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Gas chromatographic headspace analysis of sevoflurane in blood

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ABSTRACT

We have developed a rapid, simple and precise gas chromatographic headspace analysis for sevoflurane in blood which circumvents problems associated with the high volatility and low blood/gas partition coefficient of this anesthetic drug. Blood standards are easily prepared by volumetric addition of a saturated aqueous solution of sevoflurane. Likewise, internal standardization is achieved using a saturated aqueous solution of halothane. Chromatographic conditions are similar to those commonly used for the analysis of blood ethanol. A simple method is also described for the preparation of stable and precise aliquots of quality control materials for this assay.

INTRODUCTION

Sevoflurane [$H_2FC\text{-}O\text{-}CH(CF_3)_2$, flurometh-yl-1,1,1,3,3,3-hexafluoroisopropyl ether, SF] is a relatively new, non-flammable inhalation anesthetic agent [1]. Several authors have used gas chromatography to measure liquid/gas partition coefficients [1,2], solid/gas partition coefficients [3], inhaled and exhaled gas concentrations [1,4–7], and body fluid concentrations of SF [6]. Also, many reports exist of the chromatographic analysis of related volatile anesthetics in blood [8–12]. However, the high volatility and low liquid/gas partition coefficient of such compounds cause infrequently mentioned analytical difficulties during the determination of their concentrations in liquids. Preparation of liquid standards at concentrations in the therapeutic range (roughly 100 μg/ml) requires accurate delivery of small volumes of liquid anesthetic (*e.g.* 10 μl SF in 100 ml solvent = 151 μg/ml). During this delivery great care must be exercised to prevent evaporation of the highly volatile compound. Also, since partition coefficients vary with specimen type, headspace analysis is best standardized using the same matrix as the specimen (*e.g.* blood) [13]. Therefore large volumes of body fluid specimen would be required to achieve accurate addition of liquid anesthetic. Furthermore, any step in the preparation, storage or transfer of the liquid standard which exposes the liquid to a gas phase will introduce error via partitioning of anesthetic from the liquid to the gas phase (aqueous-based SF solutions have liquid/gas partition coefficients <1 [1,2]). Such problems also exist for any compound which has properties desirable of an internal standard (*i.e.* properties like those of the anesthetic except for chromatographic re-

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tention). Finally, the preparation and reproducible aliquoting of quality control materials presents similar liquid/gas partitioning problems.

We have developed some simple solutions to these analytical problems. Standards are prepared using saturated aqueous solutions of SF and the internal standard, halothane (HT), as stock standards. Reproducible concentrations can be produced by simply controlling the temperature — no accurate delivery of liquid anesthetic is required. Accurate standards in the desired biological matrix (*e.g.* blood, plasma) are prepared from the stock standards directly in the headspace tube using a syringe and simple cannula system. We have also developed a rapid method for reproducible aliquoting of quality control materials using evacuated blood collection tubes.

To further simplify the analysis we have selected chromatographic conditions that are identical to those widely used for the analysis of blood alcohol. High sensitivity and precision are achieved with headspace analysis because of the low liquid/gas partition coefficients of these compounds. While we have developed this method for the analysis of SF [14], many of its aspects can be applied to the analysis of other highly volatile compounds having low aqueous solubility.

EXPERIMENTAL

Chemicals

SF was kindly supplied by Maruishi Pharmaceutical (Osaka, Japan). HT (Fluothane) was obtained from Ayerst Labs. (New York, NY, USA). Deionized water was obtained using a reverse osmosis system (Continental Water Conditioning, El Paso, TX, USA).

Standard preparation

Saturated stock standards of SF and HT were prepared by adding approximately 100 μl of each to separate 10-ml draw Vacutainer red-top (no additives) evacuated blood collection tubes (Becton Dickinson, Rutherford, NH, USA) containing about 10 ml deionized water. These were capped and vigorously shaken. They were then placed in a 25°C (± 0.5°C) water bath (TekBath, Baxter Scientific Products, McGaw Park, IL, USA) for about 30 min. They were then centrifuged at 25°C in a refrigerated centrifuge (Model J-6B, Beckman Instruments, Brea, CA, USA) at approximately 2000 *g* for 2 min. This was required to prevent droplets of liquid anesthetic from remaining at the water meniscus. The tubes were then returned to the water bath. The concentrations of the stock standards prepared in this way did not differ from those prepared after a 24-h equilibration.

