User Manual: Extractables Screening Protocols for Fused Filament Fabricated ABS Containing Additive-manufactured Devices

Tool Reference

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Extractables Screening Protocols for Fused Filament Fabricated ABS Containing Additive-manufactured Devices

A: Protocol for Sample Extraction

1. Label the empty vials (borosilicate) with the date of extraction, solvent & replicate number, and material information.
2. Cut[1] the medical device or medical device materials into representative samples
    a. Do not prerinse or preclean materials prior to use
    b. Cut the materials into representative samples with scissors and/or a cold chisel (1/2", 12 mm)
    c. Clean scissors and/or chisel prior to use and between each material using water, acetone, and hexane (repeat 3 times) then dry with a Kimwipe
3. Weigh 200 mg of representative sample with an analytical balance capable of 0.1 mg measurement and then transfer to a clean, inert vials.
    a. Do not preclean vials or reuse vials
4. Prepare each representative sample in triplicate (e.g., 3 vials without material -blank, 3 vials with material-sample)
5. Add 1.0 mL of extraction solvent (e.g., LC/MS grade solvent) to each sample and blank vial using a calibrated pipette. Repeat for all 3 solvents (e.g., isopropanol, hexane, and water)[2].
6. Seal the vials using the respective caps with an inert liner (e.g., PTFE). Record the pre-extract observation.
7. Place the sealed vials in a vial tray in the incubator shaker, close the cover and set the time and temperature to 24 hrs. and 50 °C, respectively. Agitate the vials at 60 rpm.
8. After 24 hrs., remove vials and allow to sit in the fridge for 1 hr. prior to transferring of the extract into a separate vial.
9. Check the sample vials for any swelling of the materials[3]/ precipitations[4]/ particulate formation[5].
10. Remove the medical device material from the vials using clean tweezers and rinse the tweezers between each sample (Step 1c).
    a. Transfer the required amount of extract volume into LC/MS or GC/MS vials for analysis.
    b. Analyze the extracts within 24 hrs. Keep the extracts refrigerated until ready to use.

[1] Cut the samples only, if necessary, based on extraction logistics or provided sample dimensions. Analyzing the complete device without manipulation is preferred.
[2] Follow the ISO 10993-12 recommendation to get the sample to extraction volume ratio.
[3] Nondestructive swelling is acceptable if the extraction solvent is recoverable for the analysis.
[4] If a precipitate is formed, a re-dissolution is recommended prior to sample analysis per ISO-10993-18 (2020) guidelines.
[5] Particulates should be removed prior to extract analysis. Care should be taken to ensure that the particulate removal method will not alter the extractables profile of the device.

B: Protocol for Chemical Analysis

Protocol for volatile and semi volatile extractable analysis by GC/MS

1. Evaluate the GC/MS performance with vendor recommended standard operating procedures (tune evaluation with GC/MS tuning standard - perfluorotributylamine (PFTBA).
2. Prepare reference standard (as listed in table 1) calibration samples in isopropyl alcohol with the concentration range between 100 to 5000 μgL^{-1}. Inject standard samples and determine the linear dynamic range for the samples. GC/MS parameters are listed on Table 1.
3. Bring the refrigerated extracts to room temperature (~30 min). Transfer 100 µL from each sample and blank extract to GC/MS vials and close the caps.
4. Pre inject few of the extracts to the GC/MS system to check if samples need to be diluted. A 10-50X dilutions may be required to bring the concentrations within the dynamic range.
5. Inject blank samples in between each extract sample runs.

Table 1: GC-MS instrument parameters

Parameters:	Set values
Instrument	Agilent 7890B GC-5977B MS
Column	Agilent DB-5MSUI 30m x 0.25um x 0.25um
Inlet Conditions	200 °C, split (5:1), purge flow 3.0 mL/min, on at 2 min
Inlet Liner	Split Liner
Helium (Typically ≥99.9995%) Flow Rate	1.2 mL/min
Oven Conditions	Initial temperature of 50 °C for 3 minutes then ramped to 315 °C at a rate of 12 °C/min and held at 315 for 15 minutes
Injection Volume	1 µL or 0.5 µL (water)
Transfer Line Temperature	250 °C
Ionization Mode	EI
Mass Range	m/z 50.0 to 1050
Ion Source Temperature	250 °C
MS Quad temperature	150 °C
Reference standards used for semi quantification	bis(2-Ethylhexyl) phthalate (DEHP) Dimethyl phthalate Dibutyl sebacate Diphenyl phthalate
Calibration range	100 to 5000 μgL^{-1}

GC/MS data analysis

- Use Agilent Unknown Analysis software for the identification and semi-quantification of the compounds detected in GC/MS analysis of the sample extracts. Details on how to use the Unknown analysis software can be found on their website.
- Use NIST 1A v17 Mass Spectral Library (National Institute of Standards and Technology, Gaithersburg, MD) for the identification.

