1249 • Describe the approach used to calculate the UF for each analytical method (e.g.,
1250 GC-MS, LC-MS positive/negative modes) or extraction processing condition
1251 (e.g., dilution/concentration). An UF is calculated to account for variation

¹⁰⁴ Note that not all the examples in ISO 10993-18 Second Edition 2020-01, Annex E are recognized by FDA. See FDA's [Supplementary Information Sheet](#) for ISO 10993-18 Second Edition 2020-01.

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1252 observed in the RFs for each analytical method (e.g., UFs for GC-MS and LC-MS
1253 should be determined separately). We do not recommend determining a single UF
1254 across multiple detectors nor should the UF be based on the percentage of positive
1255 detections for a set of reference standards. A default UF value for GC-FID and
1256 GC-MS as low as 4 can be used without further justification.¹⁰⁵ A UF value for
1257 LC-MS analysis can be much higher than for GC-MS due to greater RF
1258 variability. RFs of analytes measured in LC-MS can span a relatively wider range
1259 (i.e., magnitudes greater than 1000-fold) compared to GC-MS analysis.¹⁰⁶
1260 Therefore, a default UF value for LC-MS has not been established, though
1261 methods describing how to calculate one are available. For example, the formula
1262 $UF = 1/[1-(RSD)]$ can be used, where RSD is the relative standard deviation of
1263 RRFs of an RF database representing a wide range of chemical properties.^{107, 108}

1264 **(1) Substances of Toxicological Concern**

1265 If applicable, a justification should be provided to indicate cohort of concern
1266 substances are not expected to be present and that a TTC can be applied without
1267 targeted evaluation for excluded compounds. See ISO/TS 21726 for additional
1268 information about the cohort of concern. An example of a rationale is one that
1269 addresses the material suppliers and manufacturing process.

1270
1271 When information gathering reveals that the presence of one or more toxic
1272 substances is possible (e.g., based on findings in the TRA), then appropriate
1273 studies (e.g., targeted analysis) may be needed to determine the quantity of such
1274 substances present in the device. For example, to quantify that manufacturing
1275 fixatives such as formaldehyde and glutaraldehyde are below acceptable levels,
1276 targeted analysis using derivatization can be performed.^{109, 110}

1277 **(2) Reporting Thresholds for Elemental Analysis**

1278 Elemental analysis (e.g., ICP-MS) is a targeted approach for the determination of
1279 elemental concentrations, so we recommend reporting the quantity of each
1280 analyzed element with a concentration above the LOQ for that element. The LOQ
1281 for each analyzed element should be low enough to quantify that element at or

¹⁰⁵ Jenke D, Odufu A. Utilization of internal standard response factors to estimate the concentration of organic compounds leached from pharmaceutical packaging systems and application of such estimated concentrations to safety assessment. *J Chromatogr Sci*. 2012 Mar;50(3):206-12.

¹⁰⁶ Jordi MA, et al. *J Pharm Biomed Anal*. 2018 Feb 20;150:368-376.

¹⁰⁷ Jenke D. A general strategy for the chemical aspects of the safety assessment of extractables and leachables in pharmaceutical drug products: the chemical assessment triad. *PDA J Pharm Sci Technol*. 2012 Mar-Apr;66(2):168-83.

¹⁰⁸ Jordi MA, et al. *J Pharm Biomed Anal*. 2018 Feb 20;150:368-376.

¹⁰⁹ Known knowns are analyzed by targeted analysis. See Little JL, Cleven CD, Brown SD. Identification of "known unknowns" utilizing accurate mass data and chemical abstracts service databases. *J Am Soc Mass Spectrom*. 2011 Feb;22(2):348-59. and Milman BL. General principles of identification by mass spectrometry. *TrAC, Trends Anal Chem*. 2015;69:24-33.

¹¹⁰ Collaborative trials have demonstrated low reliability in identification of spiked compounds analyzed using non-targeted approaches, e.g., Sobus JR, et al. *Anal Bioanal Chem*. 2019 Feb;411(4):835-851.

