Validation of a HS–GC–FID method for the Quantification of Sevoflurane in post-mortem matrices and comparison with a GC-MS method for forensic purposes

S. Mohamed[1], R. Barone[1], F. Rossi[1], M. Garagnani[1], R. Roffi[1], A. Giorgetti[1], P. Fais[1], G. Pelletti[1]

[1]Department of Medical and Surgical Sciences, Unit of Legal Medicine, Laboratory of Forensic Toxicology, University of Bologna, via Irnerio 49, 40126, Bologna, Italy.

INTRODUCTION

Sevoflurane (SVF) was introduced into clinical practice in 1990 as an inhalation anesthetic agent, and today is one of the most commonly used anesthetics. It is dangerous and potentially lethal, causing cardiorespiratory depression, hypotension, and malignant hyperthermia [1]. Due to the danger given by a possible exposure, voluntary or not, and the recent increase in the diversion of muscle relaxants and anesthetics, its identification and quantification could be useful in a forensic setting [2]. The aim of the present study is to develop and validate two methods for the detection and the quantification of sevoflurane through a single preparation of post-mortem biological fluids and organs, using gas chromatography coupled to flame ionization detection (GC–FID) and gas chromatography coupled to mass spectrometry (GC-MS). A cross validation study between the two methods has been performed.

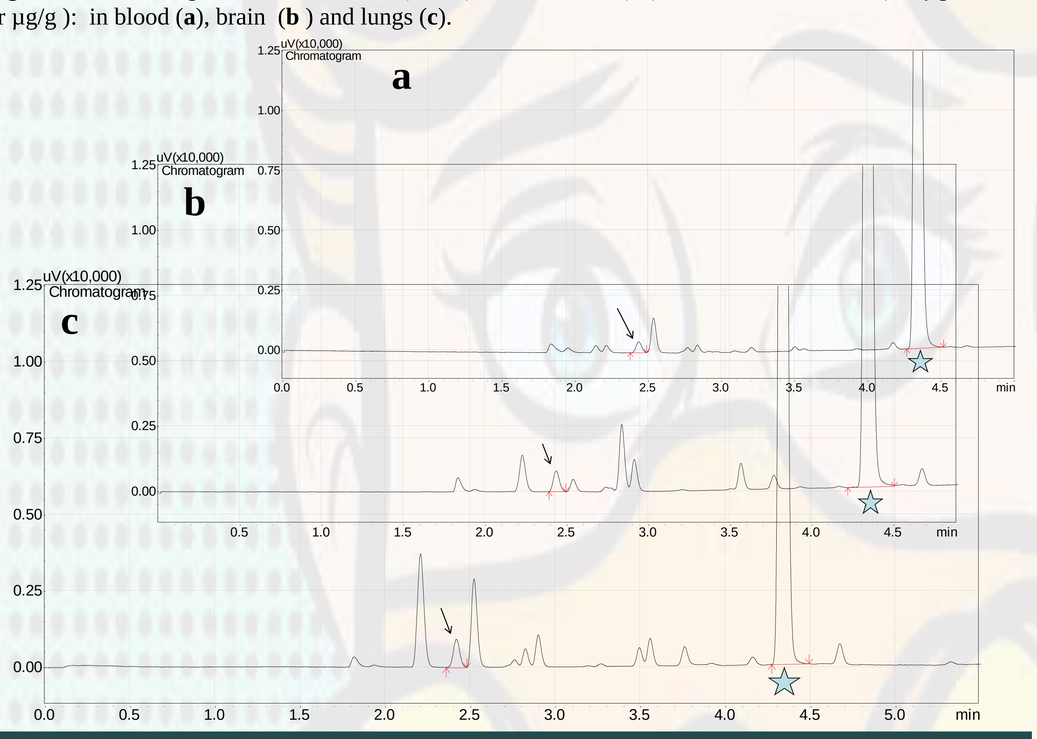
MATERIALS and METHODS

Methods were validated according to International Guidelines [3]. A Shimadzu GC-2010 gas chromatography system equipped with FID was used for the separation and quantitation of the compounds analyzed. GC-MS analyses were performed using GC-2010 Ultra coupled with a GC–MS–QP 2010 Ultra autosampler AOC 6000 (Shimadzu, Milano). Both instruments were equipped with a Zebron capillary column ZB-624 (30 m, 0.32 mm ID, 1.80 µm film thickness). A 0.5 mL (blood) or 0.5 g (homogenized brain and lungs) of samples were added with 1 g of NaCl and 0.5 mL of the IS (n-butanol) solution were pooled in a 10 mL headspace vial. The vial was immediately sealed with a rubber stopper and an aluminum crimp seal, shaken for 30 s and kept for 50 min at 40 °C in the heater. A 0.5 mL aliquot of headspace was withdrawn with a gas-tight syringe for analysis and injected onto the GC–FID and GC-MS. Comparison between methods was performed with a Bland-Altman plot. The method was applied to a real forensic case and to 20 negative controls.

