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Chemical Analysis for Biocompatibility Assessment of Medical Devices

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Draft Guidance for Industry and Food and Drug Administration Staff

DRAFT GUIDANCE

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Document issued on September 20, 2024

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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Draft Guidance for Industry and Food and Drug Administration Staff

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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FDA is issuing this draft guidance to describe recommended methodological approaches for chemical analysis for biocompatibility assessment of medical devices.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

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

II. Background

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

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

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

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[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.

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

III. Scope

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

1. Screening for unspecified extractables (i.e., non-targeted analysis) or testing for specified extractables (i.e., targeted analysis) to evaluate certain biocompatibility endpoints (i.e., acute, subacute, subchronic, and chronic systemic toxicity, genotoxicity, carcinogenicity, and reproductive/developmental toxicity) in conjunction with TRA.

2. Chemical equivalency comparison to a device with previously demonstrated biocompatibility as part of a biological equivalency evaluation.

The methods in this guidance are intended to be generally applicable for chemical characterization of devices. However, for some types of devices (e.g., ophthalmic, respiratory, hemodialyzers) different methods may be needed due to the materials used in the device or based on historically established approaches. If there are device-specific FDA guidances or FDA-recognized consensus standards that address chemical characterization for a particular device type (e.g., ISO 11979-5[4], ISO 15798[5], and ISO 16672[6] for ophthalmic implants, device specific guidance on contact lenses,[7] ISO 18562-3[8] and ISO 18562-4[9] for gas pathway devices, and ISO 7405[10] for dental materials), those recommendations and methods should be followed.

Some types of devices commonly raise additional considerations when performing extractables 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.*

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

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

IV. Information Gathering[12]

A. Device Components and Materials

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

In certain cases (e.g., products made from animal-derived tissues[13] or when reporting a change for a clinical study or marketing application), additional information describing the manufacturing process is often needed, such as the manufacturing steps and manufacturing materials. For example, this information may help demonstrate that manufacturing materials are removed or limited to an amount that does not adversely affect the biocompatibility of the device, or to support a justification that a device change is unlikely to adversely impact the biocompatibility of the device.

When performing chemical equivalence studies, a description of the changes to the device (e.g., materials of construction, manufacturing methods, device geometry) that could affect the equivalence determination should be provided. For example, for changes in device material, formulation, or material supplier/vendor, a list of materials of construction (e.g., base polymer, plasticizers, stabilizers, surfactants, color additive, adhesives) relevant to the change for the previous and proposed material should be provided. Likewise, for changes in device manufacturing, a list of manufacturing 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)"

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

B. Test Articles, Control Articles, and Blanks

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

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

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

C. Test Article Processing Prior to Extraction

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

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

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

- 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.

V. Test Article Extraction

A. Extraction Conditions

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

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

[a] We recommend that exaggerated conditions exceed both time and temperature of clinical use.[16]

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

[c] See ISO 10993-18 Table 2 for examples where exhaustive extraction would not typically be warranted.

[16] See also ISO 10993-18.

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

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

B. Number of Extraction Replicates

For each solvent, we recommend that extractions be performed in triplicate[17] and the analyses be conducted on each separate extraction, unless otherwise justified. For example, it may be acceptable to conduct a single replicate for particular device types if this is a historical practice or if a different number of replicates is recommended in device-specific FDA guidance(s) or FDA-recognized consensus standards.

Triplicates can be particularly important to:

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

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

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

C. Extraction Volume

[17] See also ISO 10993-18.
[18] See also ISO 10993-18.

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

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

D. Extraction Temperature and Time

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

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

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

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

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

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

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

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

E. Particulates

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

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

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

[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.

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

F. Additional Considerations for Exhaustive Extraction

(1) Determining the Endpoint of Exhaustive Extraction

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

If NVR analysis is used to demonstrate exhaustive extraction has been achieved, we recommend consideration of the information provided in Appendix B, Section IX.B.

(2) Combining/Pooling Extracts

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

VI. Chemical Analysis

Justification and explanation of the chemical analysis plan should be provided. In general, we recommend profiling of extractables through a non-targeted analysis with subsequent use of targeted analysis to identify and quantify appropriate extractables, as necessary. Further considerations are provided in Appendix C.

A. Suitability of Detection Methods

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

- 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]

B. Reference Standards

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

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[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, Dufaÿ 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.

C. Calibration, Sensitivity, and Quantification

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

D. Chemical Identification

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

VII. Data Reporting

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

VIII. Appendix A: Information Gathering Steps, Further Considerations

A. Information for Device Components and Materials

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

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

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

- name of device components
- duration and type of tissue contact (e.g., direct, indirect, none) of each component
- materials comprising each component
- material-specific information
  - chemical name
  - trade name (if relevant)
  - supplier
  - component contribution to the total device by surface area (cm^2) or, if surface area is not relevant, the amounts (e.g., by mass for textiles)
  - material standards
  - technical data sheets (e.g., from a supplier of a component or material) with information on surface finish and manufacturing processes, if available
  - other material-specific information, such as formulation information including known impurities, safety data sheets, and certificates of analysis

B. Information for Test Articles, Controls and Blanks

We recommend the following information be provided:

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

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

A. Extraction Solvents

(1) Considerations Regarding Alcohol/Water Mixtures

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

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

(2) Other Considerations

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

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

Solvents with high boiling points (e.g., dimethyl sulfoxide (DMSO)) may confound subsequent analytical steps, such as sample concentration (see Appendix C, Section X.A.(2)) due to loss of analytes during the concentration 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.