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1282 below the relevant chemical-specific toxicological threshold. A toxicological
1283 threshold given in units of $\mu\text{g}/\text{day}$ can be converted into concentration units to
1284 compare with the LOQ using a calculation analogous to the AET equation (Eq. 1).
1285 For example, $\text{threshold} [\mu\text{g}/\text{mL}] = \text{threshold} [\mu\text{g}/\text{day}] \times A/BCD$, where A, B, C,
1286 and D have the same meaning as Eq. 1 (UF = 1 because ICP-MS is a targeted
1287 approach).
1288

1289 **B. Method Justification**

1290 We recommend including a justification that includes data supporting how the approach
1291 is fit for the intended purpose of the study. Generally, this means the specific method has
1292 been qualified for identifying and quantifying analytes with a range of properties above
1293 the reporting threshold. The methods should be capable of identifying and quantifying
1294 analytes that are expected based on the materials of construction and manufacturing
1295 processes, as well as non-targeted analytes with a range of chemical properties.
1296 Justifications should address the following:

- 1297 • Extraction conditions should generate extracts that will not underestimate tissue
1298 exposure.
- 1299 • Analytical methods (chromatography, ionization, and detection methods) should
1300 be capable of identifying and quantifying all analytes above the reporting
1301 threshold.
- 1302 • System suitability and calibration demonstrate that the analytical methods are
1303 functioning as expected (i.e., the method has been set up and implemented
1304 properly, the method as set up is capable of performing at the same level it
1305 performed at during its qualification, and the method has performed acceptably
1306 throughout its use).
- 1307 • Quantification method is of sufficient sensitivity and avoids underestimation of
1308 analyte quantities.
- 1309 • Identification method results in identifications with justified confidence levels
1310 (e.g., by using quality assurance/quality control (QA/QC) approaches).¹¹¹
1311

1312 **C. Extraction Conditions and Results**

1313 We recommend providing the following information about the extraction conditions and
1314 results:

- 1315 • identity of extraction vehicles
- 1316 • number of replicate extractions per solvent
- 1317 • number of test articles (devices) used in each extraction
- 1318 • surface area or weight of each test article
- 1319 • volume of solvent, including confirmation that the test article is completely
1320 covered by solvent
- 1321 • extraction temperature

¹¹¹ Sussman EM, *et al.* *ACS Biomater Sci Eng.* 2022 Mar 14;8(3):939-963.

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- 1322
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- number and duration of extraction cycles
 - physical appearance of extract and test articles before and after extraction, including photographs
 - color changes
 - increases in turbidity
 - particulates
 - test article integrity changes/destruction

1330 **D. NVR Analysis**

1331 We recommend providing the following information about the NVR analysis:

- 1332
- 1333
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- 1341
- number of replicates
 - extraction cycle time and temperature
 - volume of extract used for analysis
 - time, temperature and pressure used to dry the samples, as the amount of non-volatiles can vary based on these parameters
 - method precision and sensitivity in units of mass/device, based on calibration with the vessel/crucible used for drying (i.e., not based on balance specifications alone)
 - NVR expressed in total mass for the sample (e.g., for extractions of multiple devices) and mass per device for each replicate for each extraction cycle

1342 **E. System Information**

1343 We recommend providing sufficient information to describe the analytical system

1344 operation, including instrument configurations and operating parameters, such as

- 1345
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- instrumentation manufacturer, model, and principal components
 - injection volume, split ratio (if applicable)
 - chromatography mobile phase, description of mobile phase gradient, flow rate, run time, and/or heating rate, as applicable
 - chromatography stationary phase manufacturer, type, and dimensions
 - detection method hardware, software, and principal software settings (e.g., MS peak picking algorithm settings)
 - ionization apparatus, principal modes and settings utilized

1354 **F. Calibration Data**

1355 We recommend providing sufficient data to demonstrate suitability of the calibration

1356 method across the range needed for quantification, such as the following:

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- calibration curve(s) in a graphical format
 - calibration equation and statistics describing the goodness of fit
 - optionally, extracted ion chromatograms that demonstrate integration of the calibration points

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G. Chromatographic Data

The following chromatograms should be provided, with sufficient labeling to discern RT and relative signal intensity:

- GC-MS—provide total ion chromatograms (TIC) for each control article and test extract that is analyzed^{112, 113}
- LC-MS—base peak chromatograms (BPC) and (optionally) TIC for each control article and test extract that is analyzed¹¹⁴
- Chromatograms for LC-UV and/or additional LC detection methods (when performed) should be provided with a matching RT axis to permit comparison to LC-MS data¹¹⁵
- Internal reference standards should be labeled for easy identification
- Optionally: Peaks above the AET are labeled to allow easy cross-reference to tabulated results¹¹⁶

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H. Extractable Identities and Amounts