Working standards were prepared directly in the headspace tube by pipetting 1 ml (Medical Laboratory Automation, Mount Vernon, NY, USA) of the desired specimen (water, blood, etc.) into an open 10-ml red-top tube. The tube was then capped. A 19-gauge 1-inch needle (Becton Dickinson) was then inserted through the stopper. Another such needle was inserted through the stopper of the stock standard. These served as cannulas for the finer gauged needle of a 50-μl fixed needle syringe (Hamilton, Reno, NV, USA). The syringe needle was passed through the cannula into the liquid phase of the stock standard. A 40-μl volume of the stock standard was then quickly transferred to the working standard headspace tube. The cannula system allowed rapid transfers without chance of syringe needle damage. The cannulas were then removed and the working standard tube vortex-mixed for about 15 s (S8220 Deluxe Mixer, Baxter Scientific Products) and briefly centrifuged.

An alternative means of stock standard preparation was used in order to determine the concentration of the saturated solution of SF. A 10-μl volume of liquid SF was added to 10.0 ml of water in a red-top tube using a 10-μl syringe (Hamilton) and a class A volumetric pipet. This was mixed by inversion. Assuming additive volumes and a density of 1.505 g/ml [1], the nominal concentration of SF was 1.50 mg/ml. Working standards were prepared by transferring 40 μl of this solution to a headspace tube containing 1 ml of water, followed by the addition of internal standard.

Sample preparation and quantitation

A 1-ml volume of sample or quality control material was quickly transferred to an empty 10-ml red-top headspace tube and capped. Working standards were prepared in headspace tubes as described above. Saturated HT solution (internal standard) (20 μl) was then added to each standard, sample or quality control tube using the cannula system described for the delivery of the SF stock standard. All tubes were vortex-mixed for approximately 15 s and placed in a 25°C water bath. Concentrations for samples and quality control materials were determined by comparison of their SF/HT peak-height ratios with those of the working standards.

Gas chromatography (GC)

We used an Aerograph Series 2100 gas chromatograph (Varian, Walnut Creek, CA, USA), with flame ionization detection. The oven was operated isothermally at 165°C, and the detector and injector ports were set at 200°C. The 6-feet 1/4 inch O.D., 2 mm I.D. glass column was packed with 80–100 mesh Porapak S (Supelco, Bellefonte, PA, USA). Prepurified nitrogen was used as the carrier gas (45 ml/min), and compressed air (300 ml/min) and prepurified hydrogen (45 ml/min) were used as flame gases. Electrometer settings of $512 \cdot 10^{-12}$ A/mV produced approximately half-scale pen deflection with the working standard. Peak heights were measured to the nearest millimeter.

All headspace equilibrations were performed at 25°C. This temperature was chosen since it was the lowest we could consistently control with a simple heating bath. While the liquid/gas partition coefficients of these compounds approximately double upon changing the temperature from 37 to 25°C, peak heights only decrease by approximately 10%. This results from the large vapor/liquid volume ratio in the headspace tube (11.2 ml vapor:1 ml liquid). Moreover, peak-height ratios for both aqueous and blood samples remained constant upon changing the temperature from 37 to 25°C.

Peak-height ratios equilibrated rapidly after the vortex-mixing step. The ratio rose approximately 5% in the first 20 min and then stabilized. For routine analysis, samples and standards were allowed to equilibrate for 1 h. Long-term equilibration (days) caused a slow rise in peak-height ratio (discussed below). However, since standard and sample ratios changed consistently, measured concentrations remained constant for incubation periods of at least 24 h.

A 1.00-ml volume of headspace vapor was injected with the use of a Pressure-Lok series A 2-ml gas syringe (Dynatech Precision Sampling, Baton Rouge, LA, USA). The syringe was filled and emptied twice while the needle remained in the headspace tube, then filled a third time which was used for the injection. After injection the syringe was filled and emptied three times to reduce possibilities of carry-over [15].

