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# Impact of phase ratio, polydimethylsiloxane volume and size, and sampling temperature and time on headspace sorptive extraction recovery of some volatile compounds in the essential oil field

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**Abstract**

This study evaluates concentration capability of headspace sorptive extraction (HSSE) and the influence of sampling conditions on HSSE recovery of an analyte. A standard mixture in water of six high-to-medium volatility analytes (isobutyl methyl ketone, 3-hexanol, isoamyl acetate, 1,8-cineole, linalool and carvone) was used to sample the headspace by HSSE with stir bars coated with different polydimethylsiloxane (PDMS) volumes (20, 40, 55 and 110 μL, respectively), headspace vial volumes (8, 21.2, 40, 250 and 1000 mL), sampling temperatures (25, 50 and 75 °C) and sampling times (30, 60 and 120 min, and 4, 8 and 16 h). The concentration factors (CFs) of HSSE versus static headspace (S-HS) were also determined. Analytes sampled by the PDMS stir bars were recovered by thermal desorption (TDS) and analysed by capillary GC–MS. This study demonstrates how analyte recovery depends on its physico-chemical characteristics and affinity for PDMS (octanol–water partition coefficients), sampling temperatures (50 °C) and times (60 min), the volumes of headspace (40 mL) and of PDMS (in particular, for high volatility analytes). HSSE is also shown to be very effective for trace analysis. The HSSE CFs calculated versus S-HS with a 1000 mL headspace volumes at 25 °C during 4 h sampling ranged between $10^3$ and $10^4$ times for all analytes investigated while the limits of quantitation determined under the same conditions were in the nmol/L range.


*Keywords:* Headspace; Headspace sorptive extraction; PDMS stir bar; Phase ratio $\beta$; $K_{O/W}$; PDMS volume; Sampling temperature; Sampling time

## 1. Introduction

High-capacity headspace sorptive extraction (HSSE) is a high-concentration-capacity headspace sampling technique (HCC-HS) deriving from stir bar sorptive extraction (SBSE), which was introduced by Sandra and co-workers [1] in 1999. HSSE was first applied to headspace sampling by Tienpont et al. [2] and Bicchi et al. [3] in 2000 and is based on the static headspace (S-HS) approach. In HSSE, an analyte (or analytes or a fraction) is sorbed onto a thick film of polydimethylsiloxane (PDMS) coating a glass coated iron stir bar. The stir bar is suspended in the headspace volume from where the analytes are sorbed (sampled) by the PDMS coating. After sampling the stir bar is placed in a glass tube and transferred to a thermo-desorber from where the analytes are thermally recovered and then analysed by GC or GC–MS. Literature reports a number of HSSE applications: Kreck et al. [4] applied HSSE in combination with enantio-MDGC–MS to determine chiral monoterpenes in tea tree, eucalyptus and thyme essential oils. Demyttenaere et al. [5,6] compared HSSE and HS-SPME for the detection of volatile metabolites from toxigenic fungi, while Cavalli et al. [7] compared S-HS, HS-SPME, HSSE and direct thermal desorption in the analysis of the volatile fraction of French olive oil.

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Table 1
List of abbreviations and acronyms and meanings

| Acronym | Abbreviation |
|---|---|
| CF | Concentration factor |
| HCC | High-concentration-capacity techniques |
| HSSE | High-capacity headspace sorptive extraction |
| HS-SPME | Headspace solid-phase microextraction |
| FSOT capillary column | Fused-silica open tubular capillary column |
| PDMS | Polydimethylsiloxane |
| SBSE | Stir bar sorptive extraction |
| S-HS | Static headspace |
| β | Phase ratio |
| ST | Short–thin stir bar (l: 1 cm, thickness: 0.5 mm) |
| LT | Long–thin stir bar (l: 2 cm, thickness: 0.5 mm) |
| SK | Short–thick stir bar (l: 1 cm, thickness: 1.0 mm) |
| LK | Long–thick stir bar (l: 2 cm, thickness: 1 mm) |
| LOQ | Limit of quantitation |

HSSE is based on sorption, i.e. the partition of an analyte between the sample and the bulk of a polymeric retaining phase. The model proposed by Zhang and Pawliszyn for HS-SPME [8] was also extended to HSSE to explain the accumulation of an analyte from a solid or liquid matrix onto the PDMS coating [3]. HSSE recovery depends on the overall partition coefficient, $K$, of the analyte between the PDMS stir bar and the matrix itself. In its turn, $K$ depends on the analyte partition coefficient between PDMS stir bar and sample headspace, $K_1$, and on the partition coefficient between headspace and sample matrix, $K_2$. HSSE has been shown to achieve very high concentration capabilities mainly because of the high volume of polymeric coating [3,9] that ranges between 20 and 110 μL depending on the length and thickness of the stir bar.

To the best of the author's knowledge, an in-depth study to evaluate the influence of sampling conditions on HSSE recovery of an analyte has not yet been reported. This article aims to evaluate (a) how analyte volatility and solubility, PDMS and vial volumes (i.e. $\beta$), sampling time and temperature influence the HSSE recovery of six high-to-medium volatility components with different octanol–water partition coefficients ($K_{O/W}$), i.e. isobutyl methyl ketone, 3-hexanol, isoamyl acetate, 1,8-cineole, linalool and carvone, dissolved in water, and (b) how effective is HSSE concentration capability for trace analysis. Table 1 lists the acronyms adopted in the present article together with their meaning.

