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A Rapid, Sensitive Method for Quantifying Enflurane in Whole Blood

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Thermal conductivity detection (TCD) in conjunction with gas–liquid chromatography was employed for the analysis of enflurane in *n*-heptane extracts of whole blood. Enflurane was estimated to be 98 per cent extracted from whole blood by *n*-heptane. Analysis of enflurane standards ranging in concentration from 4.1 to 813 μM demonstrated linearity, with a correlation coefficient of 0.9999. As little as 8 μM enflurane may be detected in whole blood. TCD gas–liquid chromatography is a rapid and sensitive method for quantifying enflurane in whole blood. (Key words: Anesthetics, volatile: enflurane. Measurement techniques: chromatography.)

ENFLURANE is a widely used halogenated anesthetic. Assessment of the pharmacokinetics, metabolism, and toxicities of enflurane has necessitated the development of a sensitive but rapid procedure for the quantification of enflurane in whole blood. Previously, enflurane concentrations in biological fluids have been determined by gas chromatographic methods employing flame ionization[1] or electron capture[2] detection. However, these methods have been limited by extremely large solvent responses and a narrow linear range, respectively. The use of thermal conductivity detection (TCD) avoids the problems associated with the other two methods. The linear range of TCD is extremely wide, and solvent responses can be minimized, allowing for large injection volumes. This report describes the use of TCD for the analysis of enflurane in human blood samples over a wide concentration range.

Methods

Immediately upon collection, 1 ml of whole blood (obtained from patients undergoing enflurane anesthesia) was added to 2 ml of water-saturated *n*-heptane (spectra grade) in two dram vials equipped with Teflon® cap liners. The biphasic system was shaken for a period of 5 min and allowed to settle for a minimum of 10 min. A 100-μl aliquot of the *n*-heptane phase was injected into a Varian model 1440 gas-liquid chromatograph equipped with thermal conductivity detection (TCD) and a Varian CDS 101 electronic integrator. Separation was attained with a 6-foot ⅛ inch OD stainless steel column packed with SE-30, 5 per cent, on Varaport 30® (100–120 mesh). The injection port and detector were maintained at 150 and 200 C, respectively. The column temperature was maintained at 80 C for the initial 2 min following injection. Subsequently, the column temperature was programmed to increase at a rate of 50 C/min until a temperature of 200 C was attained. The column

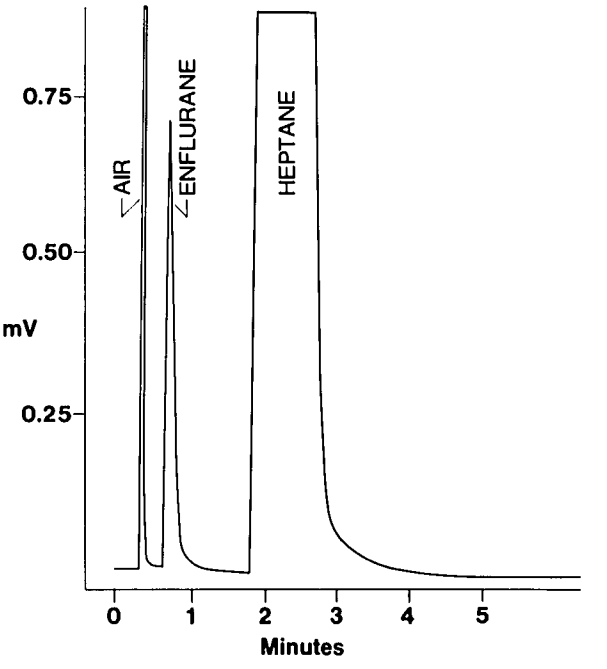

FIG. 1. Typical chromatogram resulting from thermal conductivity detection (TCD) gas chromatographic analysis of *n*-heptane extracts of whole blood containing enflurane.