Direct injections were also performed in order to compare results with headspace analysis. A solvent flush technique was used whereby 0.5 μl of water was introduced into a 5-μl Hamilton microliter syringe, followed by 0.5 μl of air, then 2.0 μl of sample, and finally by 1 μl of air. These contents were then emptied directly onto the gas chromatograph. Much lower electrometer settings were required to achieve half-scale deflection with direct injection ($8 \cdot 10^{-12}$ A/mV).

Quality control

Quality control specimens were prepared by adding approximately 50–500 μl of liquid SF to about 1000 ml of either deionized water or outdated blood bank plasma in a 1-l plastic bottle. These were mixed by inversion and refrigerated. Once cool the cap was removed and replaced with the stopper arrangement shown in Fig. 1. New 15-ml red-top evacuated blood collection tubes were then inverted and punctured with the needle. Rapid vacuum-driven filling occurred. About sixty such tubes were prepared for every batch of quality control. A key factor in the production of the aliquots was the reproducible fill volume. This provided a constant liquid concentration even though there remained in each tube a substantial gas phase (about 6 ml). Also, the rapid filling of sixty tubes (about 10 min) with cold solution reduced the variability between

tubes due to change in concentration of the reservoir as its volume decreased. These quality control tubes were then randomized as to the order of preparation and frozen at −20°C. For analysis the tube was thawed in a 25°C water bath. A 1-ml volume was quickly transferred from the tube to an empty 10-ml red-top headspace tube and capped.

Validation of precision

Within-run and total precision were assessed using the National Committee for Clinical Laboratory Standards (NCCLS) guidelines [16]. This protocol provides a correct estimate for the total precision because it properly weights the between-day, between-run (inter-assay), and within-run (intra-assay) components. The "one run per day" modification we used (appendix C of ref. 16) also provides the separate estimate of the within-run precision standard deviation. Each quality control material was analyzed in duplicate for twenty days. Standard headspace tubes were prepared each day in duplicate from a single freshly prepared saturated aqueous solution of SF. Single tubes of quality control materials were thawed and two aliquots were transferred to headspace tubes for the duplicate analysis. A 20-μl volume of freshly prepared saturated aqueous HT was added to all controls and standards.

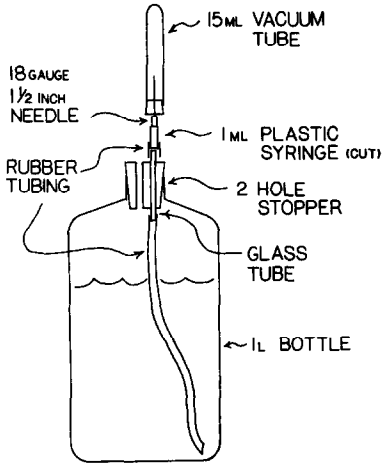

Fig. 1. Diagram of the device used to aliquot quality control materials into individual tubes.

The concentrations of the controls were determined by comparison of their SF/HT peak-height ratios with the average ratio of the standards.

Validation of accuracy

Linearity of the assay was assessed by preparing several headspace tubes with various concentrations of SF. A 1-ml volume of deionized water was pipetted into each of eighteen headspace tubes. The volume of saturated aqueous SF to be added to each tube was then removed using MLA pipets. Thus, if 10 μl of the SF solution were to be added, 10 μl of water were removed from the tube. Then aqueous saturated SF was added to each headspace tube using the cannula system. The volumes used were 5, 10, 20, 40, 70, 100, 150, 200, and 400 μl, with resultant nominal concentrations between 12 and 944 μg/ml. Aqueous saturated HT (20 μl) was added to each tube. Two headspace tubes were prepared for each concentration. Measured concentrations were determined using the 40-μl addition as the standard. Regression analysis was performed using the nominal concentration as the independent variable (x) and the measured concentration as the dependent variable (y).

Recovery studies were performed with whole blood, plasma and serum specimens remaining after routine clinical analysis in order to quantitate the effects of biological matrix on headspace partitioning. Working standards were prepared using these materials and the peak heights and peak-height ratios were compared with those of water working standards. Hematocrits were determined using the Sysmex NE8000 (TOA Medical Electronics, Kobe, Japan), and lipid measurements were performed on the Paramax 720ZX (Baxter). Total lipid was calculated as the sum of plasma or serum cholesterol plus triglycerides.

