Appendix A
Volatile Extraction and Analysis by DHS-GC/MS

Tool Reference

RST Reference Number: RST24MC04.01

Date of Publication: 10/17/2025

Recommended Citation: U.S. Food and Drug Administration. (2025). *Dynamic Headspace Gas Chromatography Mass Spectrometry (DHS-GC/MS) Method for Sensitive Detection of Volatiles in Saline Extracts* (RST24MC04.01). https://cdrh-rst.fda.gov/dynamic-headspace-gas-chromatography-mass-spectrometry-dhs-gcms-method-sensitive-detection

For more information

Catalog of Regulatory Science Tools to Help Assess New Medical Devices

Disclaimer

About the Catalog of Regulatory Science Tools

The enclosed tool is part of the Catalog of Regulatory Science Tools, which provides a peer-reviewed resource for stakeholders to use where standards and qualified Medical Device Development Tools (MDDTs) do not yet exist. These tools do not replace FDA-recognized standards or MDDTs. This catalog collates a variety of regulatory science tools that the FDA's Center for Devices and Radiological Health's (CDRH) Office of Science and Engineering Labs (OSEL) developed. These tools use the most innovative science to support medical device development and patient access to safe and effective medical devices. If you are considering using a tool from this catalog in your marketing submissions, note that these tools have not been qualified as Medical Device Development Tools and the FDA has not evaluated the suitability of these tools within any specific context of use. You may request feedback or meetings for medical device submissions as part of the Q-Submission Program.

For more information about the Catalog of Regulatory Science Tools,
email RST_CDRH@fda.hhs.gov.

Appendix A: Volatile extraction and analysis by DHS-GC/MS

1. **Sample preparation**
   a. ***Standard sample mixtures***
      i. Prepare 0.9% saline (0.15 M) sample by diluting the saline stock (5 M) with LC/MS grade water in a clean and rinsed volumetric flask (2 L).
      ii. Prepare the volatile standard mix by diluting the stock standard with the 0.9% saline in cleaned volumetric flasks.
      iii. Conduct serial dilution of the volatile standard mix to generate the appropriate calibration levels using stock standard mix and saline For example prepare stock 1 (2000 ng mL-1) from 8260B MegaMix Calibration Mix (from Restek, Bellefonte, PA) by diluting 0.01 mL of original standard to 10 mL with 0.9% NaCl or saline Then, prepare stock 2 (200 ng mL-1, 50 mL) by diluting 5 mL of stock 1 with 0.9% saline to yield 10X diluted solution. From stock 2, prepare calibration series by transferring 0.01, 0.1, 0.5, 1.0, 2.5, 5.0, 10, and 20 mL to volumetric flasks (50 mL) and diluting those separately with saline to generate 0.04, 0.4, 2, 4, 10, 20, 40, and 80 ng mL-1 concentrated solutions, respectively.).
      iv. Transfer 5 mL of each concentration of calibrant to a labelled clean headspace sample vial and close it with the screw caps using a rubber septum.
   b. ***Medical device materials***
      i. Label the empty vials (borosilicate) with the date of extraction, solvent & replicate number, and material information (indicate how many replicates were used for your study).
      ii. Cut the medical device or medical device materials into representative samples. (Cut the samples only, if necessary, based on extraction logistics or provided sample dimensions. Analyzing the complete device without manipulation is preferred).
      iii. Do not prerinse or preclean materials prior to use.
      iv. Cut the materials into representative samples with scissors and/or a cold chisel (1/2”, 12 mm).
      v. 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.
      vi. Weigh 3 g of representative sample with an analytical balance capable of 0.1 mg resolution or better and then transfer to clean, inert vials. Do not preclean vials or reuse vials.
      vii. Prepare each representative sample in triplicate (e.g., 3 vials without material -blank, and 3 vials with material-sample)
      viii. Add 15.0 mL of 0.9% saline (0.15 M) to each sample and blank vial using a calibrated pipette.
      ix. Seal the vials using the respective caps with an inert liner (e.g., PTFE). Record the pre-extract observation.
      x. 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 150 rpm.

   xi. After 24 hrs, remove vials and allow to sit in the room temperature for 1 hr prior to transferring 5 mL of the extract into a separate headspace vial.

2. **Sample analysis by dynamic headspace gas chomarography mass spectrometry (DHS-GC-MS)**
   i. Optimize the manufacturer recommended parameters for the system you will be using to conduct DHS GC/MS analysis. Following table summarizes the optimized contions for the the GERSTEL DHS system consisting a multipurpose sampler (MPS) with a robotic arm and TDU coupled to a cooled injection system (CIS). These system components are controlled by GERSTEL MAESTRO software incorporated with Agilent MassHunter platform (system model number will be helpful here).

