Recommended Practice

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Guidelines for solid-phase micro-extraction (SPME) of volatile flavour compounds for gas-chromatographic analysis, from the Working Group on Methods of Analysis of the International Organization of the Flavor Industry (IOFI)

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1. Introduction

The present guidelines refer to the method of sampling flavour volatiles from aqueous solutions which are free of non-volatile compounds, or from the headspace of liquid or solid samples, prior to gas-chromatographic (GC) analysis, using a commercial solid-phase micro-extraction (SPME) device. Guidelines for the use of SPME for quantitative analyses are also given.

2. Principle

The method[1] is based on the extraction of volatiles from a liquid or a gas phase by a sorbent coated on, or bonded to, the surface of a quartz fibre (Figure 1). When the fibre is dipped into a solution, solutes are partitioned between the solvent and the sorbent. When the fibre is only in contact with the gas phase of a sample in a closed vial, there is a three-phase (at least) partitioning between the fibre, the headspace and the sample. After equilibration, the fibre is inserted into the hot injector of a gas chromatograph to volatilize the analytes.

3. Equipment

3.1. Fibres. Several types of fibre coating are commercially available, with various film thicknesses, for example:

(a) Polydimethylsiloxane (PDMS): non-polar coating.
(b) Polyacrylate (PA): medium-polar coating.
(c) Carbowax (polyethylene glycol): polar coating.
(d) Mixed coatings, for example:
  (i) Carboxen/PDMS.
  (ii) PDMS/divinylbenzene (DVB).
  (iii) Carbowax/DVB.

Volatiles are solubilized in the fibre coating, except for those containing Carboxen or DVB, which function by adsorption. Mixed phases containing DVB or Carboxen are not recommended for quantification.

The extraction selectivity depends on the respective polarity of the analytes and the fibre. For the extraction of polar compounds, a polar fibre should be chosen, and vice versa for non-polar compounds. A greater film thickness (100 µm) is preferred for low molecular weight volatiles, and a thinner film for higher molecular weight compounds. Some phases are chemically bonded to the fibre, and thus can even be dipped directly into a solvent.

3.2. Sample vials. For trace analyses, deactivation of the glass surface with a silylating reagent may be necessary (e.g. 5% dimethylchlorosilane in toluene). PTFE-faced septa are recommended.

3.3. Thermostatic device to maintain the vial at a given and accurate temperature, equipped with a means of agitation. This can be a magnetic stirring device, but should preferably be a system for mechanical shaking, to avoid sorption of analytes in the magnetic-bar coating.

3.4. Injector inserts. GC suppliers often propose injector inserts dedicated to SPME injections. This option is highly recommended. In any case, narrow-bore low-volume inserts should be used, with the injector operating in splitless mode. Wide-bore inserts, such as those used in classical split/splitless injections, are not suitable.

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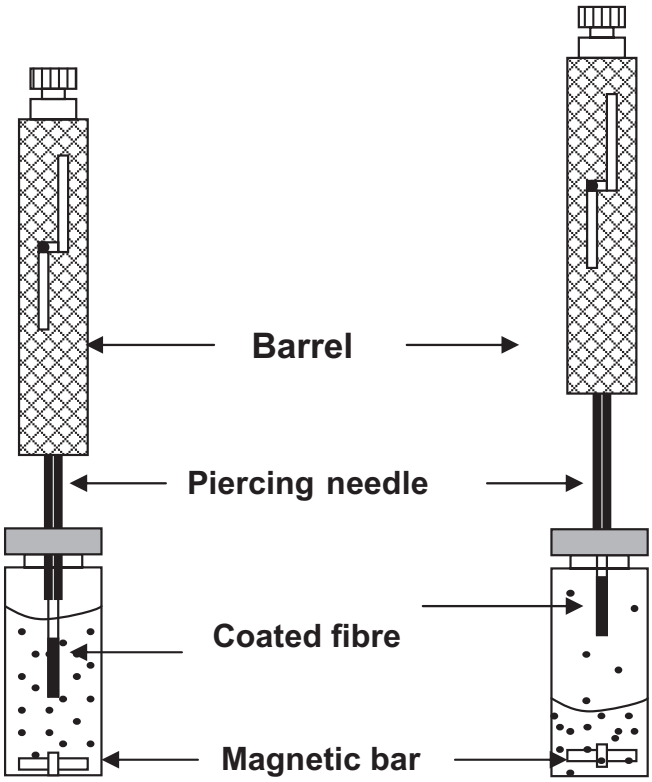

Figure 1. SPME sampling by direct extraction (left) or headspace extraction (right)

4. Sampling Procedure

4.1. Put a clean magnetic stirring bar (if this is the chosen means of agitation) and a given and accurate volume (or mass) of the sample in a sample vial (3.2) and seal the latter hermetically with an inert septum.

