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# Standardized SPME-GC-MS Protocol for the Detection of Volatile and Semi-Volatile Compounds in Human Serum

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**Protocol status:** Working
**We use this protocol and it's working**

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## Abstract

This protocol describes the standardized method for the extraction and analysis of volatile and semi-volatile compounds in human blood serum using Solid Phase Microextraction (SPME) with direct fiber immersion, followed by Gas Chromatography-Mass Spectrometry (GC-MS). The method is optimized for use with a DVB/CAR/PDMS fiber and a GC-MS system equipped with an HP-5 column. It enables reproducible, non-targeted metabolic profiling for applications in disease biomarker discovery and environmental exposure assessment. The method is optimized for biological samples and can be applied to studies of disease biomarkers, such as Chagas.

## Guidelines

**Ethical compliance**: The serum samples analyzed in this study were collected and handled under approval of the institutional ethics committee, following established ethical guidelines and with informed consent from all participants. Users intending to apply this protocol must ensure that their own sample collection complies with ethical standards, including review and approval by an Institutional Review Board (IRB) or equivalent ethics committee, and with international guidelines such as the Declaration of Helsinki.

## Materials

**Materials**

- Human serum samples (≥500 µL each)
- 10 mL headspace vials with PTFE/silicone septa

**Reagents**

- Methanol (HPLC grade)
- Helium gas (99.999% purity)
- Calibration standards (optional)

**Equipment**

- SPME fiber: DVB/CAR/PDMS (Divinylbenzene/Carboxen/Polydimethylsiloxane) (80 µm / 23 ga, Supelco or equivalent)
- GC-MS system (e.g., Agilent 7860 GC with 5977B MSD)
- GC column: HP-5MS, 30 m × 0.25 mm i.d., 0.25 µm film thickness
- Vortex mixer
- Thermostatic water bath or agitator block (40–50 °C)
- Centrifuge
- Micropipettes and tips

**Equipment Setup**

- Injection mode: splitless
- Injection temperature: 270 °C
- Temperature program:
  - 50 °C (2 min hold)
  - Ramp 8 °C/min to 100 °C
  - Ramp 15 °C/min to 280 °C (4 min hold)
  - Ramp 20 °C/min to 300 °C (10 min hold)
- Carrier gas: helium, 1 mL/min
- MS mode: Full scan (m/z 50–650)

## Safety warnings

**SAFETY INFORMATION**

- Treat all human serum samples as potentially infectious material; follow institutional biosafety level 2 procedures.
- Methanol is toxic and flammable; use in a fume hood and wear PPE (lab coat, gloves, safety glasses).
- Dispose of all biological and chemical waste following institutional and national regulations.

## Ethics statement

This study did not involve the use of animals. Human serum samples were collected and handled under the approval of the institutional ethics committee, in compliance with national and international ethical guidelines. All participants provided informed consent prior to sample collection.

## Before start

- Ensure that all serum samples were collected with proper ethical approval (IRB or equivalent) and informed consent from participants.
- Serum samples should be stored at **–80 °C** until analysis. Thaw samples on ice immediately before processing to avoid degradation of volatile compounds.
- Verify that the **SPME fiber (DVB/CAR/PDMS, 80/23 mm)** is properly conditioned according to the manufacturer's instructions before use.
- Confirm that the **GC-MS system** is calibrated, the column is installed correctly, and high-purity helium gas is available as the carrier.
- Perform all sample handling in accordance with **biosafety guidelines** for human-derived material.

## Procedure

1 Thaw serum samples on ice.

2 In a 1.5 mL tube, mix:
- 100 µL of blood serum
- 200 µL of HPLC-grade methanol

3 Vortex for 30 seconds.

4 Let stand at room temperature for 15 minutes to allow protein precipitation.

5 Centrifuge at 12,500 rpm for 10 minutes.

6 Transfer the clear supernatant to a 0.5 mL Eppendorf tube.

7 Store the supernatant at 4°C if not analyzed immediately (up to 24 hours).

8 Precondition the DVB/CAR/PDMS fiber as per manufacturer instructions.

9 Introduce the DVB/CAR/PDMS SPME fiber directly into the serum supernatant.

10 Allow extraction to proceed for **30 minutes** at room temperature.

11 Immediately transfer the fiber to the GC inlet for thermal desorption.
*Optional*: For volatile-only profiling, perform headspace extraction at 40 °C.

12 Desorb the fiber in the GC injector at 270 °C for 5 min in splitless mode.

13 Run GC-MS with the specified temperature program.

14 Acquire mass spectra in full scan mode (m/z 50–650).

15 Perform peak deconvolution.

16 Identify compounds using the NIST library (Salek et al 2013).

17 Normalize peak areas and calculate retention indices (Sun and Stremple 2003).

18 Compare VOC profiles between Chagas-positive and negative groups.

## Troubleshooting

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| Problem | Possible Cause | Solution |
|---|---|---|
| **Low signal** | Fiber not conditioned | Recondition fiber |
| **High noise** | Solvent/equipment contamination | Run blanks, clean injector liner |
| **Poor reproducibility** | Variations in extraction conditions | Standardize timing and temperature |

## Critical Notes

20 Perform all extractions in triplicate to assess reproducibility.

21 Regularly clean the injector port and replace the septum to avoid carryover.

22 Ensure fibers are properly conditioned before use.

23 Store samples and extracts cold to avoid degradation.

## Protocol references

Sun, G. & Stremple, P. *Retention index characterization of flavor, fragrance and many other compounds on DB-1 and DB-XLB*. J&W Scientific, 91 Blue Ravine Road, Folsom (2003).

Salek, R.M. *et al.* The role of reporting standards for metabolite annotation and identification in metabolomic studies. *GigaSci* **2**, 13 (2013). https://doi.org/10.1186/2047-217X-2-13