## 2. Experimental

### 2.1. Materials and reagents

(a) *Solvents and chemicals*: solvents were all pesticide-grade from Riedel-de Haen (Seelze Germany).
(b) *Standards*: pure standard samples of isobutyl methyl ketone, 3-hexanol, isoamyl acetate, 1,8-cineole, linalool and carvone were from Riedel-de Haen.

Standard stock solutions in cyclohexane (1 mM) of each analyte were prepared and stored at −18 °C. A standard working solution with analyte concentrations ranging from 40 μM for *i*-butylmethylketone to 1.3 μM for 1,8-cineole, linalool and carvone (see Table 2) was prepared by diluting suitable volumes of each standard stock solution with water and used for all experiments. A set of calibration standard solutions in cyclohexane used for quantitative analysis was also prepared in a suitable range of concentrations.

### 2.2. Sample preparation

#### 2.2.1. HSSE sampling

Two series of experiments were carried out:

(a) In the first set, the headspaces originating from 2 mL of the standard mixture in vials of different volumes (8, 21.2 and 40 mL) at different temperatures (25, 50 and 75 °C) and for different sampling times (30, 60 and 120 min) were submitted to HSSE using stir bars of different lengths and PDMS volumes and thicknesses. In particular, the following PDMS stir bars were used: 20 μL (l: 1 cm, thickness: 0.5 mm, short–thin (ST)), 40 μL (l: 2 cm, thickness: 0.5 mm; long–thin (LT)), 55 μL (l: 1 cm, thickness: 1.0 mm; short–thick (SK)) and 110 μL (l: 2 cm, thickness: 1 mm; long–thick (LK)). PDMS stir-bars are marketed under the name 'Twister' (Gerstel, Mülheim a/d Ruhr, Germany).
PDMS stir bars were suspended into the vapour phase and the headspace sampled by HSSE under the different conditions reported above. The stir bar was kept correctly positioned in the headspace volume by using an appropriate length of harmonic stainless steel wire, one end of which clamped the PDMS coating, while the other end was inserted into the vial septum cap. After sampling, the PDMS stir bar was removed from the vapour phase,

Table 2
Characteristics of the analytes investigated

| Compounds | $M_r$ | $T_{eb}$ | Vapour pressure (Pa) | Henry law constant (atm m$^3$/mol) | $K_{o/w}$ | Water solubility (mg/mL) | Analyte concentration (μM) |
|---|---|---|---|---|---|---|---|
| Isobutyl methyl ketone | 100.2 | 116.5 | 2653.0 | $1.4 \times 10^{-4}$ | 20.4 | 19.0 | 40.0 |
| 3-Hexanol | 102.2 | 134.8 | 639.9 | $4.0 \times 10^{-5}$ | 44.6 | 16.1 | 35.0 |
| Isoamyl acetate | 130.2 | 142.5 | 746.5 | $5.9 \times 10^{-4}$ | 182.0 | 2.0 | 6.9 |
| 1,8-Cineole | 154.2 | 176.4 | 253.3 | $1.1 \times 10^{-4}$ | 316.2 | 3.5 | 1.3 |
| Linalool | 154.2 | 198.0 | 26.6 | $2.1 \times 10^{-5}$ | 933.0 | 1.6 | 1.3 |
| Carvone | 150.2 | 228.5 | 13.3 | $1.4 \times 10^{-4}$ | 1174.0 | 1.3 | 1.3 |

inserted into a glass tube and then introduced in a thermodesorber for capillary GC (cGC)–MS analysis (see Section 2.3). Each experiment was repeated three times. Blank runs of the stir bar were done before and after each analysis and no memory effects occurred for the target solutions.

(b) In the second set, HSSE was applied to sample the headspace of 2 mL of the standard solution at 25 °C in 250 and 1000 mL vials for 30, 60 and 120 min and 4, 8 and 16 h using SK and LK stir bars. The same operative conditions as reported above were used.

#### 2.2.2. S-HS sampling

Two series were carried out:

(a) In the first set, the S-HS obtained from 2 mL of the standard solution in vials of different volumes (8, 21.2 and 40 mL) at different temperatures (25 and 50 °C) and for different sampling times (30, 60 and 120 min) was sampled. One milliliter of the vapour phase obtained under these conditions was automatically injected into the S-HS–cGC–MS system and analysed under the same conditions reported for HSSE (see Section 2.3). Each experiment was repeated three times.

(b) In the second set of experiments, the S-HS resulting from 2 mL of the standard solution in 250 and 1000 mL vials after 30, 60, 120 min and 4 h at 25 °C was sampled. The operative conditions reported above were used.

