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Evaluation of isoflurane in air by thermal desorption–gas chromatography☆

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ABSTRACT

A method to quantify the exposure of operating room personnel to isoflurane has been developed. Thermally desorbable passive samplers were used to adsorb isoflurane. The anaesthetic concentration was then determined by gas chromatography. Several adsorbents were evaluated, Chromosorb 106 being the most adequate for isoflurane adsorption and thermal desorption. Additionally, a suitable calibration method for thermal desorption–GC was developed. Finally, a laboratory evaluation was carried out to determine the isoflurane sampling rate for the chosen sorbent. The effects of humidity and storage time on both the system response and reproducibility were also checked.

INTRODUCTION

There is growing evidence that occupational exposure to anaesthetic gases represents a health hazard. In general, the numerous epidemiological investigations agree fairly well that there is an excess risk of spontaneous abortion, but no agreement exists on carcinogenic and teratogenic effects [1]. It has been reported that one of the most serious shortcomings of these studies is the lack of quantitative information on the nature, degree and length of exposure to anaesthetic agents that could allow any cause–effect relationship between exposure and adverse effect to be found [2].

Isoflurane (Forane, 1-chloro-2,2,2 trifluoroethyl-difluoromethyl-ether), either alone or with nitrous oxide, has become one of the most widely used inhalation anaesthetics in operating theatres [3]. Therefore, it is important to develop simple methodologies for the determination of the exposure to isoflurane of personnel working in these environments.

The most common method for the determination of personnel exposure to gases and organic vapours is to draw air, by means of a portable pump, through a tube containing a solid sorbent able to retain the specific compound of interest. This way of sampling, called active, is not useful for the operating theatre staff, since it is undoubtedly uncomfortable. Alternatively, passive sampling does not require air-taking devices, so it offers a good and unobstrusive way of sampling.

In passive sampling, the environmental pollutant diffuses across a convection-free air zone within the sampler to a collection medium, where it is adsorbed. In most passive samplers, the adsorbed vapour is desorbed with a solvent

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and quantitatively measured by gas chromatography. Owing to the relatively low concentrations of isoflurane in operating theatres, solvent desorption techniques are insufficiently sensitive, since only a small amount of the collected isoflurane would be analysed. Thus, thermal desorption methods can offer some advantages, the most important being their higher sensitivity, since the whole sample is available for analysis.

The aim of this study was to develop a method to quantify the exposure of operating room personnel to isoflurane by means of thermally desorbable passive samplers. A suitable sorbent was selected, the performance of diffusive samplers for isoflurane was evaluated and the effects of humidity and storage time on sorbent capacity were checked under controlled laboratory conditions.

Finally, the use of a controlled atmosphere device for the calibration of the thermal desorption–gas chromatography system is also described.

EXPERIMENTAL

Passive samplers

The passive samplers used were the standard stainless-steel tubes for an ATD50 thermal desorption system (Perkin-Elmer, Beaconsfield, UK) (89 mm × 6.4 mm O.D.). The tubes were packed with 150 mg of a solid sorbent. Prior to use, and during storage, the tubes were protected with storage caps; when used, a diffusion cap was fitted to allow controlled exposure to the environment.

Thermal desorption–gas chromatography system

All passive samplers were desorbed by means of the ATD50 system, directly connected to a Perkin-Elmer Model 8700 gas chromatograph by a heated transfer line. Thermal desorption was done in two stages: first, the tube was heated with the carrier gas flowing through it, transferring the desorbed vapours from the sample tube to a cooled trap (packed with Tenax TA, SKC, Valley View, PA, USA). Afterwards, when the entire sample had been collected, the trap was rapidly heated to desorb the volatile materials, which were rapidly injected into the GC column via the heated transfer line.

Adsorbent selection

The solid sorbents studied were packed in the standard sample tubes and placed between the injector and the flame ionization detector of the gas chromatograph, like a column, and a given flow of carrier gas (dry nitrogen) was passed through it. A known amount of isoflurane (Forane, Abbot Laboratory, Madrid, Spain) was injected, and the volume of carrier gas required up to the beginning of the elution peak of isoflurane was calculated. Measurements were made in the 40–180°C temperature range.

Three sorbents were considered, Tenax TA, Chromosorb 102 and Chromosorb 106 (SKC).

