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Journal of Chromatography A, 1071 (2005) 131–134
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Short communication

Simultaneous determination of unmodified sevoflurane and of its metabolite hexafluoroisopropanol in urine by headspace sorptive extraction–thermal desorption–capillary gas chromatography–mass spectrometry

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Available online 10 November 2004

Abstract

Unmodified sevoflurane and its metabolite, hexafluoroisopropanol (HFIP), have both been proposed as biomarkers of exposure in post-shift urine for operating room personnel exposed to inhalation anaesthetic sevoflurane. We used headspace sorptive extraction (HSSE) and thermal desorption–capillary GC–MS to assess sensitively both compounds in the urine matrix (after a HFIP deconjugation step). In GC–MS splitless mode, calibration plots (∼15–650 μg/L) were linear ($r^2 > 0.9910$) and the limits of detection (1 μg/L for both biomarkers) showed increased sensitivity for HFIP with respect to the previously described headspace GC–MS method. The method was suitable for biological monitoring of both biomarkers of exposure to sevoflurane.

Keywords: Sevoflurane; Hexafluoroisopropanol; Biomarkers of exposure; Urine; Biological monitoring; Headspace sorptive extraction; Capillary GC–MS

1. Introduction

Sevoflurane is a halogenated inhalation anaesthetic that is widely used in day surgery and paediatric surgery. It is partially excreted in urine as the unmodified halide; in addition, up to 4–6% of intake is thought to be rapidly excreted as hexafluoroisopropanol (HFIP), mostly in form of inorganic fluoride and the glucuronide complex [1,2] (Fig. 1).

Recently, we proposed a headspace gas chromatography–mass spectrometry (GC–MS) method for biological monitoring of sevoflurane and other unmodified volatile anaesthetics (isoflurane, halothane, nitrous oxide) in post-shift urine of operating theatre personnel at μg/L level [3]. This method provided good correlations with personal monitoring measurements of inhaled ppm concentrations [4]. HFIP has also recently been proposed as an exposure biomarker for operating room personnel [5,6]. However, quantification of HFIP in urine is difficult due to the need for a pre-analytical deconjugation step using acid hydrolysis or β-glucuronidase. Moreover, existing GC analytical methods allow quantification of >100 μg/L of HFIP [5] in comparison with a level of detection (LOD) of ∼0.1 μg/L for sevoflurane [3].

In recent years, stir-bar sorptive extraction (SBSE) and headspace sorptive extraction (HSSE) techniques have been developed based on sample pre-concentration over a polydimethylsiloxane (PDMS) hydrophobic film [7,8]. Briefly, a magnetic stir bar coated with PDMS (0.5 or 1 mm d_f) is placed in a liquid (for SBSE) or gaseous (HSSE) phase for absorption of volatile and semivolatile compounds. Heating the stir bar releases the extracted analytes into a GC–MS system for

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doi:10.1016/j.chroma.2004.09.039

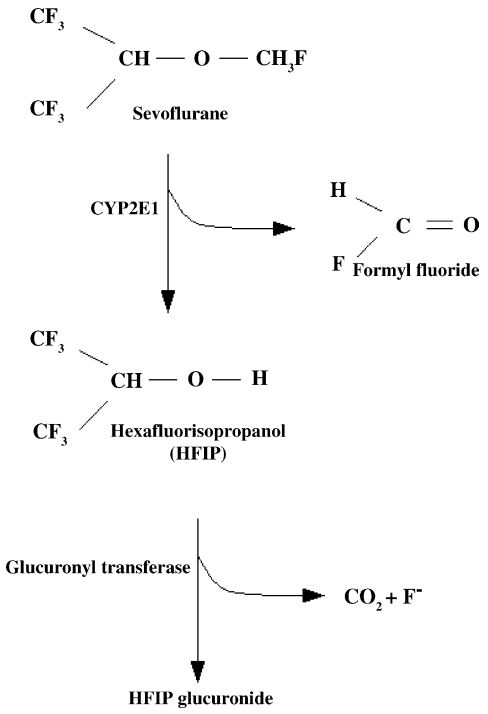

Fig. 1. Metabolic pathway of sevoflurane.

subsequent analysis, which provides very low detection limits (down to the ng/L level).

The aim of the present work was to develop a sensitive HSSE method for simultaneous biological monitoring in post-shift urine of sevoflurane and its metabolite, HFIP.