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

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

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

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

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

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

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

(4) Extraction Media for Elemental Analysis

Elemental analysis is commonly performed on polar extracts (e.g., deionized water). However, it also can be performed using saline or an acidic solution (e.g., diluted hydrochloric acid (HCl) or nitric acid (HNO_3) solutions), if justified.[33, 34, 35] For example, when certain elemental analytes are expected based on the device materials or manufacturing processes, there may be a preferred extraction media to maximize analyte release. Likewise, if corrosion of metallic device components is a concern, then testing conditions specific to the metal should be considered. For example, phosphate-buffered saline (PBS) has been recommended for studies on nitinol, where corrosion and release of nickel from the bulk is a concern.[36]

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

B. Considerations for Determining the Endpoint of Exhaustive Extraction

If NVR analysis is used to demonstrate that exhaustion has been achieved, we recommend the following:

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[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, Wünsch 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.

- 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.

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

We also recommend the following additional points when determining the exhaustive endpoint:

- 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

differences in extraction conditions may alter equilibria due to changes in the partitioning of analytes.[41, 42]

[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.

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

A. Extract Processing

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

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

(1) Solvent Exchange

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

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

- 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]

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

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]

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

- 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).

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:

- 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

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.

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

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

We recommend the following alternative approaches be considered:

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

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

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

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

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

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

We recommend the following alternative approaches be considered:

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

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

Issue: Laboratory has not qualified a method for analysis of a solvent that is proposed.[66]

We recommend the following alternative approach be considered:

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

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

- Perform solvent exchange.
- Dilute the extract in an analytically expedient solvent.

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.

(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

[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).

(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.

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

(3) Primary Detection Methods

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

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

(4) Secondary Detection Methods

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

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

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

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

(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.

C. Reference Standard Selection

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

We recommend the method-specific[73] surrogate reference standards chosen be described and justified.

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

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

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

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

The following can be considered in developing a justification for the use of reference standards (either surrogate reference standards or authentic reference standards) for ICP 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.

- 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]

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.

D. Calibration, Sensitivity, and Quantification

(6) Calibration

We recommend a description of the calibration method be provided in your test report, which includes:

- 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.

(7) Sensitivity

We recommend a rationale be provided to support that the sensitivity for each reference standard is adequate for the study. For example, if LOQs[79] are used for sensitivity, the LOQ for each reference standard should be lower than the reporting threshold (e.g., AET).

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.

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]

(8) Semi-Quantification Method

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:

- nearest RT [81]
- 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.

E. Chemical Identification

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).

- Analytical instrumentation, methods, libraries and standards should be adequate to identify and semi-quantify extractables, and generate chemistry data for TRA.
- 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.
- Additional structural elucidation of extractables (e.g., for TRA) based on identification level and supporting information (e.g., orthogonal data).[85, 86]
- 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]
- 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.

We recommend the following guidelines for the identification levels (i.e., unknown, tentative, confident, confirmed)[90,91, 92] for compounds above the reporting threshold (e.g., AET).

We recommend that the following extractables be reported as Unknown:

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

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

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

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

- A library match or expert interpretation of mass spectral data;
  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, Valkenborg D, Lemière 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.

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

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

- 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.

Supporting information[96, 97] for identification can include one or more of the following:

- 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)
- MS^n elucidation of fragments

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

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

- 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.

reference standards.[98] This is considered achievable on most quadripole time-of-flight (qTOF), triple TOF, and Orbitrap instruments.[99]

- Database matches should meet the above mass accuracy criteria at minimum.
- A minimum instrument mass resolution of 10,000 is recommended to achieve identification of co-eluting substances.[100]

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

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

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

- 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]

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

- 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.