We also performed a correlation study between direct injection and headspace analysis. On five separate days approximately ten blood samples each day were prepared for both types of analysis. Various concentrations of SF were produced by mixing blood with saturated aqueous

SF in 4-ml Vacutainer tubes. A particular volume of blood was first pipetted into the tube and stoppered. The cannula system was then used to deliver a volume of saturated aqueous SF that would produce a total nominal volume of 4 ml (assuming additive volumes). Blood standards were prepared by combining approximately five additional blood specimens, delivering 3.8 ml of this mixture to a 4-ml tube, and adding 200 μl of saturated aqueous SF (nominal concentration 118 μg/ml). A 100-μl volume of saturated aqueous HT was then added to each tube using the cannula system. The cannula system was also used to allow access of the direct injection syringe to the contents of these tubes. After direct injection 1 ml of each specimen was quickly transferred to a headspace tube for analysis. The tube was vortex-mixed, equilibrated, and analyzed by headspace analysis in the usual manner.

Studies designed to ascertain the extent of binding or partitioning of the anesthetics to the stopper or interior of the headspace tube [3] were performed with the help of vapor saturated with the anesthetics. Approximately 100 μl each of liquid SF and liquid HT were placed in a single 10-ml red-top tube and capped. The tube was vortex-mixed, briefly centrifuged, and placed in the 25°C water bath. After about 20 min, 1 ml of this saturated vapor was transferred using the gas tight syringe to an empty capped headspace tube, thereby producing vapor concentrations about ten times lower than the saturated concentration. Since SF and HT have different liquid/gas partition coefficients [2], the absence of a liquid phase in the headspace tube eliminated the variable of partitioning. While similar vapor concentrations could be produced in many tubes using this method, we achieved more precise data by eliminating the variable of tube-to-tube variation. This was accomplished by making repeated headspace injections from a single headspace tube. Since each injection removed vapor from the headspace tube, in the absence of absorption effects the peak heights of the volatiles should decrease by an amount determined by the injection volume and the volume of the headspace tube. The interior volume of a capped headspace tube and the needle deadspace volume of the injection syringe were determined gravimetrically (Mettler Instrument, Hightstown, NJ, USA) after filling with deionized water. These data indicated that each 1-ml reinjection should yield peak heights that were 7.2% lower than those of the previous injection. Time-dependent absorption phenomena should therefore yield peak-height decreases greater than 7.2%. While peak-height data required this comparison for correct interpretation, peak-height ratios should remain constant with reinjection as long as there is either lack of absorption or equal absorption of the two volatiles. After removal of each 1 ml for injection, the headspace tubes were equilibrated to ambient pressure by briefly inserting a syringe needle through the headspace stopper.

RESULTS

Fig. 2 illustrates typical chromatograms. Fig. 2A demonstrates the lack of interfering peaks of a headspace injection for a blank blood speci-

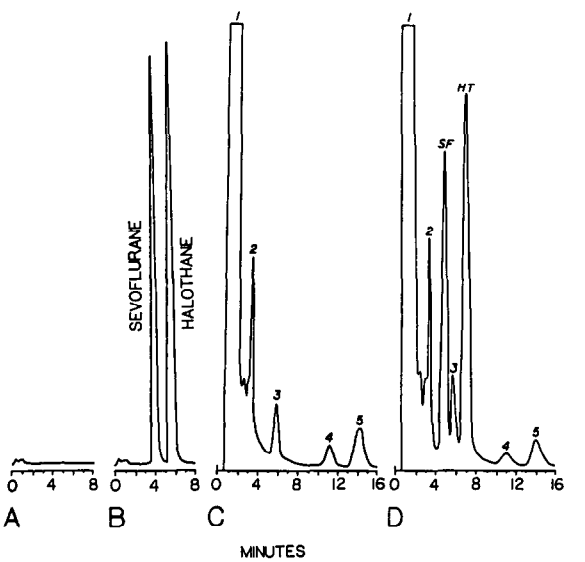

Fig. 2. (A) Headspace chromatogram of a blank blood specimen (1-ml injection, electrometer $512 \cdot 10^{-12}$ A/mV). (B) Headspace chromatogram of an aqueous working standard. (C) Direct injection chromatogram of a blank blood specimen (2-μl injection, electrometer $8 \cdot 10^{-12}$ A/mV). Peak 1 is the solvent peak (water) and peaks 2–5 are constant artifact peaks. (D) Direct injection chromatogram of a blood specimen with a sevoflurane concentration of 120 μg/ml.