Calibration method for the thermal desorption–gas chromatography system

Calibration standard tubes were prepared by generating an isoflurane standard atmosphere, and injecting known volumes of this through the sampling tube. Standard atmospheres of isoflurane vapour in air were dynamically generated using the syringe injection technique, as previously described [4], and were checked by active samplers consisting of activated charcoal tubes connected to the atmosphere by means of a sampling pump. In all cases the concentration measured by the activated charcoal tubes agreed to the theoretical concentration calculated from airflow and syringe speed within 10% [5], so this value of the theoretical concentration can be considered as the true concentration of isoflurane in the test atmosphere (X_{ref}). The coefficient of variation, as determined by on-line chromatography, was 4%.

Fig. 1 illustrates the system used to generate the controlled atmosphere as well as to obtain calibration standards. To do the latter, a servomotor-driven gas syringe of 50 ml capacity alternately draws from the standard atmosphere and expels the sample through the adsorbent tube, by means of both an inversion of the polarity of the motor that powers the syringe and automatic changes in the position of a three-way valve. Different volumes of air were drawn through the adsorbent tubes to obtain different calibration standards over the range required.

Determination of sampling rate and performance of diffusive samplers for isoflurane

Diffusive sampling was carried out with the

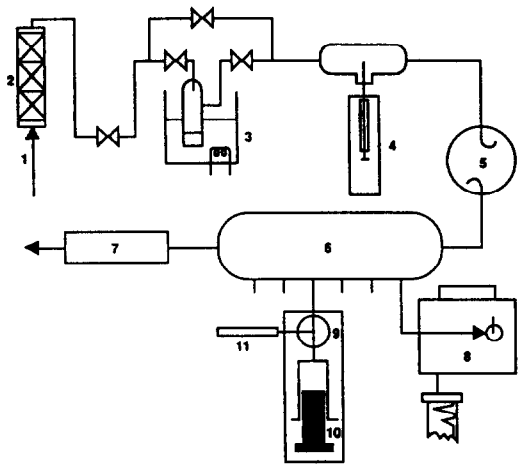

Fig. 1. Scheme of the system used to generate the controlled atmosphere and to obtain calibration standards. 1 = Air intake; 2 = filter; 3 = humidifier; 4 = automatic injector; 5 = mixing chamber; 6 = sampling chamber; 7 = humidity sensor; 8 = gas chromatograph; 9 = three-way PTFE valve; 10 = servomotor-driven syringe; 11 = adsorbent tube.

ATD50 sampling tubes packed with Chromosorb 106 having diffusive caps without membranes.

The experiments with isoflurane were performed according to the scheme shown in Table I, which is a concise version of the Instituto Nacional de Seguridad e Higiene en el Trabajo (INSHT) protocol [5]. This requires the generation of controlled atmospheres having three different concentrations of the pollutant (for isoflurane 1.3, 13 and 26 ppm were used) and the simultaneous exposure of six samplers for each one of the five experiments. The humidity of the final atmosphere could be adjusted by introducing water bubblers into the incoming airflow. A temperature of 20–25°C, a relative humidity (RH) of 40–45% and a relative air speed on the surface of the diffusive monitors of 0.7–1 m/s were used.

TABLE I

CONCISE VERSION OF THE INSHT PROTOCOL TO EVALUATE THE PERFORMANCE OF THE PASSIVE SAMPLER FOR ISOFLURANE

Concentration (ppm)	Sampling time		
	0.5 h	2 h	8 h
1.33	X		X
13.25		X	
26.50	X		X

Thermal desorption of the isoflurane collected was carried out in two stages with nitrogen as a carrier gas (68.95 kPa), an oven temperature of 240°C, 6 min of desorption time, trap lower and upper temperatures of −30 and 240°C, respectively, and a transfer line temperature of 100°C. The GC analyses were performed with a fused-silica capillary column (25 m × 0.2 mm I.D., free fatty acid phase 0.3 μm film thickness) (Hewlett-Packard, Palo Alto, CA, USA), using nitrogen at 68.95 kPa as carrier gas. The oven and detector temperatures were 90 and 200°C, respectively.

