ELSEVIER

Journal of Chromatography B, 732 (1999) 115–125

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Solid-phase microextraction gas chromatographic–mass spectrometric method for the determination of inhalation anesthetics in urine

Diana Poli, Enrico Bergamaschi, Paola Manini*, Roberta Andreoli, Antonio Mutti

Laboratorio di Tossicologia Industriale, Dipartimento di Clinica Medica, Nefrologia e Scienze della Prevenzione, Università degli Studi di Parma, Via Gramsci 14, 43100 Parma, Italy

Received 9 April 1999; received in revised form 9 June 1999; accepted 9 June 1999

Abstract

Solid-phase microextraction (SPME) has been applied to the headspace sampling of inhalation anesthetics (i.e. nitrous oxide, isoflurane and halothane) in human urine. Analysis was carried out by gas chromatography–mass spectrometry using a capillary column with a divinylbenzene porous polymeric stationary phase. A SPME divinylbenzene–Carboxen–polydimethylsiloxane coated fiber, 2 cm long, was used, and its performances were compared with those of a Carboxen–PDMS in terms of sensitivity, extraction efficiency, extraction time, fiber coating–urine distribution coefficient. For both fibers, linearity was established over four orders of magnitude, limits of detection were below 100 ng/l for nitrous oxide and below 30 ng/l for halogenated. Precision calculated as %RSD was within 3–13% for all intra- and inter-day determinations. The method was applied to the quantitative analysis of anesthetics in the urine of occupationally exposed people (operating room personnel).

Keywords: Nitrous oxide; Isoflurane; Halothane

1. Introduction

Occupational exposure to inhalation anesthetics involves operating room personnel, anesthesiologists, surgeons and operating room nurses. Nitrous oxide (N_2O) is currently used alone or in combination with other halides anesthetics, namely isoflurane (forane), halothane (fluothane), enflurane (ethrane) and sevoflurane (sevorane). Adverse effects are documented both in experimental animals and in patients receiving anesthesia; spontaneous abortion and liver damage are suspected toxic effects in occupationally exposed people [1]. Environmental concentrations of nitrous oxide below 50 or 25 ppm are recommended by the NIOSH (National Institute of Occupational Safety and Health) when used alone or in combination with other halogenated agents, respectively. The currently adopted threshold limit value (TLV) time-weighted average concentration for 8-h exposure (8-h TWA) for halogenates is 2 ppm, or 0.5 ppm in the case of mixtures including N_2O [2]. The threshold value proposed by the American Conference of Governmental Hygienists (ACGIH) for nitrous oxide and halothane is 50 ppm [3]. Besides environmental

*Corresponding author. Tel.: +39-521-290-344; fax: +39-521-291-343.
E-mail address: medlav@unipr.it (P. Manini)

0378-4347/99/$ – see front matter
PII: S0378-4347(99)00274-1

monitoring, which is useful in evaluating the degree of airborne contamination as well as the compliance with the hygienic standards, individual exposure and the related health risk can be better assessed by biological monitoring, i.e. measuring the concentrations of anesthetic gases or their metabolites in biological media. Alveolar air concentrations [4], blood [5] and urine concentrations [6–8] of anesthetics have been proposed as suitable biomarkers of exposure. It has been demonstrated that small amounts of anesthetics are eliminated unchanged in urine and that there is a relationship between air and urine concentrations collected after 4-h exposure [8]. Although the analysis of exhaled breath is another accepted method of biological monitoring [9], urine sampling is usually preferred for practical reasons. Indeed, urine collection is non-invasive, does not require particular procedures to standardize sampling (as in the case of breath) and samples can be easily stored.

Analytical methods used for the biological monitoring are mainly based on the gas chromatographic analysis of the headspace of the end-shift urine spot samples [7,10]. Packed stationary phase coated columns (GLC) and solid sorbent columns (GSC) are used for the chromatographic separation of liquid anesthetics and permanent gases, respectively. Combined column systems with different column packing materials and lengths allow the separation of complex mixtures, including permanent gases, nitrous oxide and a volatile liquid anesthetic [10]. Only few methods make use of capillary columns, owing to the difficulty to retain nitrous oxide and to their low capacity which means low sensitivity. In fact, the volume of gas injected, together with the system used for detection (flame ionization detector, thermal conductivity detector, electron capture detector or mass spectrometry) are the main factors which determine the sensitivity of the GC method.

The goal of the present work was to develop a method for the simultaneous analysis of nitrous oxide and halogenated anesthetics, namely halothane and isoflurane, based on the use of capillary gas chromatography. To overcome sensitivity problems, solid-phase microextraction (SPME) has been evaluated as a suitable sampling technique. Solid-phase microextraction has been demonstrated to be a very effective, highly sensitive solvent-free sampling technique [11]. It allows rapid sample preparation with preconcentration of a wide range of analytes, it can be used for headspace sampling or for the direct extraction of the analytes by direct immersion from the matrix. Reproducibility during the fiber lifetime and among fibers from different batches makes SPME a reliable technique, suitable for quantitative purposes [12]. Up to date, a considerable number of applications has been published not only in the environmental field, but also in pharmaceutical, clinical and food analysis. Water has been the most investigated matrix [12–14], but more recently the application field has been extended to soils [15], fuels [16], foods and beverages [17] and biological fluids [18].