### 2.3. HSSE-thermal desorption-cGC–MS analysis

Analyte thermal desorption from the PDMS stir bar was achieved with a TDS-2 unit from Gerstel installed on a Agilent 6890 GC unit. For the TDS the following parameters were used: desorption programme: from 40 to 250 °C (5 min) at 60 °C/min; flow mode: splitless, transfer line: 250 °C. A Gerstel CIS-4 PTV injector was used to focus cryogenically the analytes thermally desorbed from the stir bar. The PTV was cooled to −50 °C using liquid $CO_2$; injection, PTV; injection temperature, from −50 to 280 °C (5 min) at 600 °C/min. Inlet was operated in the split mode, split ratio, 1:20.

Capillary GC–MS analyses were performed on an Agilent 6890 GC-5973N MS system (Agilent, Little Falls, DE, USA). Chromatographic conditions: temperature programme: from −30 °C (1 min) to 50 °C at 40 °C/min then to 220 °C (5 min) at 5 °C/min. A fused-silica open tubular (FSOT) OV-1 column ($d_f$ 0.3 μm, 25 m × 0.25 mm i.d.) [Mega, Legnano (Milano), Italy] was used. Carrier gas: helium, flow-rate: 1.0 mL/min. MS was in the electron impact ionization (EI) mode at 70 eV. Ion source temperature: 250 °C. The HS components were identified by comparison of their mass spectra with those of authentic samples or with data in the literature.

### 2.4. S-HS–cGC–MS analysis

The S-HS equipment was by Chromtech (Idstein, Germany) and it was installed in a CTC-Combi-PAL-Autosampler (Bender and Hobein, Zurich, Switzerland) in its turn assembled on a cGC–MS system consisting of an Agilent model 6890 Series Plus/5973N MS system. The CTC-Combi-PAL-Autosampler (Bender and Hobein) included an incubator oven with one heated vial position and shaker (Agitator) (Chromtech). All S-HS sampling steps were automatically controlled by the CTC-Combi-PAL software. A 2.5 mL gas-tight syringe was used. cGC–MS conditions were as reported in Section 2.3.

### 2.5. HSSE recovery determination

Reference data for HSSE recovery of each analyte were obtained by calibration curves made by directly introducing 1 μL of standard solution on deactivated glass wool placed in a thermal desorption tube and then introducing it in the thermodesorber for cGC–MS analysis (see Section 2.3). Each analyte was quantified by a target ion (T.I.). Recoveries were calculated by comparing T.I. areas after HSSE sampling to those resulting from the direct TDS-cGC–MS analysis. A linear analytical response/concentration relationship was found for each analyte within the working ranges (see below).

## 3. Results and discussion

A series of parameters were here investigated to evaluate how they may affect the HSSE recovery of six analytes with different structures, volatilities and affinities for PDMS (i.e. $K_{O/W}$). The compounds investigated are characteristic of many essential oils: isobutyl methyl ketone (e.g. *Piper* genus), 3-hexanol (e.g. *Rosa* species and basil), isoamyl acetate (e.g. banana fruits aromas), 1,8-cineole (e.g. *Eucaliptus* genus), linalool (e.g. *Lavanda* genus), carvone (e.g. *Mentha* and *Carum* genus). Table 2 reports the characteristics of the analytes in question.

The following parameters were considered: (a) HSSE sampling temperature, (b) headspace phase ratio $\beta$ (headspace volume/sample volume), (c) HSSE sampling time and (d) volume of PDMS coating stir bars of different lengths and thicknesses.

All experiments were carried out by submitting to HSSE a sample consisting of a constant volume (2 mL) of a standard solution with analyte concentrations ranging from 40 μM for isobutyl methyl ketone to 1.3 μM for 1,8-cineole, linalool and carvone (see Table 2), thus allowing us to keep constant the analyte absolute amounts in all experiments. These analyte concentrations were chosen to obtain cGC–MS detectable signals for each analyte for all experiments. Each experiment was repeated three times and the average T.I. area values were considered for data elaboration. Repeatability was measured by analysing six times the standard solution under investiga-

Table 3
Recovery and R.S.D.s% determined on six analyses of the analytes investigated sampled by HSSE with SK stir bar in 1000 mL vial volume

| Compounds | CFs ± R.S.D.% |
|---|---|
| Isobutyl methyl ketone | 1.4 ± 6.5 |
| 3-Hexanol | 7.9 ± 8.3 |
| Isoamyl acetate | 19.2 ± 0.5 |
| 1,8-Cineole | 31.7 ± 5.1 |
| Linalool | 3.7 ± 0.4 |
| Carvone | 9.2 ± 4.8 |

tion with SK stir bar in 1000 mL vial volume; the resulting R.S.D.s are reported in Table 3 and are in agreement with those determined in a previous article where HSSE repeatability was evaluated for a standard mixture of volatile compounds [3].

The first group of experiments was carried out under the following conditions: stir bars of different lengths and PDMS thicknesses coated with PDMS volumes of 20 μL (l: 1 cm, thickness: 0.5 mm, short–thin), 40 μL (l: 2 cm, thickness: 0.5 mm; long–thin), 55 μL (l: 1 cm, thickness: 1.0 mm; short–thick) and 110 μL (l: 2 cm, thickness: 1 mm; long–thick) were applied to sample the headspace of 2 mL of the standard mixture in vials of different volume (8, 21.2, 40, 250 and 1000 mL) at different temperatures (25, 50 and 75 °C) and for different sampling times (30, 60 and 120 min). For SK and LK stir bars, further experiments at 25 °C with 250 and 1000 mL vials and sampling time of 4, 8 and 16 h were also carried out.