4.2. Insert the SPME needle through the vial septum, such that:

(a) For direct extraction the fibre is immersed in the solution (Figure 1, left). This direct extraction method is only applicable to solutions which are free of non-volatile compounds. Solutions containing non-volatile compounds may irreversibly contaminate the fibre coating.

(b) For headspace extraction the fibre coating is maintained in the headspace without any direct contact with the sample (Figure 1, right). Headspace extraction is applicable to samples containing non-volatile compounds that could contaminate the fibre by direct contact.

4.3. Start the stirring or shaking of the vial and maintain the vial and SPME assembly:

(a) At an accurately thermostated temperature (3.3).
(b) With stirring or shaking in a reproducible manner.
(c) For an accurately-measured period of time.

4.4. Remove the fibre from the vial and insert it into the GC injector. Immediately expose the fibre to the injector temperature for an accurate period of time. Then rapidly remove the SPME needle from the injector.

5. Quantitative Use of SPME

The use of SPME to quantify a volatile compound first requires the establishment of a calibration curve, and then comparison of GC peak areas of the sample to those of the calibration curve. Only fibres coated with *ab*sorbing phases (e.g. PDMS) should be used for quantification purposes.

5.1. Calibration curves. Prepare model solutions of the compound to be quantified in the concentration range of the same compound in the sample. *The medium used for the calibration must be the same as the sample matrix*, but free of the target compound.

5.2. When no matrix free from the target analyte is available, quantitation can also be carried out by the method of standard additions.

5.3. Determine the time to equilibration (i.e. the duration of fibre exposure to the sample), which varies from minutes to hours and from one compound to another.

5.4. Sample and analyse successively the model solutions (5.1) according to the procedure described in 4.1–4.4, using exactly the same experimental procedure (extraction and desorption times, temperature, sample and vial volumes, and stirring rates).

5.5. Plot the GC areas for the compounds from the various calibration solutions as a function of their concentrations. *The concentration range of the standards must correspond to a linear portion of the calibration curve*. Otherwise, adapt the concentration range.

5.6. Sample and inject the unknown samples under exactly the same conditions as in 5.4.

5.7. Determine the concentration of the unknown compound by comparing its GC area with the corresponding value on the calibration curve.

5.8. Quantitative analysis is feasible in non-equilibrium situations if all SPME sampling/desorption conditions (see 5.4) are strictly identical for all samples and standards. In this respect, a SPME auto-sampler is highly recommended.

6. Recommendations

6.1. Analyses of the blank fibre should be performed before the first SPME extraction of each sampling series, and repeated randomly within a series.

6.2. The extraction yield, from a given sample, differs greatly from one compound to another. Hence, the relative amounts of the components of a mixture cannot be estimated from the normalized GC area percentages. *An individual calibration curve must be established for each compound*.

6.3. Salting out. For aqueous samples, the addition of salt generally decreases the water solubility of organic compounds, and improves the extraction yield.

6.4. Stirring or shaking shorten extraction times; for quantitative purposes, *the stirring or shaking rate must be identical for all samples and calibration solutions*.

6.5. Temperature. For quantitative purposes, *the temperature must be reproducible and accurate for all samples and calibration solutions*. Its value influences the extraction yield. An increase of the temperature:

- Decreases the recovery if $k_{fl} > 1$ (exothermic extraction)
- Increases the recovery if $k_{fl} < 1$ (endothermic extraction)

where k_{fl} is the fibre-to-liquid or fibre-to-sample partition coefficient.

6.6. For headspace extraction, sensitivity increases if V_f/V_g or V_l/V_g increases, where V_l, V_g, and V_f are the volumes of liquid, gas, and fibre phase, respectively.

6.7. The time-to-equilibration of the extraction step varies with compounds. It must be checked to ensure reproducibility.

6.8. The fibre deteriorates with use, and care should be taken to monitor its performance (for example, using calibration solutions) and physical appearance.

6.9. *Because of the large diameter of SPME needles, injector septa are rapidly damaged and must be changed frequently, especially for quantitative work.* Pre-drilled septa are advisable.

6.10. When the gas volume within the vial is low, or when the air-to-sample partition coefficient is low, the recovery of analytes from the headspace is almost equal to that of a direct extraction from the solution. Therefore, *SPME sampling in the headspace is never representative of the headspace composition.*

Reference

1. J. Pawliszyn. *Solid Phase Microextraction: Theory and Practice*. Wiley-VCH: New York, **1997**.