2. Experimental

2.1. Chemicals

Nitrous oxide (N_2O, boiling point, −88.16°C) technical grade was purchased from Sapio (Milan, Italy). Isoflurane (2-chloro-2-difluoromethoxy-1,1,1-trifluoroethane, Forane, Abbot, Latina, Italy) and halothane (2-bromo-2-chloro-1,1,1-trifluoroethane, Fluothane, Zeneca, Cheshire, UK) are liquid with boiling temperature of 48.5 and 50.2°C, respectively, and with vapour pressure of 238 and 243 mmHg at 20°C. HPLC-grade dichloromethane (boiling point, 39.75°C) was used as the internal standard (I.S., LabScan, Dublin, Ireland).

2.2. Calibration standards

Standard gaseous solutions were prepared weekly filling 10-l sample-bags 232 Series (SKC, Eighty Four, PA, USA) with 4.96±0.07 l of air and by adding the analytes with gas-tight syringes. The stock solution (standard A) was obtained introducing in a sample-bag 10 ml of nitrous oxide, 5 μl of isoflurane and 5 μl of halothane (final concentrations, $8.25\times10^{-5}M$, $8.316\times10^{-6}M$, $9.579\times10^{-6}M$, respectively). In the same way, the I.S. gaseous solution was prepared introducing in a second sample-bag 10 μl of CH_2Cl_2 (final concentration $3.145\times10^{-5}M$). The more dilute standard B was obtained adding 10 ml of standard A in a third sample-bag (final concentrations, $1.66\times10^{-7}M$, $1.7\times10^{-8}M$, $1.9\times10^{-8}M$ for nitrous oxide, isoflurane and

halothane, respectively). Standards were stabilized at least 8 h to have an homogeneous solution. Direct splitless injections of gaseous standard solutions were performed using 10- and 100-μl gastight syringes (Hamilton, Reno, NV, USA).

Two series of calibration samples were prepared in triplicate for SPME analysis. A total of 10 ml of urine with 1 g NaCl and 200 μl of H_2SO_4 9N were collected in 20-ml glass vials. The vials were sealed with Teflon-lined septa and hole caps. Then, 20 μl of internal standard (5.343 μg/l) and different volumes of calibration standards (50, 100 and 500 μl of standard B and 5, 20, 50, 200 and 400 μl of standard A) were added to each vial in order to obtain different calibration samples with concentration of anesthetics in the range 0.0366–145.2 μg/l for nitrous oxide, 0.0156–61.2 μg/l for isoflurane and 0.0187–75.48 μg/l for halothane.

A different series of calibration samples was prepared for static headspace sampling, with concentrations of anesthetics in the range 4.5–363 μg/l for nitrous oxide, 3.8–306 μg/l for isoflurane and 4.7–377 μg/l for halothane.

2.3. General SPME procedures

SPME studies were carried out by using two different kinds of fiber coatings: 75 μm Carboxen–PDMS and 50–30 μm DVB–Carboxen–PDMS (Supelco, Bellefonte, PA, USA). New fibers were conditioned for at least 2 h under a helium stream at the desorption temperature of 240°C in the GC injection port equipped with a 0.75-mm inlet liner for SPME (Supelco). SPME headspace extraction procedure was performed manually. The fibers were suspended at about 2.0 cm above the solution 15 min for Carboxen–PDMS and 20 min for DVB–Carboxen–PDMS. Fresh samples were used for each measurement. After sampling, the fiber was immediately inserted into the GC injection port for thermal desorption (for 16 min at 240°C). No carryover was observed using this approach.

2.4. Static headspace

For comparative purposes, a Hewlett-Packard HP 7694 static headspace sampler (Palo Alto, CA, USA) equipped with a 3-ml sample loop was used. Conditions: oven temperature, 60°C; loop temperature, 70°C; transfer line temperature, 80°C.

2.5. Gas chromatography–mass spectrometry

GC–MS analysis was carried out on a Hewlett-Packard HP 6890 gas chromatograph coupled with a HP 5973 Mass Selective Detector. The analytes were separated on a RT-QPLOT column, 30 m×0.32 mm I.D. (Restek, Bellefonte, PA, USA). Helium was used as carrier gas. The temperature program was: 40°C hold 3 min, 15°C/min to 130°C, hold 1 min, 10°C/min to 180°C, hold 2 min. The temperatures of injector and MS detector were 240 and 230°C, respectively. MS acquisition was performed in SIM, by monitoring the signal of the following ions according to the timetable (dwell-time in parentheses): at 1.8 min, m/z 30 (300) for nitrous oxide; at 10 min, m/z 49 (120), 84 (160) for dichloromethane; at 12.5 min, m/z 51 (300) for isoflurane; at 14.0 min, m/z 117 (300) for halothane. A solvent delay of 1.8 min was set to protect the filament from oxidation.

2.6. Sample preparation and analysis

Urine samples from occupationally exposed workers (operating room personnel) were collected at the end of a 4-h workshift. The subjects voided rapidly in unpolluted areas (significant variations in urinary concentration of anesthetics do not occur if collection time is less than 5 min [6]). About 10 ml of urine were transferred in 20-ml glass vials containing 1 g NaCl and 200 μl of H_2SO_4 9N, with airtight plugs. The vials, stored at 4°C until analysis, were equilibrated for about 1 h at room temperature and thus analyzed by SPME and HS sampler.