Sampling rates (diffusive uptake rates, *SR*) were calculated for each of the samplers, according to the following equation derived from Fick's law

$$SR = \frac{m}{c \cdot t} \quad (1)$$

where m is the amount of isoflurane adsorbed (ng), c is the atmosphere concentration (ppm, v/v) and t is the exposure time (min).

A set of four experiments was carried out at two different isoflurane concentrations (1.33 and 26.5 ppm) and two exposure times (90 and 480 min), to determine the effect of humidity on the uptake capacity of the adsorbent solid. In addition, samplers containing different amounts of isoflurane were stored for 2 weeks at room temperature to test storage stability.

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RESULTS AND DISCUSSION

Adsorbent selection

It has been reported that non-constant sampling rates are a serious drawback of the use of passive samplers for thermal desorption [6]. Some papers claim that the observed variations are functions of time solely, so that the effective

sampling rate during an actual exposure can be calculated from calibration graphs of uptake rate *versus* time [7,8]. Other studies predict reduced sampling efficiency as a function of both concentration and time [6,9,10]. In general, it is accepted that the phenomenon of decreasing uptake rates is associated with the relative strength of the adsorbent.

Adsorbents for thermal desorption should show, on the one hand, high adsorptivity to ensure that the rate of sample concentration is constant over the period of exposure and, on the other hand, sufficiently low adsorptivity to allow complete thermal desorption. So, it is very important to select the more adequate adsorbent to each pollutant, especially in this case because isoflurane is a very volatile compound.

Three adsorbents were checked: Tenax TA, Chromosorb 102 and Chromosorb 106. The plots of the logarithm of the "initial specific retention volume" *versus* reciprocal absolute temperature for the three adsorbents are shown in Fig. 2. From this plot the specific retention volumes at 20°C can be calculated, being 45.2, 7.3 and 0.2 l/g for Chromosorb 106, Chromosorb 102 and Tenax TA, respectively.

These calculated values seem to be low, since it has been reported that to ensure the adsorbent is strong enough the retention volume should exceed 60 l per g of adsorbent at room temperature. However, in special cases, weaker adsorbents may be used [7], and it is to be considered that the values reported here are for initial, not overall, specific retention volumes.

The values obtained indicated that Chromosorb 106 was the strongest adsorbent of the three evaluated, while Tenax had the lowest adsorption capacity, under the experimental conditions used in the present work. However, the use of Tenax for isoflurane collection has been described previously [11].

Based on the above-mentioned results, Chromosorb 106 was initially chosen as the adsorbent. Its strength was then tested in order to ensure that it would be able to give a constant sampling rate. Thus, sampling rates were calculated for a wide range of concentrations (between 1.3 and 27.2 ppm) and times (between 30 and 480 min) for a set of six samplers for each of the experiments.

Fig. 3 shows the average values of sampling rates for all six samplers under the different conditions tested. There were no significant changes in sampling rate even at high exposure dose, when it has been claimed that sampling rate should decrease for a non-ideal adsorbent [12]. So, Chromosorb 106 was definitively selected for isoflurane uptake.

Calibration methods for the thermal desorption–gas chromatography system

The ATD50 was used with an on-line gas chromatograph, and the relationships between sample amount on the tube and integrated peak

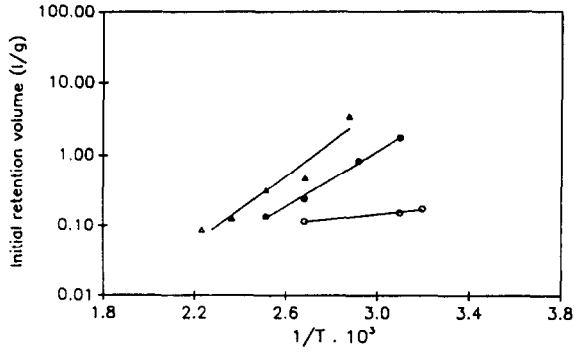

Fig. 2. Determination of adsorbent strength for isoflurane on (○) Tenax TA, (●) Chromosorb 102 and (△) Chromosorb 106.

