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Enflurane as an internal standard in monitoring halogenated volatile anaesthetics by headspace gas chromatography–mass spectrometry

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Abstract

Recently, we proposed the use of a run-only headspace-GC–MS method for the biological monitoring of ppb concentrations of unmodified volatile anaesthetics (isoflurane, sevoflurane and halothane, plus nitrous oxide) in post-shift urine of operating theatre personnel. The adoption of enflurane (a volatile anaesthetic no longer used in clinical practice) as a proper and viable internal standard improves intra-day and inter-day accuracy in halide quantitation, providing a GC–MS reference method useful in the practice of biomonitoring of exposure of operating theatre personnel to modern volatile anaesthetics (isoflurane, sevoflurane, halothane).

Keywords: Headspace analysis; Internal standards; Anesthetics; Enflurane

1. Introduction

Gas chromatography–mass spectrometry (GC–MS) technique is a powerful tool for biomonitoring of occupational exposure to many toxicants or hazardous substances. Automation of sampling and injection greatly reduces sources of variability. Nevertheless, it is commonly accepted that the headspace technique coupled to GC–MS has a lower reproducibility than liquid injection. In particular, headspace reproducibility may be affected by system-pressure variations.

Although mass quadrupole technology has greatly improved, reaching ppb sensitivity levels, linearity of MS is still poor with respect to traditional GC detection methods, such as flame ionization detection and electron-capture detection. Thus, extensive calibration has to be performed for every analytical sequence. In particular, use of proper internal standards (I.S.) is highly recommended for GC–MS quantitative analysis [1]: the technique of choice is isotopic dilution by deuterated homologues of the analytes [2,3].

We recently proposed routine use of a simple, precise and accurate, run-only headspace-GC–MS method for biological monitoring of ppb concentrations of unmodified volatile anaesthetics (halides such as isoflurane, sevoflurane and halothane, and nitrous oxide) [4,5]. Post-shift levels of urinary halogenated anaesthetics in operating theatre personnel (3–7-h exposure) correlate well with the corresponding breathing zone data [4]. Moreover, the

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method is able to reveal ~0.1 $\mu g/l_{urine}$ of each analyte and does not require λ (liquid–gas partition coefficients) determination [4].

Herein, we suggest the possibility of using enflurane as an I.S. to enhance GC–MS accuracy for quantitative determination of halide in urine headspace. Enflurane is a readily available halogenated volatile anaesthetic and is a structural isomer of isoflurane that is no longer in clinical use in Western countries.

2. Experimental

2.1. General

Enflurane 99% (CAS No. 13838-16-9), sevoflurane 99% (CAS No. 28523-86-6) and isoflurane 99% (CAS No. 226675-46-7) were purchased from Abbott Labs. (Abbott Park, IL, USA), while halothane 98% (CAS No. 151-67-7) was from Zeneca (Macclesfield, UK). Three working solutions of mixed anaesthetic halides (isoflurane, sevoflurane, halothane) in the approximate range of 6–600 ppm were prepared by dilution in low-benzene carbon disulphide (≥99.5%, Fluka–Sigma–Aldrich, Milan, Italy) and stored at 4 °C.

Calibration standards were prepared by spiking known amounts of working solutions in 20-ml presealed vials previously prepared with 10 ml of blank urine and fluxed with nitrogen (99.9%), according to our previously published method [4]. Afterwards, 1 μl of I.S. working solution (enflurane 137.9 ppm in low-benzene carbon disulphide, stored at 4 °C) was added to calibration vials and equilibrated overnight at 4 °C before headspace-GC–MS-analysis. Based on previous studies [4,6] in which the loss process was studied by simulation, PTFE vial septa were used to avoid analyte loss.

2.2. Gas chromatography

A Hewlett-Packard 7694 Static Headspace Sampler mixed and equilibrated each calibration vial for 2 h at 41 °C, then transferred 1 ml of urinary headspace to a GCD integrated GC–MS system (Agilent, Palo Alto, CA, USA), equipped with a Chrompack capillary column PoraPlotQ (27 m × 0.25 mm I.D., 8 μm thickness) (Varian, Walnut Creek, CA, USA). Helium was used as carrier gas (flow set to 1 ml/min). The injector (split ratio 20:1) and MS interface temperatures were set to 200 and 280 °C, respectively; oven temperature was initially 40 °C (4 min), then ramped to 140 °C (40 °C/min increase). A test chromatogram is reported in Fig. 1.

2.3. Intra-day and inter-day precision assays

Four-points (plus blank) calibration plots were obtained for each analyte in quintuplicate (intra-day assay). Inter-day precision was assessed by pooling a total of five intra-day determinations. The data sets obtained were split by either considering or not considering I.S. introduction (internal or external calibration); the resulting mean percent relative standard deviations (RSD) were compared using the paired Student's *t*-test (significance set at $P < 0.05$) using the Intercooled Stata 7.0 software programme (Stata, College Station, TX, USA).