3. Results and discussion

3.1. Gas chromatography of anesthetics

A capillary column with a stationary phase consisting of a divinylbenzene porous homopolymer was used for the simultaneous analysis of nitrous oxide and halogenated anesthetics. This column is produced by several manufacturers and is devoted to the analysis of light hydrocarbons, aldehydes, ketones, chlorofluorocarbons and carbon dioxide. Its applica-

tion to anesthetic gases allowed the separation of all the examined compounds in the same chromatographic run, without the need of the complex column systems described in the literature [7,10]. Fig. 1 shows the chromatogram of a standard mixture obtained with the HS–GC–MS system using the RT-QPLOT capillary column. With the temperature program used, nitrous oxide elutes at 2.20 min and is baseline separated from carbon dioxide. The elution of the halogenated compounds required a 15-min chromatographic run.

The use of gaseous instead of liquid standard solutions for all the anesthetics allowed to avoid interferences and peak overlapping arising from other solvents. In fact, the preparation of liquid isoflurane and halothane standard solutions would have required the use of a solvent, chosen considering both its solving properties and the retention time on the RT-QPLOT column. Another advantage of using gaseous versus liquid solutions was a better reproducibility in standard preparation. Isoflurane and halothane are liquid with very low boiling points, which easily vaporize at room temperature. For the same reasons, also the internal standard was handled as a gas.

3.2. SPME method development

Since no data are available in the literature about solid-phase microextraction of inhalatory anesthetics in biological matrices, this study was carried out with the aim of characterizing the partition equilibrium between urine and the SPME fiber coating from both kinetic and thermodynamic points of view. Owing to the high volatility of the compounds investigated, headspace SPME was preferred to direct extraction of the analytes from the aqueous phase. Fast equilibration times, better reproducibility, no memory effects and longer lifetime of the fiber are reported in the case of headspace sampling [13,19]. In the present study, two different fibers were tested and their performances compared in terms of affinity, efficiency and extraction time: a 75-μm Carboxen–PDMS and a new fiber, a 50–30-μm DVB–Carboxen–PDMS 2 cm long. The kinetic aspects of extraction were investigated and preliminarily optimized; then, thermodynamic parameters, such as various distribution coefficients, K_{hs}, K_{fh} and K_{fs}, and the heat of adsorption, ΔH, were calculated in order to understand and to characterize all the equilibria at all the various interfaces sample–head-

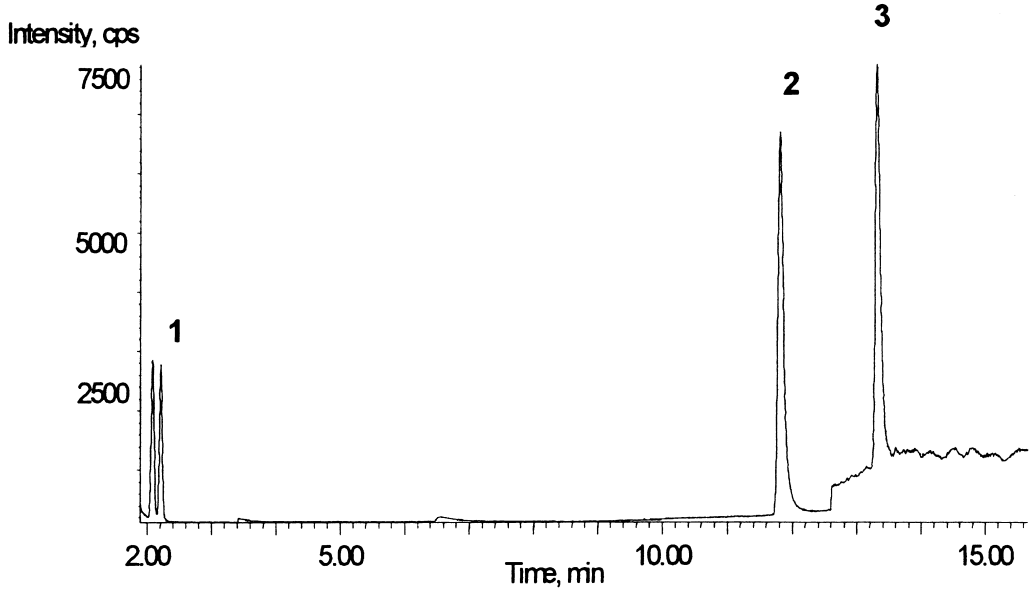

Fig. 1. HS–GC–MS chromatogram of a standard mixture of anesthetics. Peaks identification and standard concentrations: (1) nitrous oxide, 181.5 μg/l; (2) isoflurane, 153.0 μg/l; (3) halothane 188.7 μg/l. Column, RT-QPLOT 30 m×0.32 mm I.D. For other conditions: see Section 2.

space–fiber coating. Static headspace analysis was performed for comparative purposes.

3.2.1. Extraction time profile

The first step in the development of an SPME method is the determination of the time needed for the analytes to reach equilibrium between the matrix, the headspace and the fiber. The extraction time profile was established for each anesthetic and for different coating materials, by plotting chromatographic peak areas versus the extraction time. The equilibration time was assumed to be reached when a further increase of the extraction time did not result in a significant increase in the detector response. Fig. 2 shows the kinetics of the absorption of analytes obtained for the two fibers at room temperature. It can be noticed that for all compounds investigated Carboxen–PDMS and DVB–Carboxen–PDMS reached equilibrium after about 15 and 20 min, respectively. The equilibrium time is longer in the case of the DVB–Carboxen–PDMS, maybe due to its major length: 2 vs. 1 cm for Carboxen–PDMS. During preliminary investigations, the more polar Carbowax–DVB coating (65 μm) was also tested, but it needed about 30 min to reach equilibrium, exhibiting a lower sensitivity for the isoflurane and halothane as compared to other two coating materials. In addition, the Carbowax–DVB was found not to adsorb nitrous oxide at all and, therefore, it was no longer used in this study.