Recovery was then calculated through calibration curves obtained from direct injection into the cGC–MS system via TDS of the standard solutions of the analyte(s) investigated in a suitable range of concentrations (see Section 2.5) [10,11]. Quantitation by direct injection into the cGC system via TDS of the standard solutions allowed us to determine absolute recoveries related to the total amount of the analytes contained in the liquid phase. Analyses were carried out under rigorously standardised conditions to make the results from different experiments comparable.

HSSE recovery strictly depends not only on the overall set of parameters applied for each experiment but also on the solubility in water, volatility and polymer affinity of the analytes investigated. In spite of this, the influence of each parameter on recovery will first be discussed separately.

(a) *HSSE sampling temperature*: temperature is the parameter that influences headspace composition most. In general, the best recoveries for all investigated analytes with all stir bars were obtained at 50 °C, although the other sampling conditions also influenced recovery. Table 4 reports analyte recoveries obtained at different temperatures with the four PDMS stir bars from a 40 mL vial after 60 min sampling. With the exception of *i*-butylmethylketone, sampling at 25 °C gave recoveries decidedly lower than other temperatures, e.g. the recovery at 25 °C of 1,8-cineole are about three times lower than that at 50 °C in all conditions. Twenty five degree centigrade is probably too low a temperature to favour vapourisation of the analytes investigated and negatively affects the equilibrium of headspace formation ($K_2$). Sampling at 75 °C gave results comparable or slightly lower than those obtained at 50 °C. This is most probably because this temperature is quite high and it not only produced higher analyte concentration in the headspace vapour phase ($K_2$) than at 50 °C, but it also drastically increased their release from PDMS to headspace ($K_1$). The low absolute recovery for isobutyl methyl ketone at all temperatures was probably due to its high solubility in water, which influences its vapourisation and to its low affinity for PDMS (i.e. $K_{O/W}$).
At 25 °C, isoamyl acetate and 1,8-cineole were the best analytes recovered at all phase ratios, sampling times and stir bars. At 50 °C, 1,8-cineole and carvone were very well recovered. At this temperature, the most effective sampling time was 60 min with all phase ratios and stir bars; in particular, comparable results were obtained with a 40 mL vial for LT, SK and LK stir bars. At 75 °C 1,8-cineole and carvone were the best recovered. In this case too the highest recoveries were after 60 min sampling with both 40 and 21.2 mL volumes and the LT stir bar.

(b) *Headspace phase ratio, β (headspace volume/sample volume)*: the influence of headspace phase ratio β on recovery was investigated by varying the volume of the headspace vials (8, 21.2 and 40 mL) while keeping constant the sample volume (2 mL); β values of 3, 9.6 and 19 were used. Table 5 reports the analyte recoveries of the four PDMS stir bars with the β values investigated at 50 °C and after 60 min sampling. In general, the highest recovery was obtained with a vial volume of 40 mL, (i.e. with the highest β value), the only exception was isoamyl acetate. For the lower volatility analytes (1,8-cineole, linalool and carvone) recovery increased with

Table 4
Analyte % recoveries at different temperature with the PDMS stir bar from 40 mL vial after 60 min sampling

| PDMS volume (μL) | Isobutyl methyl ketone | | | 3-Hexanol | | | Isoamyl acetate | | | 1,8-Cineole | | | Linalool | | | Carvone | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 25 °C | 50 °C | 75 °C | 25 °C | 50 °C | 75 °C | 25 °C | 50 °C | 75 °C | 25 °C | 50 °C | 75 °C | 25 °C | 50 °C | 75 °C | 25 °C | 50 °C | 75 °C |
| 20 ST | 2.2 | 1.2 | 1.2 | 5.3 | 8.1 | 8.7 | 24.4 | 17.9 | 22.4 | 24.7 | 50.1 | 39.9 | 3.4 | 22.7 | 21.2 | 4.6 | 38.1 | 49.5 |
| 40 LT | 2.3 | 1.7 | 1.3 | 5.6 | 9.0 | 14.0 | 23.7 | 19.0 | 32.0 | 22.8 | 73.6 | 49.3 | 2.6 | 36.6 | 24.6 | 4.5 | 49.9 | 53.3 |
| 55 SK | 2.3 | 1.9 | 1.5 | 5.7 | 13.1 | 15.0 | 26.5 | 25.1 | 39.9 | 20.2 | 73.8 | 42.3 | 1.9 | 34.6 | 17.3 | 4.2 | 54.7 | 38.9 |
| 110 LK | 2.3 | 2.4 | 2.2 | 5.3 | 20.5 | 25.3 | 23.7 | 30.9 | 46.7 | 18.4 | 68.2 | 49.5 | 2.0 | 34.6 | 19.4 | 3.4 | 54.2 | 39.1 |