men. Ethanol and acetone, volatiles that are sometimes present in blood specimens, do not interfere since they both have much shorter retention times than SF at these chromatographic settings [13]. Fig. 2B displays a headspace injection of the aqueous working standard. HT was selected as an internal standard because its physical properties are similar to those of SF, and it is readily available. The chromatographic resolution of these compounds was found to be acceptable over the wide range of concentrations used in the linearity study. Fig. 2C and 2D, respectively, display direct injection chromatograms of drug-free blood and of a blood sample spiked with SF and HT. The very low liquid/gas partition coefficients of these compounds mean that the liquid and vapor concentrations are on the same order of magnitude. However, while headspace injection volumes can be large (1 ml), direct injection volumes are much lower (2 μl) and therefore require much lower electrometer settings. Peak 1 in Fig. 2C and D is the water peak, and peaks 2–5 represent constant artifacts observed at these low settings. Column flow-rate was reduced by approximately 25% in order to achieve resolution of the SF and HT peaks from these artifacts. The presence of peaks 4 and 5 further lengthened the time between injections from 8 min for headspace analysis to 16 min for direct injection. Flame ionization sensitivity for these compounds (as assessed by peak height) is approximately ten times lower than that for ethanol at comparable μg/ml concentrations.

The concentration of SF in the saturated aqueous stock standard was determined by comparison of peak-height ratios with the stock standard prepared using liquid SF. This experiment was conducted on three separate days and the average concentration at 25°C was 2.36 mg/ml ($\pm$ 0.11, 1 S.D.). Assuming additive volumes, the concentration of SF in the working standard (*i.e.* the standard in the headspace tube) prior to addition of internal standard was 90.8 μg/ml.

The precision with which these two types of stock standards could be prepared was also compared. Nine separate stock standards were prepared by both methods. Separate headspace tubes (*i.e.* working standards) were prepared from each of the stock standards. Saturated HT was used as the internal standard for both sets of tubes. Coefficients of variation (C.V.) for SF peak height, HT peak height and SF/HT peak-height ratio were found to be 4.6, 6.7 and 7.5%, respectively, for the saturated solution standard, and 11.0, 5.7, and 14.8%, respectively, for the alternative liquid standard. Thus, precision was improved by at least a factor of 2 by using aqueous saturated SF as the stock standard.

Table I displays the NCCLS precision data for the quality control materials. The concentration of SF in the plasma sample is only approximate since standardization was performed using water only (see Discussion regarding matrix effects). However, this small difference does not impact on the assessment of precision. The relatively low within-run C.V. with all the materials are a reflection of the ease of preparation of headspace tubes from a single specimen. A contributing factor to this precision is the fact that internal standard could be reproducibly delivered using the cannula system and saturated HT solution: the average of the daily C.V.s of the HT peak-heights for the aqueous specimens was 4.0%. The total precision data include these contributions in addition to the imprecision of standard preparation and tube-to-tube variation in the control specimens. The SF/HT peak-height ratio for the standards for the twenty days averaged 0.924 with a C.V. of only 3.5%. As regards tube-to-tube variation,

TABLE I

WITHIN-RUN AND TOTAL PRECISION FOR SEVOFLURANE QUANTITATION AS DETERMINED BY THE NCCLS PROTOCOL [16]

Each material was analyzed in duplicate for twenty days.

Material	Concentration (μg/ml)	Coefficient of variation (%)	
		Within-run	Total
Water	81	2.9	5.9
Water	109	4.2	5.8
Water	870	2.5	5.2
Plasma	78	3.4	8.9

note that the plasma samples yielded a higher total C.V. than the aqueous samples. Since the within-run precision values are similar for these two specimen types, these total precision data indicate a larger tube-to-tube variation with the plasma samples. The source of this difference is unknown. It is also worth noting that there is no significant effect of concentration on the C.V.s for the aqueous samples over the range 81–870 μg/ml. These data were taken over a total of forty days, demonstrating good stability of SF concentration in frozen samples.