3.2.2. Addition of salt, pH, stirring and temperature

It is known that salting can interfere with extraction, depending on the chemical and physical properties of the analyte and salt concentration. In general, the salting effect increases with increase of compound polarity. A substantial increase in extraction is reported to occur at salt concentrations above 1%. Saturation with salt can be used not only to lower the detection, but also to normalize random salt concentration in natural matrices [11]. In the case of inhalation anesthetics, it was found that a 10% of salt led to 26, 30 and 35% increase in sensitivity for nitrous oxide, isoflurane and halothane, respectively. The addition of a higher percentage of salt (up to 50%) was found not to further improve the amount of analyte extracted. It

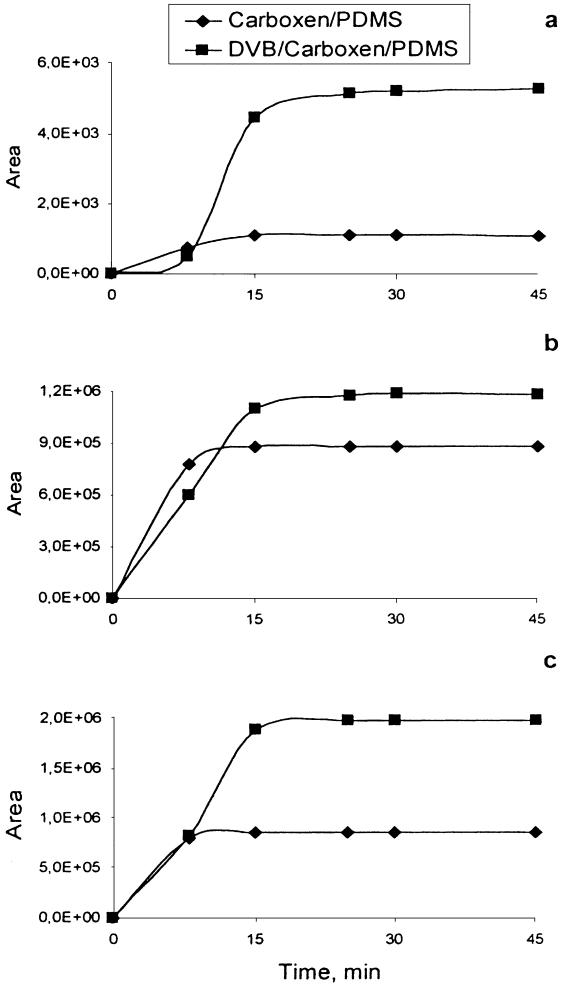

Fig. 2. Exposure time profile of nitrous oxide (a), isoflurane (b) and halothane (c) using Carboxen–PDMS and DVB–Carboxen–PDMS fibers.

has been reported that urinary tract infections can significantly interfere with the concentration of N_2O in the urine samples due to the capability of a variety of microbial species to produce this gas [20]. Since the addition of sulfuric acid is used to avoid the production of N_2O by microorganism in the urine, the effect of pH on the headspace concentration of anesthetics was not further investigated. On the other hand, pH control is recommended only when dissociable species, acids or bases, are analyzed.

In the case of highly volatile compounds, such as anesthetics, the equilibration times are short also at

room temperature and without agitation. From preliminary experiments, it appeared that no considerable gain in sensitivity was obtainable modifying these variables. Therefore, the effect of stirring and heating on the kinetic was not studied. Sampling was performed at room temperature from unstirred samples, which simplifies the method.

3.2.3. Extraction efficiency

Recovery of analytes extracted by headspace SPME was calculated from the comparison of the areas obtained through direct splitless injections of known amounts of the gaseous standard solutions performed by a gastight syringe and the results of the SPME analysis of standard urine samples containing the same amount of anesthetics. The extraction efficiencies calculated at three concentration levels for the inhalation anesthetics are summarized in Table 1. Both fibers showed a low but not negligible capability of adsorbing nitrous oxide (0.1–0.3% depending on the headspace concentration), whereas the efficiency was higher in the case of halide anesthetics. The calculated efficiencies are similar for the two fibers at low concentration levels, whereas at higher headspace concentrations the DVB–Carboxen–PDMS (2 cm long) showed a 2-fold higher capacity.

3.3. Partition coefficients

In headspace-SPME the mass (n) of an analyte extracted by the polymeric coating of the fibers is related to the overall equilibrium of the analyte in the three-phase system, liquid, headspace and fiber

$$n = (K_{fh}K_{hs}V_f c_0 V_s)/(K_{fh}K_{hs}V_f + K_{hs}V_h + V_s) \quad (1)$$

where c_0 is the initial concentration of the analyte in the sample; V_f, V_h and V_s are the volumes of the coating, the headspace and the matrix, respectively, and K_{fh} and K_{hs} are coating–headspace and headspace–sample partition coefficients, respectively [11,19].

The headspace–urine partition coefficients (K_{hs}) at room temperature were calculated for each analytes by direct splitless injections of known volumes of headspace of standard samples at known concentrations. The amounts injected (mol) were calculated by linear equations obtained by direct splitless injection of gaseous standard solutions.