Table 5
Analyte % recoveries in different vial size with the PDMS stir bar at 50 °C after 60 min sampling

| PDMS volume (μL) | Isobutyl methyl ketone | | | 3-Hexanol | | | Isoamyl acetate | | | 1,8-Cineole | | | Linalool | | | Carvone | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 8 mL | 21.2 mL | 40 mL | 8 mL | 21.2 mL | 40 mL | 8 mL | 21.2 mL | 40 mL | 8 mL | 21.2 mL | 40 mL | 8 mL | 21.2 mL | 40 mL | 8 mL | 21.2 mL | 40 mL |
| 20 ST | 1.3 | 1.5 | 1.2 | 10.1 | 10.6 | 8.1 | 33.3 | 32.5 | 17.9 | 67.5 | 68.2 | 50.1 | 30.1 | 30.4 | 22.7 | 48.2 | 50.6 | 38.1 |
| 40 LT | 1.4 | 1.5 | 1.7 | 10.6 | 11.0 | 9.0 | 29.8 | 29.6 | 19.0 | 66.6 | 75.1 | 73.6 | 28.1 | 32.1 | 36.6 | 42.6 | 51.9 | 49.9 |
| 55 SK | 2.0 | 1.8 | 1.9 | 15.7 | 14.1 | 13.1 | 42.7 | 38.1 | 25.1 | 64.1 | 67.2 | 73.8 | 27.3 | 29.7 | 34.6 | 42.3 | 50.0 | 54.7 |
| 110 LK | 2.3 | 2.4 | 2.4 | 22.7 | 25.6 | 20.5 | 42.7 | 44.9 | 30.9 | 63.5 | 71.3 | 68.2 | 27.7 | 32.8 | 34.6 | 32.7 | 42.2 | 54.2 |

Table 6
Analyte % recoveries in different time sampling with the PDMS stir bar from 40 mL vial at 50 °C

| PDMS volume (μL) | Isobutyl methyl ketone | | | 3-Hexanol | | | Isoamyl acetate | | | 1,8-Cineole | | | Linalool | | | Carvone | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 30 m | 60 m | 120 m | 30 m | 60 m | 120 m | 30 m | 60 m | 120 m | 30 m | 60 m | 120 m | 30 m | 60 m | 120 m | 30 m | 60 m | 120 m |
| 20 ST | 1.5 | 1.2 | 1.2 | 8.6 | 8.1 | 8.5 | 19.4 | 17.9 | 19.0 | 65.5 | 50.1 | 41.5 | 28.4 | 22.7 | 15.2 | 50.9 | 38.1 | 31.0 |
| 40 LT | 1.3 | 1.7 | 1.1 | 9.4 | 9.0 | 9.6 | 21.8 | 19.0 | 20.2 | 71.5 | 73.6 | 51.1 | 31.5 | 36.6 | 20.5 | 60.0 | 49.9 | 43.5 |
| 55 SK | 1.7 | 1.9 | 1.8 | 12.5 | 13.1 | 13.9 | 24.5 | 25.1 | 27.1 | 63.2 | 73.8 | 56.6 | 30.6 | 34.6 | 27.3 | 48.3 | 54.7 | 55.3 |
| 110 LK | 2.1 | 2.4 | 2.0 | 20.9 | 20.5 | 23.5 | 31.3 | 30.9 | 34.3 | 70.7 | 68.2 | 61.5 | 32.6 | 34.6 | 28.3 | 46.7 | 54.2 | 48.9 |

the PDMS volume; with a 8 mL vial volume, recoveries of these analytes were comparable with all stir bars.

(c) *HSSE sampling time*: similar considerations can be made for sampling time. Table 6 reports analyte recoveries of the four PDMS stir bars by applying the sampling times investigated at 50 °C and with a 40 mL HS vial. Good analyte recoveries with all stir bars were generally obtained after 60 min, in particular, with the less volatile analytes. Moreover, recoveries over time increased with the PDMS volumes. A sampling time of 60 min is probably a good compromise to optimise both equilibria conditioning stir bars recovery of the analytes investigated, i.e. matrix/headspace and headspace/PDMS equilibria.

(d) *Volume of PDMS and size of stir bars*: size and volume of the stir bars differently influenced analyte absolute recovery. Table 7 reports the analyte recoveries with the four PDMS stir bars after 60 min HS sampling at 50 °C with a vial volume of 40 mL. In general, under all conditions, absolute recovery increased with PDMS volume, although to a different extent depending on the analyte. On the other hand, in spite of the difference in PDMS volume, LT (40 μL) and SK (55 μL) recoveries of the less volatile compounds are comparable (or even slightly better for LT), showing that recovery was also related to the headspace/PDMS contact surface. Moreover, the most volatile analytes (isobutyl methyl ketone, 3-hexanol, isoamyl acetate) were better recovered by the thickest stir bars (SK and LK), while recoveries of 1,8-cineole and linalool were almost constant with increasing of PDMS volume.