2.4. Mass spectrometry

Detection and quantitation of each analyte were performed by SIM, using two different ion windows (see chromatogram shown in Fig. 1). In particular, the quantifier (Q_1) and qualifier (Q_2) ions were, respectively: 131 and 51 m/z for sevoflurane; 51 and 117 m/z for isoflurane; 117 and 51 m/z for enflurane (I.S.); and 117 and 196 m/z for halothane. The dwell time for each ion was set at 60 ms.

3. Results and discussion

Five-points calibration plots, including blank urines (where no detectable analyte peaks were found), were determined in quintuplicate on 5 different days, considering or ignoring I.S. Obtained plots were forced through origin (intercept set to zero) and showed good linearity (within the ranges 0.6–608 $\mu g/l_{urine}$ for sevoflurane, 0.6–598 $\mu g/l_{urine}$ for isoflurane, and 0.75–748 $\mu g/l_{urine}$ for halothane) both in the presence and absence of I.S. correction ($r^2 \geq 0.9997$ and $r^2 \geq 0.9991$, respectively, for all analytes; Table 1).

In all intra-day data sets, use of enflurane as I.S.

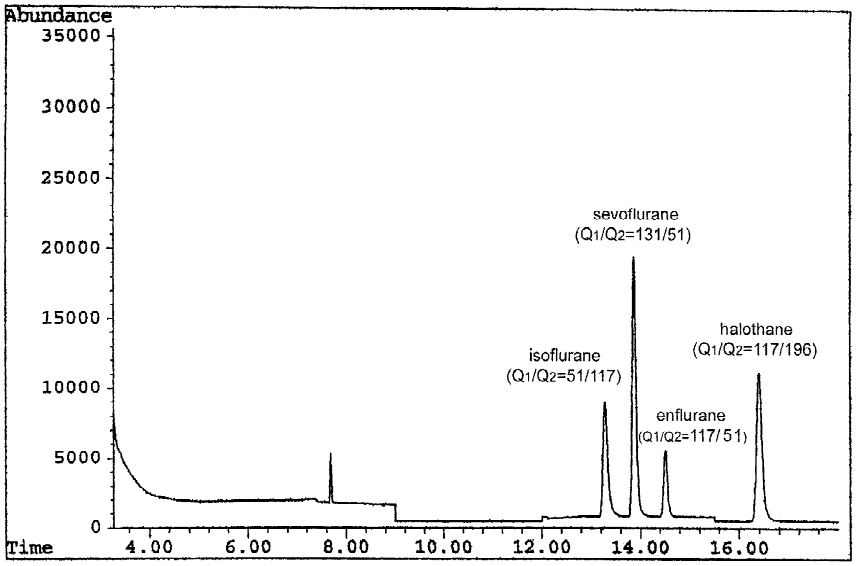

Fig. 1. Total ion chromatogram of intermediate calibration level of volatile halide anaesthetics [~60 $\mu g/l_{urine}$ of each one and 14 $\mu g/l_{urine}$ of enflurane (I.S.)]. Q_1 = quantifier ion; Q_2 = qualifier ion. Time scale in minutes; a 18-min run time was necessary for nitrous oxide determination (t_R = 3.5 min, Q_1/Q_2 = 30/44) in the same analytical run.

significantly improved mean precision (expressed as RSD). For example, in the most variable intra-day data set (reported in Table 2), I.S. adoption lowered mean RSD from >15 to <7% ($P<0.02$). Similarly, in the four other intra-day assays, the precision was rather variable and sometimes poor with no I.S., the mean RSD ranging between 4 and 13%; in these assays, when the I.S. was considered, the mean RSD fell to <4% (data not shown). All I.S.-corrected intra-day data sets showed a discrepancy between mean and median RSD, due to lack of significant improvement in the precision of the lowest concentration calibration point of each analyte (Table 2). Indeed, the lowest calibration points were close to the theoretical quantification limit of each analyte (signal-to-noise ratio ≈ 6).

Similarly, with the exception of the lowest concentration point of each analyte, inter-day RSD (5 days) was reduced when the data sets were corrected by I.S. area (≤8%, $P<0.04$; Table 3). Remarkably, when external—but not internal—calibration was used, the intra-day mean RSD values were sometimes higher than the inter-day ones (Table 4). Moreover, with external calibration, in some cases

Table 1
Linear range, slope with 95% confidence interval (CI) and correlation coefficient (R^2) for each volatile anaesthetic (n = 5) sampled by static headspace sampler and analyzed by GC–MS with and without internal standard (I.S.)