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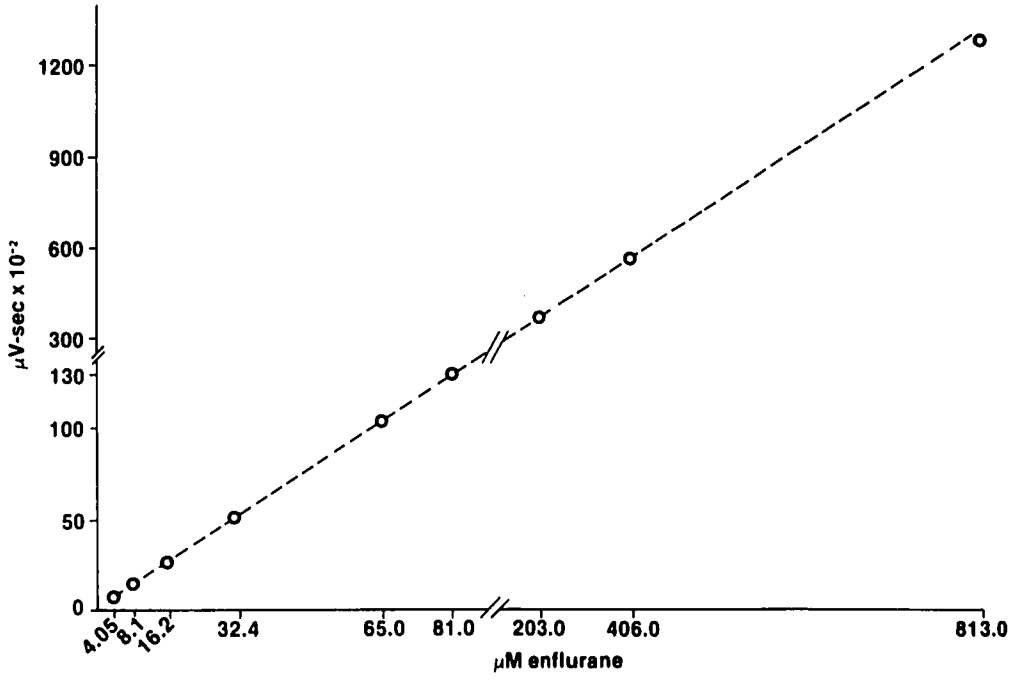

FIG. 2. Typical calibration curve derived from thermal conductivity detection (TCD) gas chromatographic analysis of enflurane standards prepared in *n*-heptane.

was maintained at 200 C for 1 min, after which time the column temperature was returned to 80 C. Under these conditions a typical chromatogram would consist only of an air peak at 0.3 min, enflurane at 0.8 min, and *n*-heptane at 1.8 min.

TABLE 1. Peak Areas Resulting from Thermal Conductivity Detection (TCD) Gas Chromatographic Analysis of Five Sets of Heptane Standards Containing Various Concentrations of Enflurane

Enflurane Concentration* (μM)	Peak Area (μV-sec × 10^{-2} ± SEM)
4.1	6.49 ± 0.22
8.1	13.50 ± 0.11
16.3	25.75 ± 0.36
32.5	50.94 ± 0.73
65.0	100.83 ± 1.98
81.3	129.69 ± 1.02
203.3	332.79 ± 0.34
406.5	639.85 ± 0.95
813.0	1288.45 ± 2.61

* n = 5 for each concentration.

TABLE 2. Determination of the Partition Coefficient of Enflurane between Whole Blood and Heptane as a Measure of Extraction Efficiency*

Initial Enflurane Concentration in Heptane (μM)	Peak Area in Heptane		Per Cent Enflurane Remaining in Heptane
	Blood Plus Heptane†	Heptane Only†	
8.1	1804	1628	+100
162.6	3177	3252	98
487.8	9047	9037	100
650.4	11739	11860	99
813.0	128369	131619	98

* Refer to methods for analytic technique.
† Peak area units are μV-seconds.