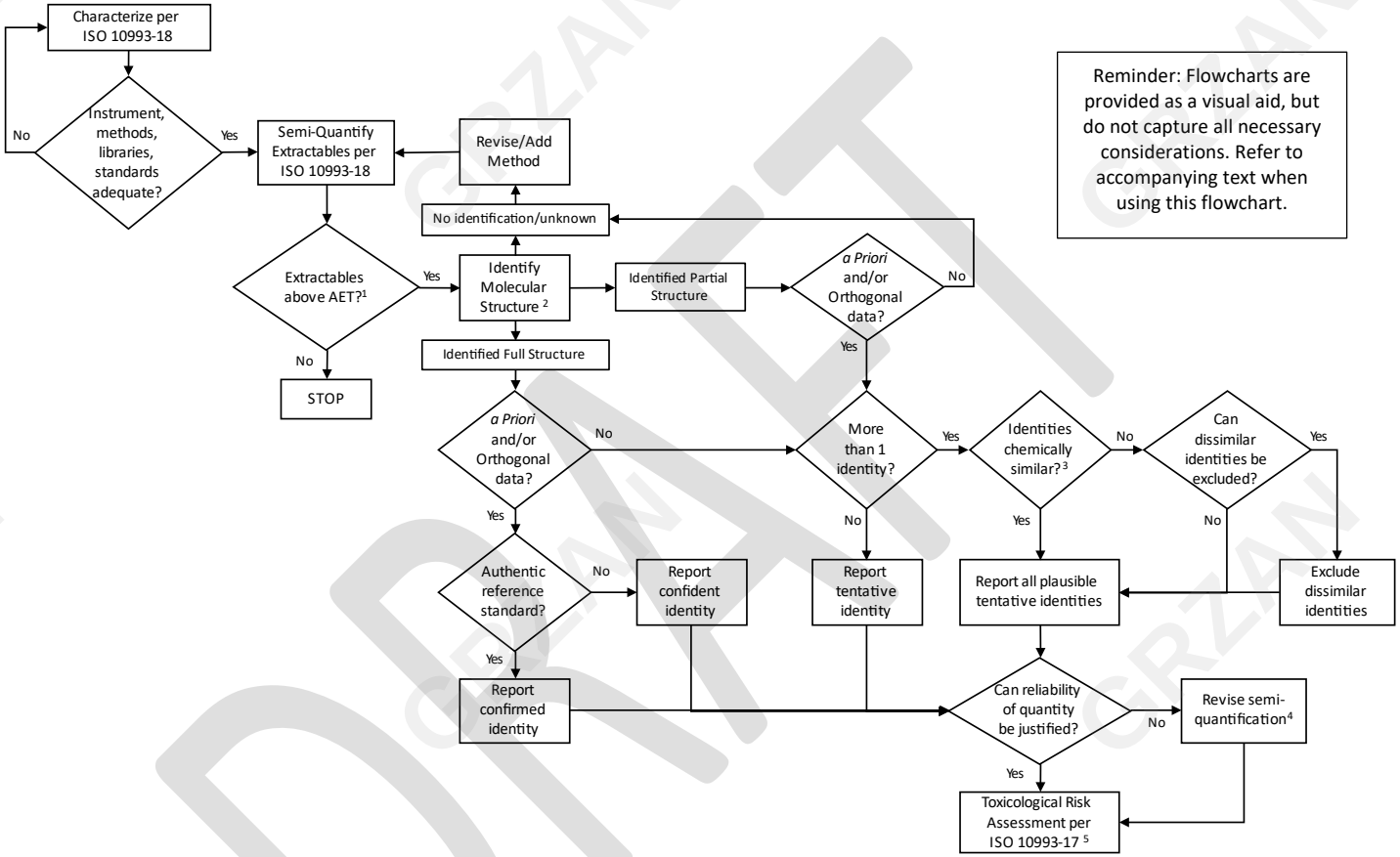


Figure 1. Diagram of process for chemical identification.

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

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

[3] "Chemically similar" means similarity between two or more chemical structures as assessed by experts.

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

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

XI. Appendix D: Reporting Considerations

A. Reporting Threshold/Analytical Evaluation Threshold Calculation

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

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

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

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

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

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

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

(1) Substances of Toxicological Concern

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

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

(2) Reporting Thresholds for Elemental Analysis

Elemental analysis (e.g., ICP-MS) is a targeted approach for the determination of elemental concentrations, so we recommend reporting the quantity of each analyzed element with a concentration above the LOQ for that element. The LOQ 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.

below the relevant chemical-specific toxicological threshold. A toxicological threshold given in units of µg/day can be converted into concentration units to compare with the LOQ using a calculation analogous to the AET equation (Eq. 1). For example, threshold [µg/mL] = threshold [µg/day] × A/BCD, where A, B, C, and D have the same meaning as Eq. 1 (UF = 1 because ICP-MS is a targeted approach).

B. Method Justification

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

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

C. Extraction Conditions and Results

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

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

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

- 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

D. NVR Analysis

We recommend providing the following information about the NVR analysis:

- 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

E. System Information

We recommend providing sufficient information to describe the analytical system operation, including instrument configurations and operating parameters, such as

- 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

F. Calibration Data

We recommend providing sufficient data to demonstrate suitability of the calibration method across the range needed for quantification, such as the following:

- 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

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 analyzed[114]
- 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 data[115]
- Internal reference standards should be labeled for easy identification
- Optionally: Peaks above the AET are labeled to allow easy cross-reference to tabulated results[116]

H. Extractable Identities and Amounts

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

- RT[117]
- 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.

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

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

- individual substance spectra (e.g., GC-MS electron ionization spectra and LC-MS "MS^2" or "MS-MS" spectra) may be helpful in supporting identification(s)[123, 124]
- individual library spectra (to support identification using a library match)[125]

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

[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.