Analyte	Range ($\mu g/l$)	Slope	95% CI	R^2
With no I.S.				
Sevoflurane	0.61–608.0	7510.9	7413.5–7608.2	0.9995
Isoflurane	0.60–598.4	13 317.9	13 081.2–13 554.7	0.9991
Halothane	0.75–748.4	7653.8	7591.8–7715.8	0.9998
With I.S.				
Sevoflurane + I.S.	0.61–608.0	0.06325	0.06297–0.0635	0.9999
Isoflurane + I.S.	0.60–598.4	0.11214	0.11114–0.11314	0.9998
Halothane + I.S.	0.75–748.4	0.06447	0.06377–0.06516	0.9997

Table 2
Intra-day precision (1 day, $n=5$) of headspace-GC–MS method for anaesthetic halide quantitation with and without internal standard (I.S.), expressed as percentage relative standard deviation (RSD)

Analyte	Amount ($\mu g/l_{urine}$)	Intra-day precision, no I.S. correction, RSD (%)		Intra-day precision, I.S. correction, RSD (%)	
Sevoflurane	0.608	16.41		17.22	
	6.08	2.64	Mean 18.22	2.70	Mean 6.19
	60.8	21.66	Median 19.03	2.17	Median 2.69
	608	32.16		2.68	
Isoflurane	0.598	3.70		3.80	
	5.98	3.70	Mean 15.33	1.49	Mean 2.43
	59.8	21.40	Median 12.55	2.08	Median 2.21
	598	32.52		2.34	
Halothane	0.748	13.57		16.02	
	7.48	3.19	Mean 17.86	2.69	Mean 5.84
	74.8	21.98	Median 17.77	3.23	Median 2.96
	748	32.69		1.41	

Mean and median RSD values are reported.

RSD increased in proportion to the amount of halogenated anaesthetics (Table 2). These phenomena could have been due to a sudden, random fall in the sensitivity of the MS detector during an analytical sequence (i.e. intra-day data set). Loss of linearity in detector response could account for the greater degree of intra-day variability when the I.S. was not used. Alternatively, the sampling precision of the headspace system could be affected by pressure variations in the headspace of the vials containing the mixture of volatile analytes at high concentrations. In any case, the present study under-

Table 3
Mean inter-day precision (5 days) with and without internal standard (I.S.) correction, expressed as percentage relative standard deviation (RSD)

Analyte	Amount ($\mu g/l_{urine}$)	Inter-day precision, no I.S. correction, RSD (%)		Inter-day precision, I.S. correction, RSD (%)	
Sevoflurane	0.608	11.39		19.66	
	6.08	13.12	Mean 10.22	2.89	Mean 8.06
	60.8	6.85	Median 10.45	4.78	Median 4.85
	608	9.51		4.93	
Isoflurane	0.598	17.66		4.89	
	5.98	14.86	Mean 10.45	4.84	Mean 4.42
	59.8	3.34	Median 10.40	3.35	Median 4.73
	598	5.93		4.61	
Halothane	0.748	15.89		13.61	
	7.48	8.30	Mean 10.25	6.10	Mean 7.52
	74.8	6.88	Median 9.11	3.37	Median 6.56
	748	9.93		7.02	

Mean and median values of RSD are reported.

Table 4
Comparison between intra- and (5 day) inter-day variability, expressed as mean percentage relative standard deviation (RSD) within the data sets reported in Tables 2 and 3 when no internal standard was used

Analyte	Mean intra-day RSD (%)	Mean inter-day RSD (%)
Sevoflurane	18.22	10.22
Isoflurane	15.33	10.45
Halothane	17.86	10.25

With external calibration, variability of intra-day precision was sometimes higher than that of inter-day precision (this did not occur when internal standard was used).

lines the utility of adopting the I.S. to prevent casual or systematic errors.

4. Conclusions

In the field of occupational health, it is desirable to have flexible methods to characterize multiple exposure biomarkers and quantify the risk linked to mixtures of airborne chemicals in work places. Static headspace sampling coupled with GC–MS analysis has been used extensively in many biomedical and industrial applications [7], and also for biological monitoring of industrial solvents (non-metabolized portion) [8]. The sampling procedure requires urine transfer into a pre-sealed PTFE vial to be accomplished within 5 min of urination in order to confine analyte loss to $<5\%$ [4,6]. Automation of analytical processes increases speed and precision, provides savings in terms of operator worktime, column lifetime and injector maintenance, and boosts overall productivity. To this end, we previously described a useful headspace sampling method for biomonitoring the exposure of operating theatre personnel to mixtures of modern halogenated anaesthetics (isoflurane, sevoflurane, halothane) and nitrous oxide [4,5].

Like the proposals of other groups for biomonitoring of volatile anaesthetics [9,10], our GC–MS method originally contemplated only external calibration. No deuterated homologue of halide volatile anaesthetics is currently available as an I.S. For this purpose, dichloromethane [11], 1-4 dioxane [12], xenon [13,14] or volatile anaesthetics such as halothane or isoflurane [15,16] have been proposed by other working groups. However, in the present work we preferred to adopt enflurane because of its chemical homology with analytes. We think that in the many parts of the world where it is no longer in clinical use, enflurane is therefore a good choice of internal standard for anaesthetics biomonitoring (in our intensive routine experience in Italy, we have yet to encounter any case of clinical usage of enflurane in the past 5 years, so we currently use it to quantify halogenated anaesthetics from clinical mixtures). Our results confirm that use of a proper, viable I.S. such as enflurane is desirable to prevent loss of reproducibility in MS response. In the absence of a deuterated homologue of halide volatile anaesthetics, enflurane dilution may be considered a reference method for quantitation of these compounds by means of GC–MS. Its use significantly enhances intra-day and inter-day precision, and is excellent for quality assurance and inter-laboratory control.

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