2. Experimental

Sevoflurane 99% (CAS no. 28523-86-6) and the internal standard (IS), enflurane 99% (CAS no. 13838-16-9), were purchased from Abbott Labs. (Abbott Park, IL, USA); HFIP 99.8% (CAS no. 000920-66-1) was from Sigma–Aldrich (Milan, Italy). Two working solutions of mixed sevoflurane and HFIP (approximately 15 and 300 mg/L for each analyte) were prepared by dilution in low-benzene carbon disulphide (≥99.5%, Sigma–Aldrich) and stored at 4 °C.

To pre-concentrate the analytes, 10 mm stir bars coated with a 0.5 mm PDMS layer (Twister; Gerstel, Mülheim a/d Ruhr, Germany) were suspended in the headspace of 20 mL pre-sealed vials using a suitable glass insert, as shown in Fig. 2. PTFE vial septa were used at each step to avoid analyte loss.

For sample preparation, 1 mL of fresh post-shift urine obtained from 10 individuals working in a urologic surgery unit was transferred to a 20 mL headspace vial pre-sealed with a PTFE–rubber septum and containing 1.5 mL of 10 M sulphuric acid and a 10 mm stir bar for HSSE as previously described (Fig. 2). After addition of enflurane, urine samples were incubated at 100 °C for 60 min to achieve acid hydrolysis of glucuronized HFIP [1]. No further sample preparation step was required.

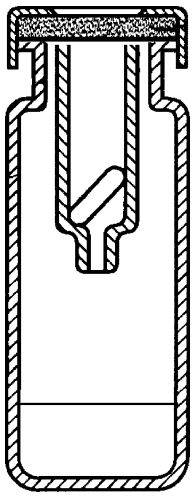

Fig. 2. System used for HSSE extraction of sevoflurane and its metabolite HFIP.

Calibration vials were prepared using spiked urine samples from individuals not exposed to anaesthetics: known amounts (approximately 15, 60 and 600 μg/L) of both analytes from working solutions were spiked into 20 mL pre-sealed vials previously prepared with 1 mL of acidified blank urine (10 M H_2SO_4, 1.5 mL) and containing a stir bar for HSSE. Afterwards, 1 μl of IS working solution (enflurane, 304 mg/L in low-benzene carbon disulphide) was added to calibration vials and equilibrated overnight at 4 °C before incubation at 100 °C for 60 min, followed by thermodesorption and programmed temperature vaporization (PTV)–GC–MS analysis [9]; operating conditions are reported in Table 1.

Table 1
Operating conditions of PTV–GC–MS

Thermodesorption (TDS-2, Gerstel)	20 °C, 60 °C/min, 260 °C (3 min)
PTV injection (CIS-4, Gerstel)	−150 °C, 720 °C/min, 260 °C (3 min)
GC separation (6890, Agilent Technologies, Little Falls, DE, USA)	Oven: 40 °C (1 min), 40 °C/min, 160 °C (10 min) Column: PoraplotQ (27 m × 0.25 mm i.d., 8 μm d_f) (Varian, Walnut Creek, CA, USA) Carrier: helium, constant pressure eluting (1 mL/min)
MS detection (5973, Agilent Technologies)	SIM: 131 and 51 *m/z* for sevoflurane; 99 and 51 *m/z* for HFIP; 117 and 51 *m/z* for enflurane (IS). Dwell time: 60 ms

3. Results and discussion

Before assessing the suitability of the HSSE method for simultaneous measurement of both analytes in urine, we predicted the log octanol–water partition coefficients (K_{ow}) using the software SRC KowWin ver. 1.66 (http://www.syrres.com/esc/wskow.htm, accessed on 1 September 2004). K_{ow} turned out to be 1.75 for sevoflurane and 1.11 for HFIP. Splitless mode provided better sensitivity with respect to a split ratio of 1:20 (Fig. 3). IS-corrected calibration plots in urine (ranging from approximately 15–650 μg/L) were linear (both, $r^2 > 0.991$). In the tested concentration range, intra-day %R.S.D. were between 8.8 and 12.8% for unmodified sevoflurane and between 7.7 and 14.3% for total HFIP.