For static headspace sampling, the amount extracted with a gastight syringe n_e is

$$n_e = (K_{hs}V_g c_0 V_s)/(K_{hs}V_h + V_s) \quad (2)$$

where V_g is the volume sampled by the gastight syringe from the headspace above the matrix. The headspace–(salted) urine partition coefficients calculated for each analyte at room temperature were 1.74 ± 0.13 for nitrous oxide, 0.42 ± 0.05 for isoflurane and 0.65 ± 0.06 for halothane. These values are slightly different from that reported in the literature [7] probably due to the salting-out effect. Thereby, the K_{fh} coating–headspace for each analyte

Table 1
Amount extracted (%) of anesthetics at equilibrium by different SPME fibers

Compound	Total amount[a] (nmol)	Amount (%) extracted	
		Carboxen–PDMS	DVB–Carboxen–PDMS
N_2O	0.41	0.2	0.2
	4.12	0.3	0.2
	32.34	0.1	0.1
Isoflurane	0.042	68.6	62.1
	0.416	58.0	51.0
	3.33	22.3	38.7
Halothane	0.048	82.2	81.2
	0.479	74.9	74.5
	3.84	29.3	63.3

[a] Total amount (nmol) of anesthetic in the sample vial.

were calculated from (1) and subsequently the K_{fs} coating–sample partition coefficients from

$$K_{fs} = K_{fh}K_{hs} \quad (3)$$

The values of log K_{fs} (for SPME) and log K_{hs} (for static headspace) are plotted in Fig. 3. For all the anesthetics and particularly for halides, it clearly appears the better efficiency of SPME as compared to static headspace sampling. The absolute response of the SPME is determined by the distribution coefficient K_{fs}.

3.4. Determination of heats of absorption ΔH_{fs}

From the thermodynamic relationship between the temperature and the distribution coefficient K [11,12]

$$d(\ln K)/d(1/T) = -\Delta H/R \quad (4)$$

the heat of adsorption, ΔH, can be determined by measuring K_{fs} at different temperature and by plotting the ln K versus the inverse absolute temperature, $-\Delta H$ being the slope of the straight line obtained. Values of K_{hs} were determined in triplicate for all anesthetics at 25, 40 and 80°C and, given that the amount of analyte extracted at each temperature was known, the corresponding K_{fh} and K_{fs} were calculated as described above. It was found that raising the temperature decreases K_{fs}. Fig. 4 shows the results obtained using the DVB–Carboxen–PDMS fiber. A linear relationship was found between ln K_{fs} and the inverse absolute temperature, with r^2 values of 0.986 and 0.999 for isoflurane and halothane, respectively. No significant relationship was found for nitrous oxide. This is probably due to the high volatility of nitrous oxide, whose headspace concentration is not influenced by changes in tempera-

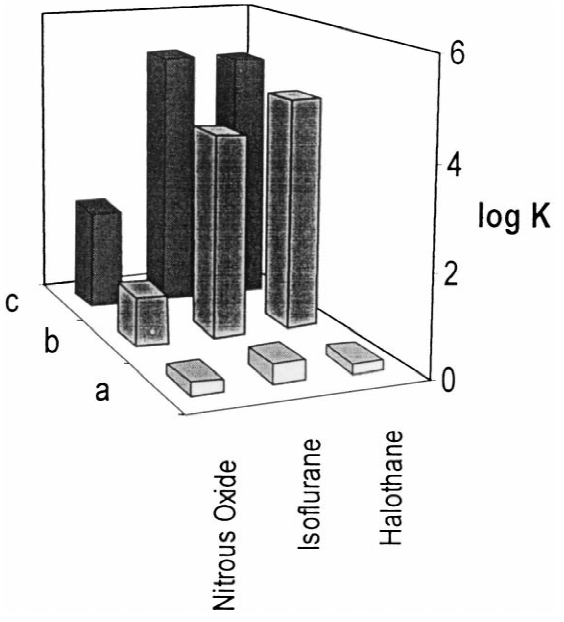

Fig. 3. Comparison among partition coefficients calculated for nitrous oxide, isoflurane and halothane at room temperature: log K_{hs} for static headspace (a) and log K_{fs} for DVB–Carboxen–PDMS (b) and Carboxen–PDMS (c).