The assay demonstrated a high degree of linearity over a wide concentration range (12–944 μg/ml). The correlation coefficient was 0.9996, with the equation of the line being: measured = (0.993 × nominal) − 1.2 (in μg/ml).

The liquid/gas partition coefficients for SF and HT are known to be dependent on the nature of the liquid phase [2,17,18]. We therefore determined the extent of the effect of the liquid matrix upon peak heights and peak-height ratios. Since both SF and HT have higher liquid/gas partition coefficients in blood, serum, and plasma than in water, lower peak heights are observed with these specimens (Table II). The ratio is higher in these specimens because of the greater increase in solubility of HT compared with SF. All differences in peak heights and peak-height ratios between the biological fluids and water were highly significant ($p < 0.0002$).

There was no observable effect of hematocrit over the range 29.5–43.0 with the blood specimens on either SF peak heights, HT peak heights, or the peak-height ratio (correlation coefficients of 0.001, 0.013, and 0.058, respectively). In addition, there was little difference in peak heights or peak-height ratios among any of the biological specimens. The application of the Student's t-test to all possible groups showed that only the SF peak heights in blood and serum were different at a low statistical significance ($p = 0.025$). Included in these data are fourteen blood specimens that were split into two parts. One part was centrifuged and the resultant plasma sample compared with its matching whole blood specimen. This reduction in variables allowed a paired t-test, which only revealed a small significant difference between HT peak heights in plasma and blood ($p = 0.008$, blood HT peak height = 143 ± 7.1, plasma HT peak height = 138 ± 7.6).

On the other hand there was some dependence of peak heights and peak-height ratios upon the total lipid concentration (measured on the plasma phase of the whole blood specimens). Fig. 3a and b display the decreases in peak heights with increasing lipid and the increase in peak-height ratio with increasing lipid. The increase in the ratio with increasing lipid is consistent with the larger difference in the HT liquid/gas partition coefficient between water and oil liquid phases [2,19]. This inter-individual variation in total lipid concentration contributes to the larger standard deviation in peak-height ratio observed with biological specimens (blood, plasma, and serum: 1.088 ± 0.040, C.V. = 3.7%, $n = 50$; compared with water: 0.933 ± 0.019, C.V. = 2.0%, $n = 6$).

The decrease in peak heights and increase in

TABLE II

EFFECT OF THE LIQUID PHASE ON HEADSPACE PEAK HEIGHTS AND PEAK-HEIGHT RATIOS

Peak heights are in arbitrary units. Values in parentheses are 1 S.D.

Liquid	Peak height		SF/HT peak-height ratio		n
	Sevoflurane (SF)	Halothane (HT)			
Water	166 (6.4)	178 (7.1)	0.933	(0.019)	6
Serum	149 (6.6)	137 (11.1)	1.088	(0.052)	11
Plasma	151 (4.2)	138 (7.3)	1.097	(0.043)	15
Blood	153 (5.0)	142 (7.3)	1.083	(0.028)	24