RESULTS

Successful validation was achieved for SVF in all the biological matrices considered. The method was linear from 1.0 to 304.0 µg/mL (blood) and µg/g (brain, lungs), with $R^2 \geq 0.99$ for all matrices. Selectivity, precision and accuracy (≤ 20%), and stability met the required technical parameters [3]. LLOQ was set at 1,0 µg/mL (blood) (Figure 1a,2a). and µg/g (brain, lungs) (Figure 1b and c, 2 b and c). The LOD was set at 0.3 µg/mL or µg/g (1/3 of the LLOQ) in all the matrices. A very good agreement was observed by Bland–Altman plot (Figure 3).

Figure 1. Chromatograms of Sevoflurane (arrow) and n-butanol (☆) in GC-FID: at LLOQ (1,0 µg/mL or µg/g): in blood (**a**), brain (**b**) and lungs (**c**).



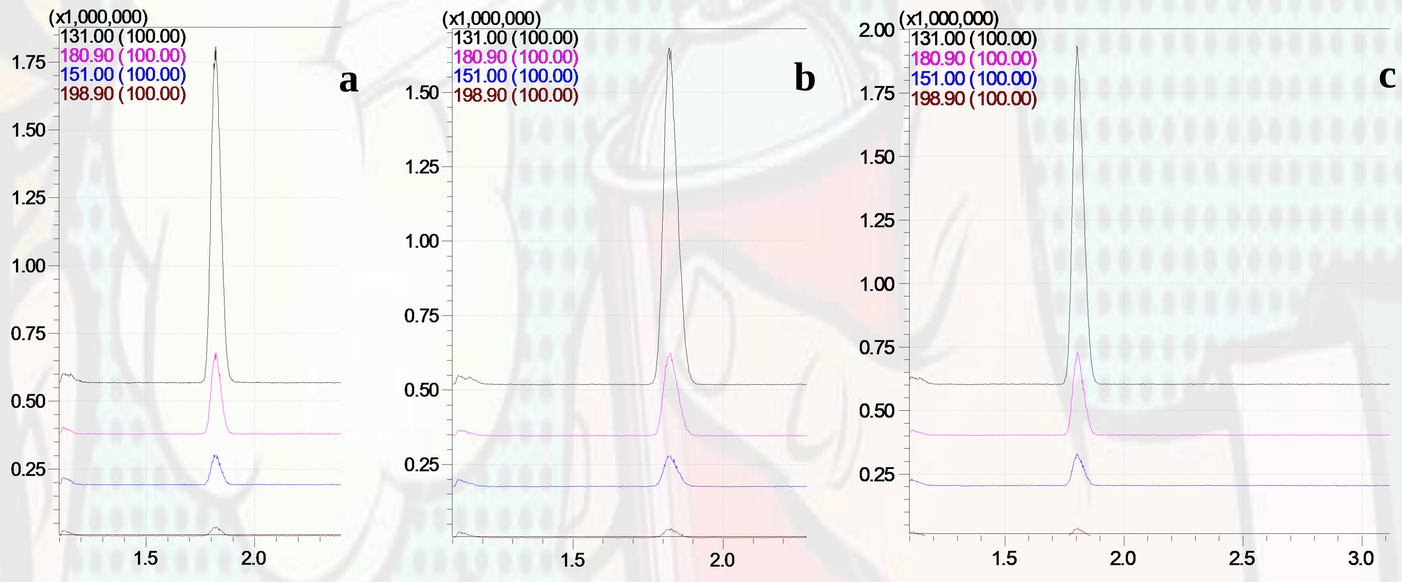


Figure 2: (a) Total Ion Chromatogram of Sevoflurane in GC-MS; Extracted Ion Chromatograms of Sevoflurane at LLOQ (1,0 µg/mL or µg/g) in blood (**b**), brain (**c**) and lungs (**d**) respectively.

CLINICAL APPLICATION

The methods were used to determine the concentration of SVF in 20 negative controls (< LLOQ) and a case of a patient undergoing surgical anesthesia after a car crash (Figure 4a,b). The patient resulted negative for alcohol, drugs and other psychoactive substances and presented SVF at a concentration of 23.3 µg/mL, a value within the concentration ranges found in patients undergoing surgical anesthesia with SVF.