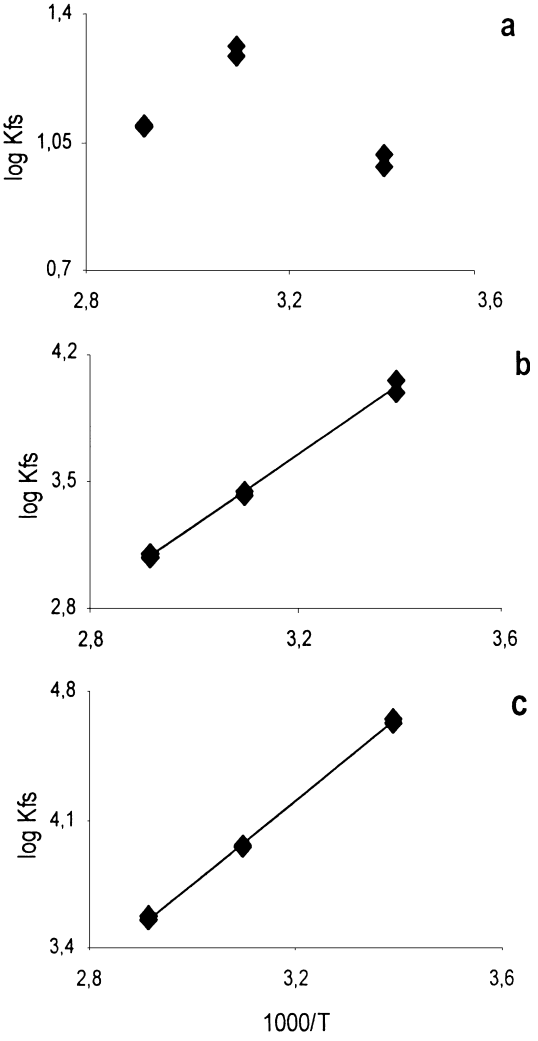

Fig. 4. Plot of ln K versus 1000/T for nitrous oxide (a), isoflurane (b) and halothane (c).

ture. From the slopes of these lines, the calculated heats of adsorption were: −20.0 and −24.2 kJ/mol for isoflurane and halothane, respectively. The decrease in sensitivity observed with increasing extraction temperature can be explained in the light of the exothermic nature of the adsorption process (negative ΔH). A similar behavior was observed using Carboxen–PDMS, suggesting that for both the fibers the Carboxen material is the component with a high affinity for halogenated compounds [21].

3.5. Linear range, detection limits and precision

The experimentally determined linear ranges, the equations, the linear correlation coefficients (r^2) and the limits of detection (LODs) for anesthetics halides and nitrous oxide are shown in Table 2. Experimental data fitted a linear model, $y = bx$, where x is the concentration and y the chromatographic peak areas to internal standard area ratio. The calibration curves were forced through origin, i.e. the intercept was set to zero because the blank values were zero. On the other hand, the intecept values a were negligible. The SPME response for both the fibers was linear over approximately three to four orders of magnitude for all the anesthetics tested, with linear correlation coefficients (r^2) above 0.988 in all cases. For comparative purposes, the same analytical parameters obtained with the static headspace sampler are reported in Table 3.

The LODs were calculated on a signal-to-noise ratio about 3 and were much lower than the values found for the analysis by headspace sampler, from 10-fold in the case of nitrous oxide to 100-fold for halides. Moreover, reliable quantitation by SPME is much below the proposed biological exposure limits for these anesthetics in the operating room personnel: nitrous oxide 28 and 15 μg/l, for airborne concentrations of 50 and 25 ppm, respectively; isoflurane, 5.3 μg/l (for an environmental exposure of 2 ppm) and 1.8 μg/l (0.5 ppm); halothane, 6.1 μg/l (2 ppm) and 3.3 μg/l (0.5 ppm) [7]. Thus, SPME is a valid alternative to the traditional methods for analysis of anesthetics in urine at very low concentrations. It should be noted that the detection limit depends also on the detection system used, and not only on the SPME performances. Further improvements of the SPME–GC method are possible in combination with electron capture detection (ECD).

Table 3
Linearity, slope, correlation coefficient and LOD for each anesthetic sampled by static headspace sampler and analyzed by GC–MS[a]

Compound	Static headspace			
	Range (μg/l)	b[b]	r^2	LOD (μg/l)[c]
N_2O	4.5–363.0	720±40	0.977	1
Isoflurane	3.8–306.0	2673±71	0.993	1
Halothane	4.7–377.4	2054±58	0.992	5

[a] Calibration fitting: $y = bx$ ($n = 15$).
[b] ± values are confidence intervals for 95% probability level.
[c] Limit of detection ($S/N = 3$) calculated under SIM conditions.

Table 2
Linear range, slope, correlation coefficient and LOD for each anesthetic extracted by Carboxen–PDMS and DVB–Carboxen–PDMS fibers and analyzed by GC–MS[a]

Compound	Range (μg/l)	b[b]	r^2	LOD (ng/l)[c]
Carboxen–PDMS				
N_2O	0.365–145.2	$(3.85\pm0.14)\ 10^{-6}$	0.988	75
Isoflurane	0.032–61.2	0.205±0.004	0.996	15
Halothane	0.037–75.48	0.220±0.003	0.999	20
DVB–Carboxen–PDMS				
N_2O	0.365–145.2	$(1.12\pm0.03)\ 10^{-6}$	0.992	100
Isoflurane	0.031–61.2	0.293±0.009	0.989	20
Halothane	0.037–75.48	0.39±0.08	0.990	30

[a] Calibration fitting: $y = bx$ ($n = 27$).
[b] ± values are confidence intervals for 95% probability level.
[c] Limit of detection ($S/N = 3$) calculated under SIM conditions.

The precision of SPME–GC–MS method was calculated in intra- and inter-day studies for both the Carboxen–PDMS and DVB–Carboxen–PDMS fibers. An average relative standard deviation (RSD%) of 3–13% was found for all determination both intra- and inter-day, which is in agreement with previous findings on other volatile compounds [12,14,18]. The %RSD values calculated at different concentrations of anesthetics in urine are displayed in Table 4.