### 3.1. HSSE concentration capability

The concentration capability of HSSE versus S-HS sampling was also evaluated by determining concentration factors (CFs), i.e. the ratio between the analyte areas obtained by HSSE sampling and the corresponding S-HS areas obtained under the same sampling conditions. CF is a useful parameter to evaluate the relative effectiveness in recovery of different stir bars for a given sample, provided that rigorous and reproducible analysis conditions are applied. Static headspace samplings and analyses were carried out on the vapour phase obtained from 2 mL of the standard solution in vials of different volumes (8, 21.2 and 40 mL) at different temperatures (25 and 50 °C) and for different sampling times (30, 60 and 120 min). Table 8 reports HSSE/S-HS CFs obtained after sampling the headspace of 2 mL of the standard solution at 50 °C for 60 min, in 8, 21.2 and 40 mL vials both statically and with the four stir bars investigated. As expected the HSSE concentration capability was influenced by volatility and solubility in water of the analytes investigated, and by sampling temperature and volume, although to different extents. A possible explanation of the lower CFs for 1,8-cineole, linalool and carvone when thick film stir bars are used can be that longer times are necessary to achieve the equilibrium with the less volatile components. This hypothe-

Table 7
Analyte % recoveries with the PDMS stir bar from 40 ml vial at 50 °C after 60 min sampling

| PDMS volume (μL) | Isobutyl methyl ketone | 3-Hexanol | Isoamyl acetate | 1,8-Cineole | Linalool | Carvone |
|---|---|---|---|---|---|---|
| 20 ST | 1.2 | 8.1 | 17.9 | 50.1 | 22.7 | 38.1 |
| 40 LT | 1.7 | 9.0 | 19.0 | 73.6 | 36.6 | 49.9 |
| 55 SK | 1.9 | 13.1 | 25.1 | 73.8 | 34.6 | 54.7 |
| 110 LK | 2.4 | 20.5 | 30.9 | 68.2 | 34.6 | 54.2 |

Table 8
HSSE/S-HS CFs values in different vial size at 50 °C after 60 min sampling

| PDMS volume (μL) | Isobutyl methyl ketone | | | 3-Hexanol | | | Isoamyl acetate | | | 1,8-Cineole | | | Linalool | | | Carvone | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 8 mL | 21.2 mL | 40 mL | 8 mL | 21.2 mL | 40 mL | 8 mL | 21.2 mL | 40 mL | 8 mL | 21.2 mL | 40 mL | 8 mL | 21.2 mL | 40 mL | 8 mL | 21.2 mL | 40 mL |
| 20 ST | 28 | 49 | 54 | 108 | 220 | 166 | 56 | 123 | 129 | 118 | 194 | 265 | 107 | 173 | 205 | 257 | 392 | 389 |
| 40 LT | 28 | 49 | 81 | 113 | 228 | 185 | 50 | 112 | 137 | 116 | 213 | 389 | 100 | 183 | 330 | 227 | 402 | 510 |
| 55 SK | 41 | 59 | 87 | 168 | 292 | 269 | 71 | 144 | 181 | 112 | 191 | 390 | 97 | 170 | 312 | 226 | 387 | 559 |
| 110 LK | 49 | 79 | 115 | 242 | 531 | 423 | 71 | 170 | 223 | 111 | 202 | 361 | 98 | 187 | 311 | 175 | 327 | 554 |

sis is confirmed by the results after 120 min sampling where CFs of these compounds increased with thick film PDMS stir bars (data not reported). The results in Table 8 show that CFs increased with the headspace volume, i.e. with the analyte dilution, further emphasising the high concentration capability of HSSE for the analyses of both traces and highly diluted samples.

Moreover, HSSE CFs increased over time until the equilibrium headspace/PDMS stir bar ($K_1$) was achieved, and decreased with sampling temperature since a higher temperature produced a more concentrated headspace. The analyte volatility also conditioned the HSSE concentration capability in function of the sampling conditions adopted. The analytes investigated behaved in the following two different ways:

- the highest CFs for the most volatile analytes (in particular, for *i*-butylmethylketone) were obtained after 60 min sampling at 25 °C in a 40 mL vial; after 120 min, CFs decreased probably because of its release from the stir bar after achieving the headspace/PDMS equilibrium. Under the same conditions but at 50 °C, CFs are almost constant over time, probably because the systems reached the equilibrium;
- in a 40 mL vial, the CFs for the less volatile analytes (in particular, 1,8-cineole) increased over time at 25 °C most probably because the headspace/PDMS equilibrium was not yet achieved. At 50 °C, CFs decreased when sampling time increased from 30 to 60 min, but increased after 120 min. In all conditions applied, CFs improved with the PDMS volume although not proportionally to the PDMS increase.

### 3.2. HSSE recovery in trace analysis

A series of experiments were also carried out to evaluate HSSE recovery when analytes in trace amounts have to be sampled in the headspace vapour phase. HSSE was by sampling at 25 °C the headspace of 2 mL of the standard solution in 250 and 1000 mL vials for 4, 8 and 16 h using SK and LK stir bars, besides the sampling times of the above experiments. These experiments were deliberately carried out under unfavourable conditions, so as to evaluate HSSE concentration capability when trace amounts must be sampled in view of applying this technique to in vitro and in vivo biological experiments. Table 9 reports the analyte recoveries with the SK and LK PDMS stir bars over time at 25 °C with a vial volume of 1000 mL. Fig. 1 reports the isobutyl methyl ketone and 3-hexanol recoveries versus time with SK and LK PDMS stir bars at 25 °C in 1000 mL HS vial.