- Match factor of >80% can be used for the tentative identification. Expert judgment must be used in selecting the best match for the detected compounds.
- Only report the presence of a compound if it is identified in at least two of the 3 extract sample replicates.
- External calibration curves of the selected reference standards can be used for semi quantification. Select "average RF of the closest standard" for the semi-quantification of the identified compounds. (semi-quantitation will be performed using the calibration curve of the standard closest to the analyte. Other options can be selected based on the user preference and the experimental logistics).
- Use blank subtraction option to remove the background interferences from the quantifiable values.
- After all the samples have been processed, export the results as .CSV file for further evaluation/report generation.

Protocol for semi volatile and non-volatile extractable analysis by LC/MS

1. Tune and calibrate the LC/MS with vendor recommended standard operating procedures.
2. Prepare reference standard calibration samples (diethyl phthalate, stearic acid, and Irganox 1010) in isopropyl alcohol with the concentration range between 100 to 10000 μgL^{-1}. Inject standard samples and determine the linear dynamic range for the samples. LC/MS parameters are listed on Table 2.
3. Bring the extracts to room temperature. Transfer 100 µL from each sample and blank extract to LC/MS vials and close the caps.
4. Pre inject a few of the extracts to the LC/MS system to check if samples need to be diluted. Dilute the samples with 1:1 hexane: Isopropyl alcohol mixture to appropriate concentration. 10-25X dilutions may be required to bring the concentrations within the dynamic range.
5. Inject blank samples in between each extract sample runs.

Table 2: LC-MS instrument parameters

Parameters:	**Set values**
Instrument	Agilent 6540 B QTOF with Agilent 1260 Nano LC with diode array detector
LC parameters	
Column	120 Poroshell Stable Bond C18 (3*100 2.7 µm)
Column Temperature	35 °C
Injection Volume	10.0 µL
Flow Rate	0.8 mL/min
Mobile Phase A	0.1% Formic Acid in H_2O (positive): 10mM Ammonium Acetate in H_2O (negative)
Mobile Phase B	0.1% Formic Acid in Acetonitrile (positive): Acetonitrile (negative)

Mobile Phase Gradient	**Time (minutes)**	**%A**	**%B**
	0.00	80.0	20.0
	4.70	0	100.0
	18.30	0	100.0
	19.00	80.0	20.0
	30.00	80.0	20.0
Stop Time	30 minutes		
MS parameters			
Ionization Mode	ESI		
Polarity	Positive and Negative Ion		
Mass Range	m/z 100-1700		
Dual AJS ESI			
Gas Temp (°C)	300		
Drying Gas(L /min)	8.0		
Nebulizer(psig)	50		
Sheath Gas Temp (°C)	400		
Sheath Gas Flow (L/min)	12		
VCap (V)	3500		
Nozzle Voltage (V)	1000		
Capillary(µA)	0.125		
Chamber(µA)	18.35		
MS TOF			
Fragmentor (V)	140		
Skimmer (V)	65		
Oct 1RF Vpp(V)	750		

LC/MS data analysis

- Use Agilent MassHunter Qualitative analysis software coupled with the Agilent Extractables and Leachables (E&L) Personal Compound Database and Library (PCDL) for the identification of the compounds detected in LC/MS analysis of the sample extracts. Details on how to use the Agilent MassHunter Qualitative Analysis software can be found on their website.
- Setup mass accuracy for <10 ppm for the identification. Match factor of >80% can be used for the tentative identification. Expert judgment must be used in selecting the best match for the detected compounds.
- Only report the presence of a compound if it is identified in at least two of the 3 extract sample replicates.
- Use a five-point calibration curve per reference standard to perform semi- quantification.
- Use blank subtraction option to remove the background interferences from the quantifiable values.