DHS Steps	Parameters	Optimized condition
DHS incubation	Incubation temperature (°C)	37
	Incubation time (min)	10
Agitation	Agitator on time (s)	60
	Agitator off time (s)	1
	Agitator speed (rpm)	500
Transfer heater	Transfer heater temperature (°C)	75
Purge phase	Purge phase volume (mL)	0.0
Trapping phase	Adsorbent material	Carbopack B/ Carbopack X
	Trapping temperature (°C)	30
	Trapping volume (mL)	750
	Trapping Flow (mL min^{-1})	50
Drying phase	Drying volume (mL)	750
	Drying flow (mL min^{-1})	50
Thermal desorption unit (TDU)	Temperature program	40 °C (initial), 0.4 min (delay), 0.0 min (initial time), 325 °C (720.0 °C min^{-1}), 5 min (hold)
	Transfer temperature (°C)	280
	Transfer temperature mode	Fixed
	Desorption mode	Splitless
	Sample mode	Remove Tube – no Standby Cooling
	Standby Temp (°C)	40
Cooled injection system (CIS)	Cryo cooling	Applied (Liquid N_2 at 22 psi)
	Heater mode	standard
	Liner Type	glass wool
	Temperature program	-120 °C (initial), 0.20 min (equilibration time) 0.00 min (initial time), 275 °C (12 °C/s), hold time (3.00 min)
	Low split	not used
System settings	Maestro runtime	30.00 min
	GC cool down time	6.00 min
	Cryo timeout	15.00 min
At Programmable Temperature Vaporizer (PTV) inlet	Split ratio	20
	Mode	Solvent vent
	Pressure	21.992 psi
	Total flow	31.747 mL min^{-1}
	Septum purge flow	3 mL min^{-1}
	Purge flow to split vent	27.378 mL min^{-1} at 0.01 min
	Vent flow	50 mL min^{-1}
	Vent pressure	16.7 psi until 0 min

ii. Evaluate the GC/MS performance with vendor recommended standard operating procedures (tune evaluation with GC/MS tuning standard - perfluorotributylamine (PFTBA).
iii. Prepare reference standard calibration samples in saline with the concentration range between 0.04 to 80 ngmL^{-1}. Inject standard samples and determine the linear dynamic range for the samples. Recommended GC/MS parameters are listed in Table 2.

Instrument	Agilent 7890B GC-5977B MS
Column	Agilent DB-5MSUI 30m x 0.25um x 0.25um DB-624 column (60 m × 250 µm × 1.4 µm film thickness) with a stationary phase of 6% cyanopropyl-phenyl and 94% dimethyl polysiloxane was used (Agilent J & W, part no. USN486321H for RSCL3-mixA and material/device extracts analysis and US2453332H 8260B MegaMix Calibration Mix) was used.
Helium (Typically ≥99.9995%) Flow Rate	1.3689 mL min^{-1}
Oven Conditions	Initial temperature of 40 °C for 2 minutes then ramped to 240 °C at a rate of 8 °C/min and held at 240 for 3 minutes for all the experiments except for the 8260B MegaMix analysis. Due to the complex nature of the MegaMix, a separately optimized oven temperature program started at 40 °C (3 min), ramped to 80 °C at a rate of 2 °C min^{-1}, increased to final temperature of 240 °C at a rate of 4 °C min^{-1} and held at 240 °C (2 min) was used. The total GC run for the MegaMix was 65 min.
MSD Transfer Line Temperature	250 °C
Ionization Mode	EI
Mass Range	m/z 35.0 to 500
Ion Source Temperature	230 °C
MS Quad temperature	150 °C

iv. After extracts are at room temperature, transfer 5 mL from each sample and blank extract to headspace vials and close the caps.
v. Include blank samples between each extract sample run.

Note: Detailed protocols for all sample analyses and processing were generated using GC-MS instruments and associated software packages. The developed methods are readily transferable to other analytical systems with equivalent capabilities." Wijeweera Patabandige, M., Hill, J., Herath, A. *et al.* Dynamic Headspace GC–MS Method to Detect Volatile Extractables from Medical Device Materials. *Biomedical Materials & Devices* **2**, 1125–1142 (2024). https://doi.org/10.1007/s44174-023-00145-1.

3. GC/MS data analysis

a. Identification

i. Use Agilent Unknown Analysis software for the identification of the compounds detected in GC/MS analysis of the sample extracts and standard mixtures. Details on how to use the Unknown analysis software can be found on the following web link
https://www.agilent.com/cs/library/usermanuals/public/G3335-90187_Unknowns_Analysis_Familiarization-en.pdf
ii. Use NIST 2017 Mass Spectral Library (National Institute of Standards and Technology, Gaithersburg, MD) for the identification.
iii. 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.

iv. Only report the presence of a compound if it is identified in at least two of the 3 extract sample replicates.
Report the identified peaks when the generated extracted ion chromatograms' (EICs') average peak area response of replicate samples is ≥1000 ion counts.

b. Quantification/Semi-Quantification

Use Agilent MassHunter Quantitative analysis software coupled with the NIST 2017 spectral library for the quantification/semi quantification of the compounds detected in GC/MS analysis of the sample extracts. Details on how to use the Agilent MassHunter Quantitative Analysis software can be found on the following web link

https://www.agilent.com/cs/library/usermanuals/Public/G3335_90061_Quant_Familiarization-EN.pdf

i. Only report the presence of a compound if it is identified in at least two of the 3 extract sample replicates.

ii. External calibration curves of the selected reference standards are used for semi quantification. Select "average response factor (RF) of the closest standard" for the semi-quantification of the identified compounds. (Other options can be selected based on the user preference and the experimental logistics). Use a five-point calibration curve per reference standard to perform semi- quantification.

iii. Before calibration curves are generated, subtract the peak areas for the blank samples from the identified compounds/standards manually for background correction.

iv. After all samples have been processed, export the results as .CSV file for further evaluation/report generation.