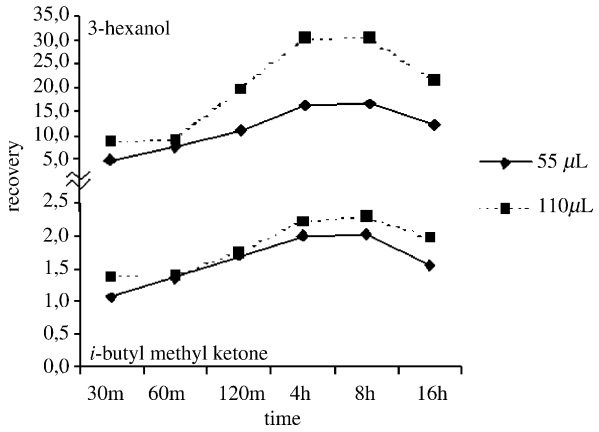


Fig. 1. Recovery (%) vs. sampling time of isobutyl methyl ketone and 3-hexanol with the SK and LK PDMS stir bars at 25 °C in 1000 mL HS vial.

Absolute recoveries were first evaluated: they drastically increased when sampling time increased from 2 to 4 h with both headspace volumes (250 and 1000 mL) and stirs bars (SK and LK), in particular, with 3-hexanol, linalool and carvone. 1,8-Cineole is the best recovered analyte under all sampling conditions. After 4 h sampling, recoveries were

Table 9
Analyte % recoveries with SK and LK PDMS stir bars over time at 25 °C in vial 1000 mL

| Time | Isobutyl methyl ketone | | 3-Hexanol | | Iaoamyl acetate | | 1,8-Cineole | | Linalool | | Carvone | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 55 μL | 110 μL | 55 μL | 110 μL | 55 μL | 110 μL | 55 μL | 110 μL | 55 μL | 110 μL | 55 μL | 110 μL |
| 30 min | 1.1 | 1.4 | 4.9 | 8.8 | 14.3 | 21.4 | 16.0 | 32.2 | 1.8 | 2.9 | 4.3 | 4.7 |
| 60 min | 1.4 | 1.4 | 7.9 | 9.1 | 19.2 | 21.5 | 31.7 | 30.5 | 3.7 | 3.5 | 9.2 | 5.7 |
| 120 min | 1.7 | 1.7 | 11.2 | 19.9 | 26.5 | 30.6 | 43.7 | 69.1 | 6.3 | 8.3 | 13.9 | 14.3 |
| 4 h | 2.0 | 2.2 | 16.4 | 30.5 | 30.2 | 40.5 | 71.3 | 73.5 | 19.3 | 13.1 | 36.0 | 21.8 |
| 8 h | 2.0 | 2.3 | 16.8 | 30.6 | 32.0 | 41.5 | 71.3 | 76.4 | 19.5 | 13.3 | 37.6 | 21.8 |
| 16 h | 1.6 | 2.0 | 12.4 | 21.6 | 26.0 | 32.4 | 49.8 | 53.9 | 13.4 | 9.4 | 39.1 | 22.0 |

Table 10
HSSE/S-HS CFs values with SK and LK PDMS stir bar over time at 25 °C in vial 1000 mL

| Time | Isobutyl methyl ketone | | 3-Hexanol | | Isoamyl acetate | | 1,8-Cineole | | Linalool | | Carvone | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 55 μL | 110 μL | 55 μL | 110 μL | 55 μL | 110 μL | 55 μL | 110 μL | 55 μL | 110 μL | 55 μL | 110 μL |
| 30 min | 580 | 750 | 1218 | 2180 | 336 | 502 | 529 | 1061 | 106 | 167 | 655 | 709 |
| 60 min | 796 | 824 | 2448 | 2831 | 1161 | 1296 | 2179 | 2097 | 494 | 468 | 1230 | 761 |
| 120 min | 1078 | 1108 | 5508 | 9755 | 1721 | 1993 | 4467 | 7059 | 1419 | 1880 | 6350 | 6510 |
| 4 h | 1190 | 1324 | 7889 | 14714 | 2294 | 3073 | 7918 | 8167 | 6130 | 4147 | 13100 | 7930 |

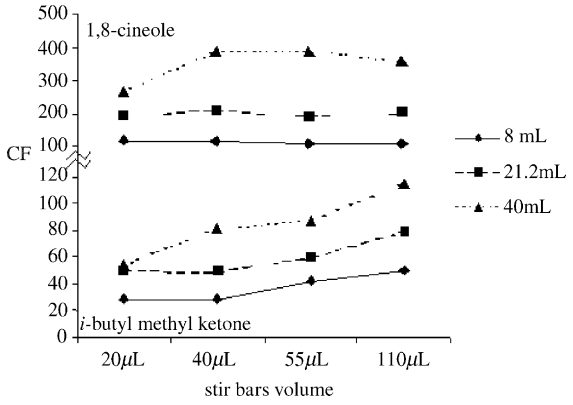


Fig. 2. CFs of isobutyl methyl ketone and 3-hexanol vs. sampling time with the SK and LK PDMS stir bars at 25 °C in 1000 ml HS vial.

generally similar with both SK and LK stir bars, and achieved their maximum values, although not increasing much further at 8 h, probably because both the standard solution/headspace and headspace/PDMS equilibria had been reached.