A calibration curve was prepared by the addition of enflurane to *n*-heptane previously saturated with water in quantities sufficient to result in five sets of standards, with each set containing standards ranging in concentrations from 4.1 to 813 μM enflurane. Enflurane analysis was performed on each set of standards by the previously described method.

Accurate enflurane concentrations in whole blood are difficult to attain due to the extreme volatility of enflurane in aqueous solutions. Therefore, extraction efficiency was estimated following equilibrium between whole blood obtained from human volunteers and *n*-heptane containing known quantities of enflurane. Duplicate enflurane standards consisting of enflurane concentrations ranging from 8.1 to 813 μM were prepared in *n*-heptane. One ml of whole blood was then added to one set of standards, while the other identical, set remained unaltered. Following shaking and equilibration for an hour at room temperature, the *n*-heptane phases were analyzed for enflurane content. Extraction efficiency was derived from the partition coefficient determined between enflurane content measured in the presence and in the absence of a whole-blood aqueous phase.

The clinical applicability of TCD gas chromatography as a method for quantifying enflurane in whole blood was assessed in four consenting adult patients receiving enflurane anesthesia for elective intra-abdominal surgical procedures. All patients studied were participants in a study approved by the Arizona

TABLE 3. Whole-blood Enflurane Concentrations from Four Patients* Prior to, during, and Following Enflurane Anesthesia

	Enflurane in Whole Blood (μM)						
			Post-anesthesia				
	Pre-anesthesia	Anesthesia†	6 Hr	12 Hr	18 Hr	24 Hr	48 Hr
Patient A	0	613	22	13	7	5	0
Patient B	0	255	13	12	3	0	0
Patient C	0	783	54	31	16	7	0
Patient D	0	241	14	12	—‡	3	1

* Receiving 1–2 MAC hours enflurane.
† Mean of ten analyses during anesthesia.
‡ Not determined.

Health Science Center Human Subjects Committee. Arterial whole blood was obtained prior to, and at predetermined intervals during and following, enflurane anesthesia. Enflurane content was determined by the method previously described.

Results

A typical chromatogram (fig. 1) resulting from TCD gas chromatographic analysis of an *n*-heptane extract of whole blood containing enflurane demonstrates the excellent peak resolution obtainable. Analysis of *n*-heptane containing enflurane in concentrations ranging from 4.1 to 813 μM resulted in a response that was both reproducible (table 1) and linear (fig. 2). Linearity was confirmed by linear regression analysis which resulted in a correlation coefficient of 0.9999. Analysis of *n*-heptane containing equivalent concentrations of enflurane both in the presence and in the absence of whole blood demonstrated that 98–100 per cent of the enflurane in *n*-heptane remained in the *n*-heptane phase when mixed with whole blood (table 2). We interpret this to mean that in excess of 98 per cent of enflurane in whole blood would be extracted into *n*-heptane. Analysis of whole blood obtained from consenting human volunteers receiving enflurane anesthesia demonstrated the direct applicability of TCD gas chromatography as a method for quantifying enflurane in whole blood during and following anesthesia (table 3).

Discussion

The method described for the analysis of enflurane in whole blood is both reliable and reproducible. Analysis time is short, providing for as many as 15 analyses/hour. In addition, linearity is significantly increased over methods of analysis employing electron capture detection,[2] and sensitivity surpasses that of methods utilizing flame ionization detection, which are limited to enflurane concentrations greater than 50 μM.[3] Enflurane in whole blood can be routinely detected by TCD gas chromatography in measurable quantities 24 hours, and occasionally 48 hours, following anesthesia, while the detection limits of other methods[3] are surpassed one hour after the termination of anesthesia. To date, in excess of 600 analyses have been conducted, with no detectable change in column separating capabilities or adverse effect on the chromatographic system resulting.

Several other volatile halogenated anesthetics, including halothane and isoflurane, may be quantified by this method following the determination of the appropriate extraction efficiencies (data not presented). Furthermore, the absence of flame detectors and radiation sources allows for operation in both the laboratory and the operating room.

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