3.6. Application

The method was applied to the quantitative determination of concentrations of nitrous oxide, isoflurane and halothane in urine, as biomarkers of occupational exposure in operating room personnel. Owing to their short half-lives, anesthetics are rapidly excreted from the human body and this could raise questions about the use of urinary concentrations of anesthetics as biomarkers of exposure in occupational medicine. Moreover, anesthetics are very volatile compounds with a poor solubility in water and, therefore, in urine. For these reasons, urine collection, storage and handling before analysis could give rise to analytical errors if such problems are not adequately considered and the procedures rigorously standardized. Nevertheless, the urinary concentration of anesthetics is routinely used for biological monitoring purposes [22] as a suitable complement to environmental monitoring, which poses special issues (e.g., sterility, application of personal samplers, etc.) in operating theatres. Whereas urinary nitrous oxide is widely used in biomonitoring programs, the much lower concentrations of halogenated anesthetics go often undetected by traditional methods, which prompted us to set up a reliable technique based on SPME. Samples were analyzed by SPME and HS sampler. Fig. 5 shows the chromatograms of two urine samples from occupationally exposed workers obtained with Carboxen–PDMS (a) and DVB–Carboxen–PDMS (b) fibers. The concentration of internal standard added to urine sample (approximately 5 μg/l) is comparable with that of all anesthetics found in the series of real urine samples (see below). The reason why nitrous oxide peak area is about 1000 lower as compared to the I.S. area is its very low mass spectrometric response factor. Despite the low value of the area/I.S. area ratio, also in the case of nitrous oxide the MS response was found to be linear over a wide range of analyte concentrations and very low RSD were found in both inter- and intra-day determinations. Undoubtedly, the best choice would have been the use of two different I.S., one for nitrous oxide with similar MS response (but which one?) and another, dichloromethane, for halides. However, such a choice would result in a more complicated method without any practical benefit. This is why we prefer to rely on a single internal standard. Quantitative results of the biological monitoring ($n=12$) were in the range 3.8–22.0 μg/l for nitrous oxide, 0.36–0.87 μg/l for isoflurane and 1.1–4.1 μg/l for halothane. Despite the very low concentrations of halogenates in urine, all the examined anesthetics were detectable after SPME enrichment. However, using static headspace

Table 4
Intra- and inter-day precision ($n=6$) of the SPME–GC–MS method calculated at two concentration levels for nitrous oxide, isoflurane and halothane

Compound	Concentration (μg/l)	Precision (%RSD)			
		Carboxen–PDMS		DVB–Carboxen–PDMS	
		Intra-day	Inter-day	Intra-day	Intra-day
N_2O	1.82	7.2	12.9	7.7	11.9
	72.6	3.4	9.4	6.3	10.5
Isoflurane	0.77	6.2	11.7	7.1	9.7
	30.6	3.2	8.4	3.8	8.7
Halothane	0.95	4.5	9.9	5.5	9.2
	37.7	3.0	6.5	4.1	7.1