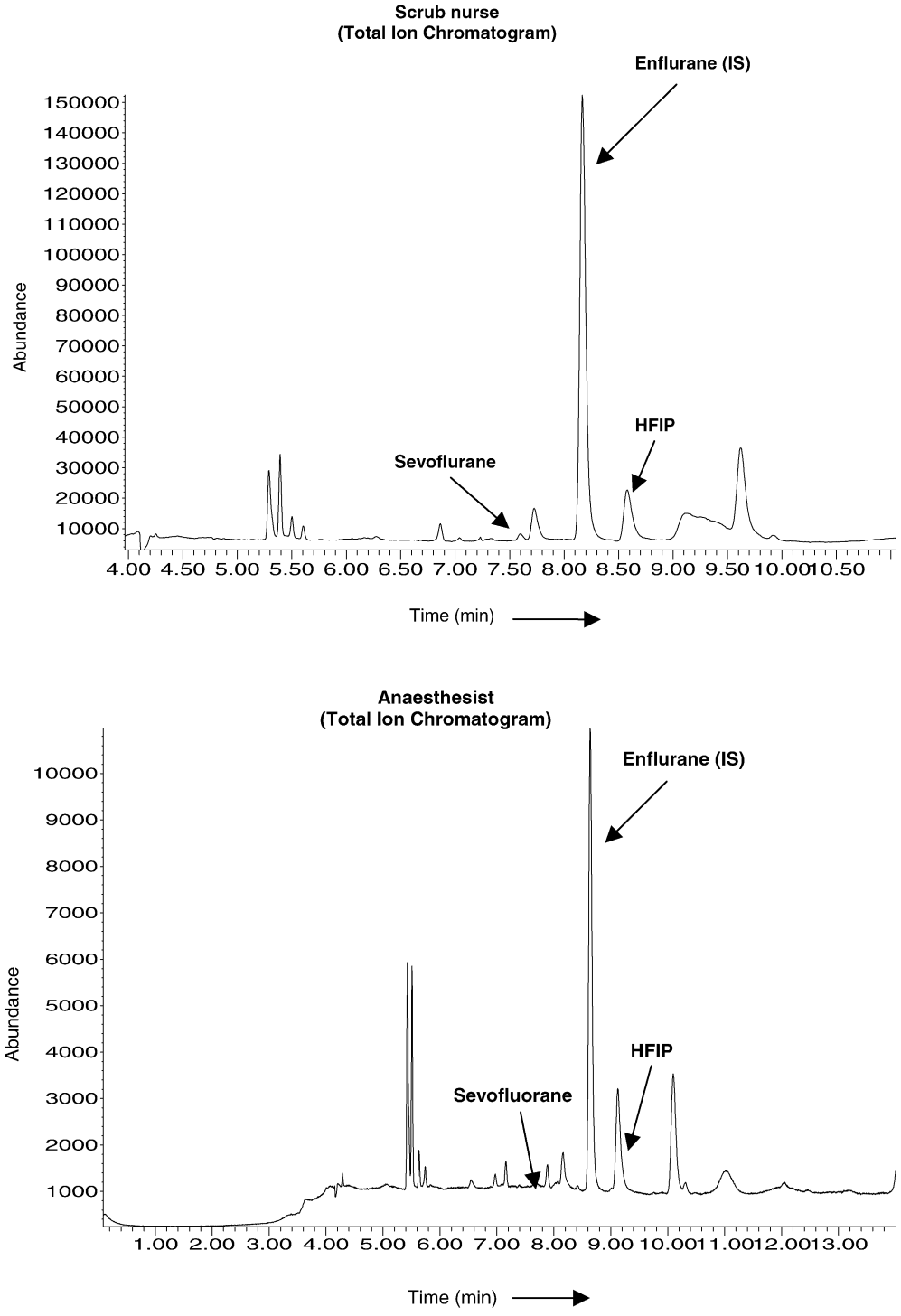

Fig. 3. Chromatograms of real post-shift urine samples in splitless mode (above) and with 1:20 split ratio (below).

With GC–MS in the selected-ion monitoring mode and splitless injection (1 min), the limit of detection (defined as a signal/noise ratio of ∼3) was ∼1 μg/L for both biomarkers under investigation (as compared with >100 μg/L in the previously described HFIP headspace GC–MS method [5]). Under the described conditions, HFIP was quantified in all samples.

The HSSE method was successfully used for biological monitoring of exposure to low doses of airborne sevoflurane. Anaesthetists ($n = 3$) appeared to have higher values of both biomarkers in post-shift urine with respect to the other workers (nurses, surgeons and auxiliary personnel; $n = 7$) (median post-shift sevoflurane and HFIP, 2 and 256 μg/L, respectively, versus <1 and 58 μg/L).

The data from this pilot-study suggest that total HFIP might be more abundant than unmetabolised sevoflurane in the post-shift urine of exposed personnel (Fig. 3) and subject to more extensive variations (data not shown). Should physiologic and/or genetic individual traits turn out to affect the biotransformation of sevoflurane, then measurement of unmodified sevoflurane in urine might provide a better reflection of recent exposure.

Acknowledgement

We are grateful to Robin M.T. Cooke for scientific editing.

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