For each solvent, identified and quantified/semi-quantified extractables above the AET should be tabulated, including:

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- RT¹¹⁷
- chemical name (e.g., International Union of Pure and Applied Chemistry (IUPAC) name)
- Chemical Abstracts Service Registry Number (CASRN), when available
- structural descriptor (e.g., international chemical identifier (InChI), simplified molecular-input line-entry system (SMILES)) or image of chemical/compound molecular structure, particularly if a CASRN is not available
- major ions observed (m/z)¹¹⁸
- type(s) of data used to establish analyte identity (e.g., library match, RT, manual spectral interpretation)
- identification confidence level (i.e., unknown, tentative, confident, or confirmed)
- amount in units of µg/device
- quantification method and reference standard
- extraction iteration (if not all extracts are pooled for analysis)

¹¹² Norwood DL, *et al. Pharm Res.* 2008 Apr;25(4):727-39.

¹¹³ Jenke D, *et al. PDA J Pharm Sci Technol.* 2013 Sep-Oct;67(5):448-511.

¹¹⁴ Nahan K, *et al. Talanta.* 2020 May 15;212:120464.

¹¹⁵ Jenke D, *et al. PDA J Pharm Sci Technol.* 2013 Sep-Oct;67(5):448-511.

¹¹⁶ Jenke D, *et al. J Chromatogr Sci.* 2012 Mar;50(3):206-12.

¹¹⁷ Jenke D, *et al. J Chromatogr Sci.* 2012 Mar;50(3):206-12.

¹¹⁸ We recommend reporting the major ions (m/z) to support the identity, see De Hoffmann E, Stroobant V. *Mass Spectrometry: Principles and Applications*, 3rd ed.; Wiley: UK, 2007.

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1394 NOTE: For tentative identifications, we recommend reporting all plausible candidate
1395 identifications.^{119, 120} For extractables reported as unknown, we recommend including
1396 supporting identification information in the data report.^{121, 122} If only an RT and semi-
1397 quantified amount are reported for an unknown extractable above the AET, then
1398 additional analytical or biological approaches may be necessary to address the impact of
1399 the unknown extractable(s) on biocompatibility endpoints of concern.
1400

1401 The following types of information could be submitted as supporting information related
1402 to substance identification on a case-by-case basis:

- 1403 • individual substance spectra (e.g., GC-MS electron ionization spectra and LC-MS
1404 “MS²” or “MS-MS” spectra) may be helpful in supporting identification(s)^{123, 124}
- 1405 • individual library spectra (to support identification using a library match)¹²⁵
1406

1407 For elemental analysis (e.g., ICP-MS), we recommend reporting a list of naturally
1408 occurring elements, indicating which elements were analyzed, and reporting the
1409 sensitivity and the amount detected for each element that was analyzed. Additionally, we
1410 recommend indicating which elements were used as reference standards and which
1411 elements they were used as surrogates for.
1412
1413

¹¹⁹ Milman BL. “Prior Data for Non-target Identification.” in *Chemical Identification and its Quality Assurance*, Milman BL ed.; Springer Berlin Heidelberg: 2011, Chapter 6, pages 141-164.

¹²⁰ Milman BL. “Non-target Identification. Chromatography and Spectrometry.” in *Chemical Identification and its Quality Assurance*, Milman BL ed.; Springer Berlin Heidelberg: 2011, Chapter 7, pages 165-234.

¹²¹ Milman BL. “Prior Data for Non-target Identification.” in *Chemical Identification and its Quality Assurance*, Milman BL ed.; Springer Berlin Heidelberg: 2011, Chapter 6, pages 141-164.

¹²² Sussman EM, et al. *ACS Biomater Sci Eng*. 2022 Mar 14;8(3):939-963.

¹²³ Jenke D. Identification and Quantitation Classifications for Extractables and Leachables. *PDA J Pharm Sci Technol*. 2020 Mar-Apr;74(2):275-285.

¹²⁴ Zhang Y, Sun S, Xing X, Du Z, Guo Q, Yu W. Detection and Identification of Leachables in Vaccine from Plastic Packaging Materials Using UPLC-QTOF MS with Self-Built Polymer Additives Library. *Anal Chem*. 2016 Jul 5;88(13):6749-57.

¹²⁵ Stein S. Mass spectral reference libraries: an ever-expanding resource for chemical identification. *Anal Chem*. 2012 Sep 4;84(17):7274-82.