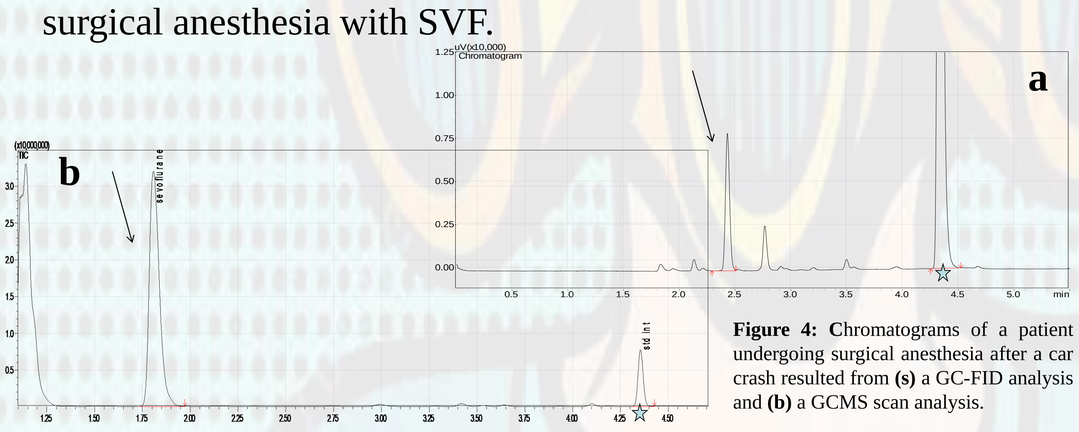


Figure 4: Chromatograms of a patient undergoing surgical anesthesia after a car crash resulted from **(s)** a GC-FID analysis and **(b)** a GCMS scan analysis.

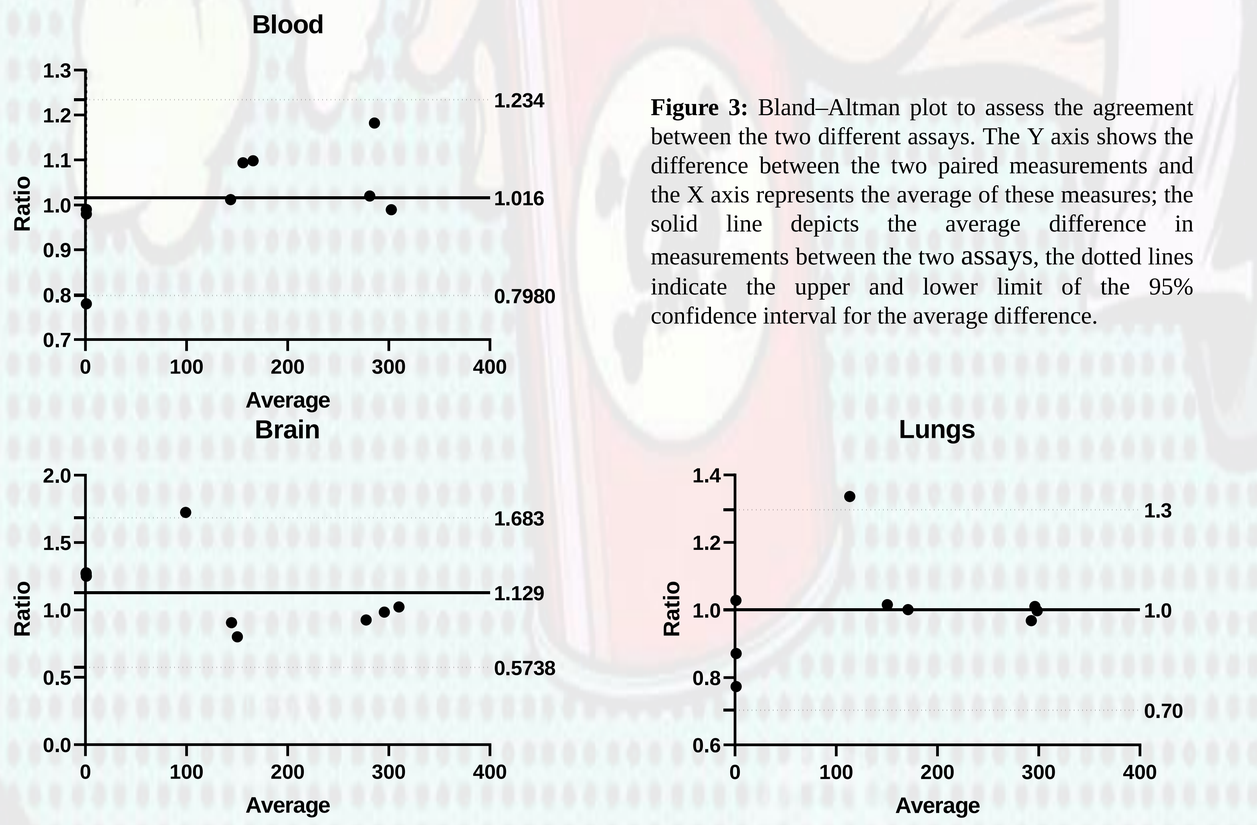


Figure 3: Bland–Altman plot to assess the agreement between the two different assays. The Y axis shows the difference between the two paired measurements and the X axis represents the average of these measures; the solid line depicts the average difference in measurements between the two assays, the dotted lines indicate the upper and lower limit of the 95% confidence interval for the average difference.

CONCLUSION

Apart from clinical and occupational monitoring, toxicologists may be asked to investigate the presence of fluorinated anesthetics in biological matrices, also for forensic purposes. The present methods are suitable for the identification and quantification of SVF in fluids and organs. These methods could be a useful tool in forensic casework, finding its application for the deaths that occur during anesthesia, deaths from anesthetic abuse and other inhalant-induced deaths.

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