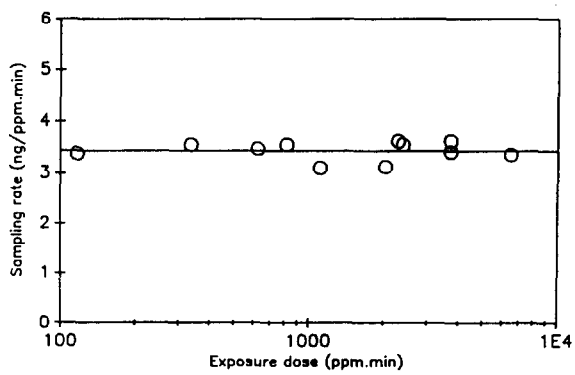

Fig. 3. Variation of sampling rate with time and concentration of isoflurane.

area had to be established. One of the main difficulties with such a system is achieving an appropriate calibration, since it cannot be carried out in a straightforward way as for normal chromatography. This is because measured concentrations are very low, of the order of a few micrograms and internal standards cannot be used, so injections have to be very precise; standards used for calibration should follow the same treatment protocol as ambient samples. Additionally, in this study, the compound to be analysed is very volatile, so a better wide-application method for calibration would be desirable. Thus, the commonly suggested methods for obtaining calibration curves cannot be used. This is because isoflurane is so volatile that it cannot be injected alone, using the ATD injector, since it evaporates from the needle, resulting in wrong injection volumes [13] and, alternatively, any solvent to be used must elute after isoflurane. This latter condition results in long analysis time and, additionally, the solvent accumulates in the cold trap, thus requiring several cleaning desorptions.

Taking into account the above-mentioned facts, the use of an isoflurane-controlled atmosphere for the generation of calibration standard tubes is proposed, according to the details described in the Experimental section and Fig. 1. This system allows for a reproducible and reliable way to transfer known amounts of sample to the GC system through the ATD apparatus. Additionally, it allows the most appropriate calibration range to be chosen, using the same isoflurane atmosphere. No solvent is required, so analysis times are shortened. This shortening is even improved by the fact that standard calibration tubes are easily prepared, thus facilitating the calibration curve checking. As no internal standard can be used, the proposed method helps to carry out the full analysis with the highest precision and reproducibility possible (as Fig. 4 shows), thus overcoming the disadvantages associated with the presently available methods for volatile compounds.

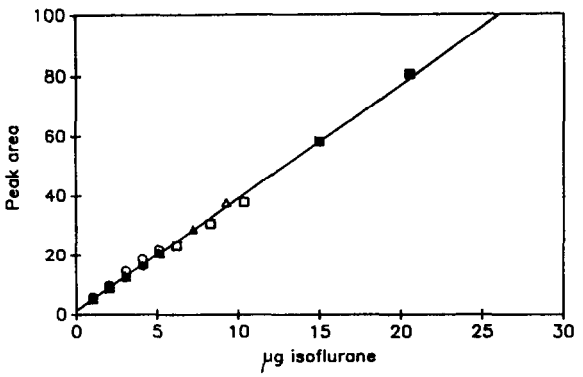

Fig. 4. Calibration curve for isoflurane using standard tubes obtained by controlled atmospheres. Different symbols correspond to different days the calibration was carried out.

Performance of the sampler for isoflurane

For a passive sampler to be appropriate, *SR* must be constant for any combination of exposure time and concentration, since it is this parameter that allows the amount of contaminant collected by the sampler to be correlated with its ambient concentration. Therefore, the sampler behaviour towards environmental isoflurane under controlled laboratory conditions has to be determined and the effect of changes in variables such as concentration of isoflurane in air, sampling time and moisture content of air require to be established. The INSHT diffusive sampler validation protocol [5] has been used to evaluate the performance of the sampler for isoflurane. This protocol is a reliable and well-established one, whose requisites match those of the well-known Health and Safety Executive (HSE) protocol [14].