Recoveries were similar, partly because the analytes diffused homogeneously throughout the whole PDMS volume as a consequence of the long sampling times, so that the contact surface is less limiting than when sampling over short times. Moreover, the low concentration of the analytes in the headspace did not saturate the PDMS thus making the amount of PDMS less critical.

### 3.3. HSSE concentration capability for trace analysis

A further series of experiments were also carried out to evaluate the concentration capability of HSSE versus S-HS with highly diluted vapour phases. CFs were determined at 25 °C by analysing the headspace produced by 2 mL of the above standard solution in a 1000 mL vial with SK and LK PDMS stir bars for sampling times of 30, 60 and 120 min and 4 h. Table 10 reports HSSE/S-HS CFs obtained after sampling the headspace of 2 mL of the standard solution in 1000 mL at 25 °C over time vials, both by S-HS and with the SK and LK stir bars. Fig. 2 reports the isobutyl methyl ketone and 3-hexanol CFs versus time with the two PDMS stir bars investigated, at 25 °C in a 1000 mL HS vial. A drastic increase of CFs over time was observed with all analytes although to different extents; with the exception of carvone and linalool, LK resulted again more effective than SK. CFs were also conditioned by the volatility of each analyte: the most volatiles achieved high CFs in shorter times while the lower volatility compounds gave the highest CF values. These results are even more interesting when considering the headspace/standard solution phase ratio (i.e. 499) that produces a strong dilution of the analytes in the vapour phase (about 500 times) compared to the original standard solution.

### 3.4. HSSE limit of quantitation (LOQ)

Further evidence of the HSSE concentration capability is given by the limit of quantitation (LOQ). LOQs were obtained by analysing ten times the standard solution (blank) by HSSE-TDS-cGC–MS in agreement with the Eurachem guide lines [12]. Table 11 reports absolute recovery and LOQs for the six analytes investigated when 2 mL standard solutions in a 1000 mL vial were submitted to HSSE sampling for 4 h at 25 °C, with SK and LK stir bars. Under these conditions, LOQs ranged from 13.4 nmol/L for isobutyl methyl ketone to 0.3 nmol/L for 1,8-cineole with SK and from 11.5 nmol/L for isobutyl methyl ketone to 0.2 nmol/L for 1,8-cineole with LK. LOQ values confirmed the high concentration capability of HSSE, in particular, for trace analysis in consideration of (a) the very high value of both the headspace/standard solution phase ratio (i.e. 499) and headspace/PDMS phase ratio (i.e. 9072 for LK and 18145 for SK) and (b) the absolute recoveries that ranged between 2.0% for isobutyl methyl ketone and 71.3% for 1,8-cineole with SK and from 2.2% for isobutyl methyl ketone and to 73.5% for 1,8-cineole with LK.

Table 11
Absolute analyte % recoveries and LOQs for six analyte investigated of 2 mL standard solution in a vial 1000 mL submitted to HSSE sampling for 4 h at 25 °C with SK and LK PDMS stir bar

| | Isobutyl methyl ketone | | 3-Hexanol | | Isoamyl acetate | | 1,8-Cineole | | Linalool | | Carvone | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 55 μL | 110 μL | 55 μL | 110 μL | 55 μL | 110 μL | 55 μL | 110 μL | 55 μL | 110 μL | 55 μL | 110 μL |
| Recoveries (%) | 2.0 | 2.2 | 16.4 | 30.5 | 30.2 | 40.5 | 71.3 | 73.5 | 19.3 | 13.1 | 36.0 | 21.8 |
| LOQ (nM) | 13.4 | 11.5 | 3.7 | 2.1 | 0.7 | 0.5 | 0.3 | 0.2 | 0.5 | 0.5 | 0.6 | 0.7 |

## 4. Conclusions

HSSE has here been shown to be an effective high concentration capacity headspace sampling technique. Recovery of analytes depends on both their physico-chemical characteristics that influence the headspace composition and on their affinity for PDMS ($K_{O/W}$), and is also affected by sampling conditions (temperatures and times and vial volumes) that must be compatible with both headspace and the sorption equilibria. Analytes recovery generally improves with increasing both PDMS volumes and stir bars contact surface: this is particularly true for high volatility compounds. In general, the best recovery for the analytes investigated were obtained by sampling the standard mixture at 50 °C for 60 min with a vial volume of 40 mL and with the 2 cm long stir bars coated with a high PDMS volume. This study has also shown that HSSE is very effective for trace analysis in particular, when sampling is with high headspace volumes and/or with unfavourable phase ratio or conditions, thus making the method interesting for application to in vitro or in vivo biological experiments. The HSSE concentration factors (CFs) calculated versus S-HS with a 1000 mL headspace volumes at 25 °C during 4 h sampling ranged between $10^3$ and $10^4$ times for all analytes investigated. The high HSSE concentration capability is also confirmed by the limits of quantitation (LOQ) determined with the standard solution under study with the same operative conditions that is in the nmol/L range for all analytes investigated.

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