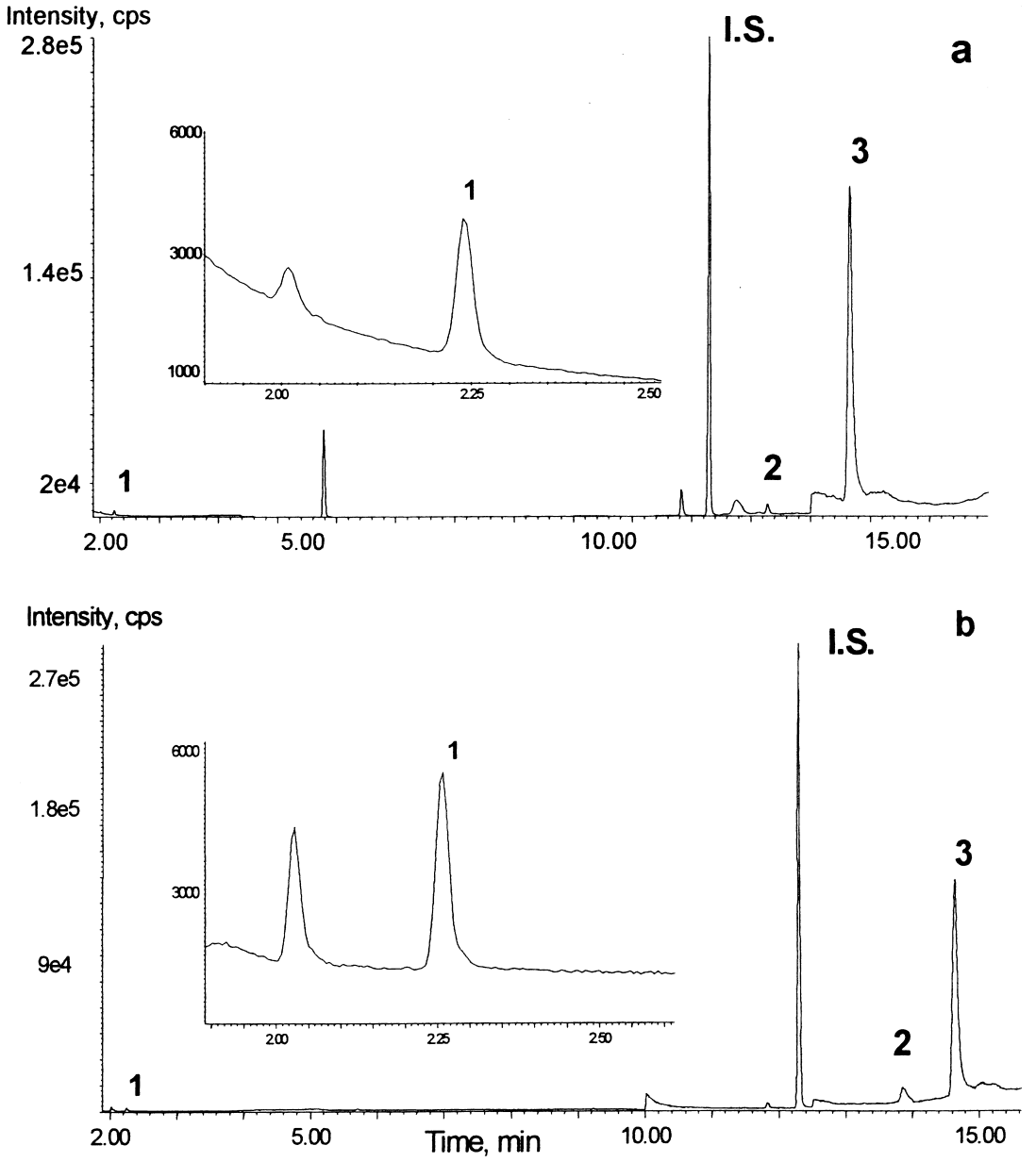

Fig. 5. SPME–GC–MS chromatograms of a urine sample from worker exposed to anesthetics obtained using (a) Carboxen–PDMS and (b) DVB–Carboxen–PDMS. Peaks identification: (1) nitrous oxide; (2) isoflurane; (3) halothane.

sampler only the nitrous oxide peak was measurable. In fact, the urine concentrations of isoflurane and halothane were below the detection limits for static headspace.

4. Conclusions

The present work demonstrated the capability of SPME technique in the analysis of anesthetics,

nitrous oxide, isoflurane and halothane. As already reported by other authors [21], Carboxen–PDMS fibers confirmed a high affinity with regards to halogenated compounds, leading to a very sensitive method, suitable for the determination of trace amounts of anesthetics in urine (15–20 ng/l). Similar performances were obtained using the new 2-cm long DVB–Carboxen–PDMS fibers, which also showed a 2-fold higher capacity at higher headspace concentrations of analytes. Both SPME fibers exhibited a lower but not negligible affinity for nitrous oxide, which is still the main component of mixtures of anesthetics. The sensitivity of SPME–GC–MS method for nitrous oxide is adequate for biological monitoring purposes, being the detection limit 100 ng/l.

Acknowledgements

This work was financially supported by Regione Emilia Romagna (Italy). We are grateful to Mr. Nicola Perchiazzi and Mr. Roberto Ferrari (Supelco, Sigma Aldrich, Milan, Italy) for their skillful assistance and support.

References

[1] C. Edling, Scand. J. Work Environ. Health 6 (1980) 85.
[2] National Institute for Occupational Safety Health (NIOSH). Criteria for a recommended standard. Occupational exposure to waste anesthetic gases and vapours. US Department of Health, Education and Welfare, Washington, DC, 77 (1977) 140.
[3] ACGIH: Exposure Indices. Proposed by American Conference of Governmental Industrial Hygienists for 1995–1996. ACGIH, Cincinnati OH, 1995.
[4] P. Nikki, P. Pfaffli, K. Ahlman, Lancet ii (1972) 490.
[5] A.D. Cattaneo, M. Rovatti, A. Pelosa, J. Zattoni, M. Tomellini, Acta Anaesth. Ital. 34 (1983) 1087.
[6] H. Sonander, O. Stenqvist, K. Nilsson, Ann. Occup. Hyg. 27 (1983) 73.
[7] M. Imbriani, S. Ghittori, G. Pezzagno, G. Ital. Med. Lav. Erg. 20 (1998) 44.
[8] M. Imbriani, S. Ghittori, G. Pezzagno, E. Capodaglio, Appl. Ind. Hyg. 3 (1988) 223.
[9] C. Prado, J.A. Tortosa, I. Ibarra, A. Luna, J.F. Periago, J. Appl. Toxicol. 17 (1997) 179.
[10] A. Uyanik, J. Chromatogr. B 693 (1997) 1.
[11] J. Pawliszyn, in: Solid Phase Microextraction. Theory and Practice, Wiley, New York, 1997.
[12] T. Nilsson, R. Ferrari, S. Facchetti, Anal. Chim. Acta 356 (1997) 113.
[13] T. Nilsson, F. Pelusio, L. Montanarella, B. Larsen, S. Facchetti, J.O. Madsen, J. High Resolut. Chromatogr. 18 (1995) 617.
[14] S.S. Johansen, J. Pawliszyn, J. High Resolut. Chromatogr. 19 (1996) 627.
[15] J.J. Langenfeld, S.B. Hawthorne, D.J. Miller, Anal. Chem. 68 (1996) 144.
[16] A. Fromberg, T. Nilsson, B.R. Larsen, L. Montanarella, S. Facchetti, J.O. Madsen, J. Chromatogr. A 746 (1996) 71.
[17] T. Peppard, X. Yang, J. Agric. Food Chem. 42 (1994) 1925.
[18] R. Andreoli, P. Manini, E. Bergamaschi, A. Brustolin, A. Mutti, Chromatographia (in press).
[19] Z. Zhang, J. Pawliszyn, J. High Resolut. Chromatogr. 19 (1996) 155.
[20] P. Apostoli, M. Gelmi, L. Alessio, A. Turano, Occup. Environ. Med. 53 (1996) 591.
[21] P. Popp, A. Paschke, Chromatographia 46 (1997) 419.
[22] R.R. Lauwerys, P. Hoet, Industrial Chemical Exposure, 1993, 98–99, 189–191, Lewis Publishers, Boca Raton, FL.