The *SR* values were calculated according to eqn. 1, and the results are summarized in Table II. For 30 min and low-level concentration the peak area was indistinguishable from background. From the results, the experimental *SR*, as a mean of all the values obtained, was calculated, resulting in a value of 3.43 ng/ppm · min. Using this experimental *SR* value, the concentration for each sampler was recalculated; its mean value, X, and the standard deviation (R.S.D.) were determined for each of the experiments carried out, as shown in Table III. The obtained results fulfil the protocol requirements, as the precision was less than 7% and the bias less than 10% for all cases. Thus, the above-

TABLE II

SR VALUES FOR DIFFERENT COMBINATION OF EXPOSURE TIME AND ISOFLURANE CONCENTRATION

Concentration of isoflurane	Sampling rates (*SR*) (ng/ppm · min)		
	90 min	290 min	480 min
X_{ref} = 1.27 ppm	3.27		
	3.29		
	3.16		
	3.66		
	3.37		
	3.53		
X_{ref} = 1.28 ppm			3.44
			3.47
			3.42
			3.66
			3.39
			3.45
X_{ref} = 12.82 ppm		3.47	
		3.54	
		3.84	
		3.82	
		3.50	
		3.54	
X_{ref} = 26.52 ppm	3.62		
	3.41		
	3.79		
	3.47		
	3.47		
	3.55		
X_{ref} = 23.13 ppm			3.02
			3.10
			3.08
			3.08
			3.34
			3.20

mentioned value for the sampling rate is valid and suitable and can be considered as the standard sampling rate of the sampler.

Humidity can influence the performance of the adsorbent, altering its adsorption capacity. The results of the experiments to check the effect of humidity on Chromosorb 106 are shown in Table IV, where it can be seen that high humidity values affected neither the collection nor the recovery of isoflurane.

TABLE III

ANALYSIS OF EXPERIMENTAL DATA TO DETERMINE THE PROTOCOL COMPLIANCE

X = Mean value of the measured concentration; X_{ref} = true concentration of the vapour in the test atmosphere; bias = $(X - X_{ref})/X_{ref} \times 100$ ($\leq$10% [5]); R.S.D. = relative standard deviation ($\leq$7% [5]).

90 min	290 min	480 min
X = 1.26 ppm X_{ref} = 1.27 ppm Bias = 0.8% R.S.D. = 6.3%		
		X = 1.28 ppm X_{ref} = 1.28 ppm Bias = 0.0% R.S.D. = 2.8%
	X = 13.53 ppm X_{ref} = 12.82 ppm Bias = 5.5% R.S.D. = 4.6%	
X = 27.46 ppm X_{ref} = 26.52 ppm Bias = 3.5% R.S.D. = 3.9%		
		X = 21.16 ppm X_{ref} = 23.13 ppm Bias = 8.5% R.S.D. = 3.6%

When dealing with automatic sampling, it is interesting to know how long samples can be stored prior to analysis without loss or alteration of the collected pollutants. Thus, it is recom-

TABLE IV

EFFECT OF HUMIDITY ON ISOFLURANE RECOVERY

Concentration (ppm)	Relative humidity (%)	Exposure time (min)	Recovery[a]
1.27	40	90	0.992 ± 0.062 (6)
1.31	98	90	0.999 ± 0.024 (6)
23.13	40	480	1.001 ± 0.036 (6)
26.27	98	480	0.999 ± 0.033 (6)

[a] Mean ± standard deviation; number of samples in brackets.

TABLE V

EFFECT OF STORAGE ON ISOFLURANE RECOVERY

Concentration (ppm)	Exposure time (min)	Recovery
2.02	90	1.001 (6) 0.998 (4)[a]
26.29	480	1.001 (6) 1.091 (2)[a]

[a] Samples analysed 2 weeks after exposure; number of samples in brackets.

mended that the effects of a period of storage, under standard and controlled conditions, after exposure to a pollutant vapour should be investigated. The results of the storage experiments, which are shown in Table V, indicate that samples can be stored for periods of 2 weeks at room temperature without loss of recovery.

CONCLUSIONS

Chromosorb 106 is a suitable adsorbent for isoflurane as it allows a constant sampling rate independent of both time and concentration. The experiments carried out to study the performance of the passive sampler —a standard ATD sampling tube containing Chromosorb 106— indicated that this sampler can be used for the determination of isoflurane in operating room ambient. Its standard sampling rate was 3.43 ng/ppm · min. Samplers can be stored, at room temperature, for periods up to 2 weeks without affecting the recovery, and there was no observable effect of humidity.

The generation of calibration standard tubes by using a controlled atmosphere and injecting known volumes of it through the sampling tube eliminates many of the problems encountered with other calibration methods and gives a good reproducibility.

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