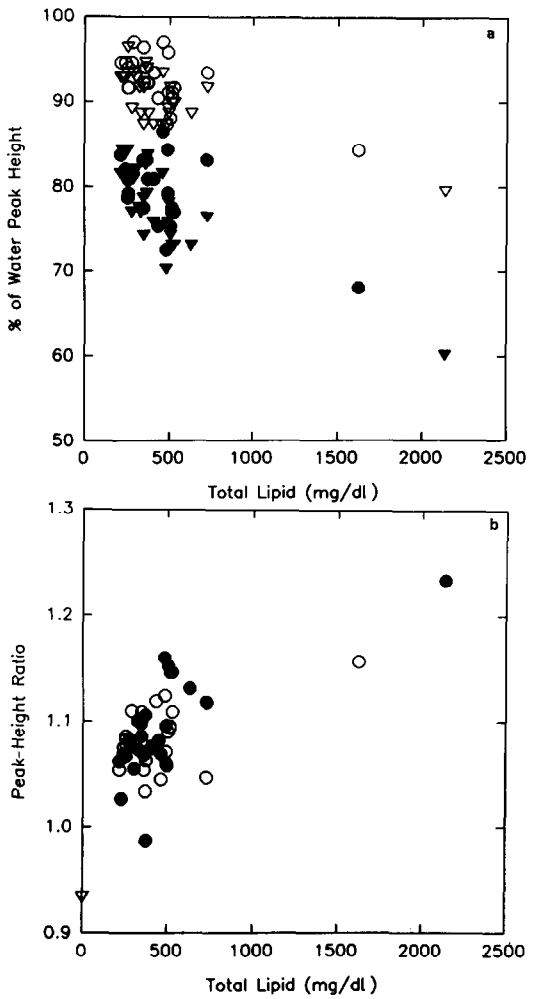

Fig. 3. Dependence of SF and HT peak heights and the peak-height ratio on the total lipid (cholesterol plus triglyceride). (A) Peak heights expressed as percentage of those in the water working standards. ▼ = HT in serum or plasma; ● = HT in blood; ▽ = SF in serum or plasma; ○ = SF in blood. (B) Peak-height ratio: ▽ = average ratio for the working aqueous standards in this experiment (n = 6); ● = serum or plasma; ○ = blood.

peak-height ratio observed with blood specimens could not be negated with the use of saturating amounts of sodium chloride in the headspace tube [13,20]. Such treatment produced no significant changes in SF or HT peak heights with either water or whole blood specimens.

Both SF and HT are known to partition into various solid materials, including rubber and PTFE [3,9,15]. We noted that when small amounts of liquid HT were stored in headspace tubes over a period of several days, the rubber stopper became visibly enlarged from absorption of the vapors. We also noticed a time-dependent decrease in peak heights of both compounds during extended headspace experiments: after 25 h, reinjection peak heights of SF and HT decreased by 16 and 27%, respectively. This is considerably greater than the calculated 7% drop in the absence of absorption effects. In addition, the difference in the percentage decrease for the two volatiles caused a change in peak-height ratio. This loss of volatiles from the vapor phase is a relatively slow process as evidenced by experiments with reinjections from tubes containing only vapor (*i.e.* no liquid phase). Fifteen injections from the same tube within a 1-h and 40-min period yielded average decreases in peak heights of 6.8% for both SF and HT. The peak-height ratio remained constant at 0.784 ± 0.005. Reinjection studies over longer periods yielded greater percentages decreases in peak heights as well as a change in peak-height ratio (Fig. 4). This increase in peak-height ratio is consistent with the reported differences between these compounds in rubber/gas partition coefficients [3]. In spite of this increase in peak-height ratio with time, when quality control specimens and standards were re-

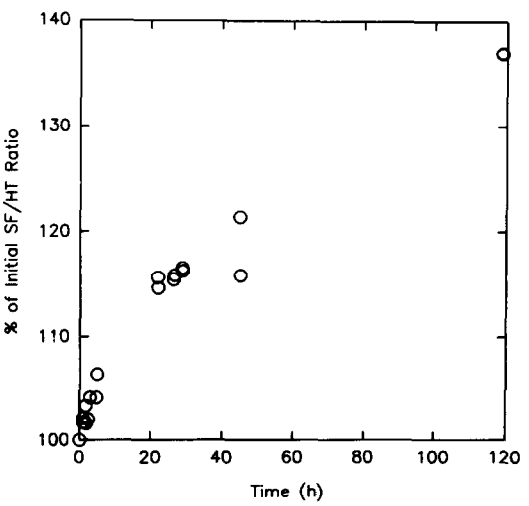

Fig. 4. Change in the peak-height ratio with time in headspace tubes containing only vapor. Repeat injections from two tubes containing SF and HT vapor with the data expressed as the percentage of the initial ratio for the particular tube.

injected a day after the original injections, all calculated concentrations were within two standard deviations of those listed in Table I. This is because the ratios for both quality control specimens and standards changed to the same extent.

Fig. 5 presents the data obtained from the split-patient correlation study. Our method for sample preparation allowed the calculation of a nominal concentration. The regression equations for the measured (y) *versus* nominal (x) concentrations in μg/ml were:

$$\text{direct injection} = (1.06 \times \text{nominal}) - 8$$
$$\text{headspace} = (1.00 \times \text{nominal}) - 6$$

Greater precision was possible with headspace analysis as evidenced by the correlation coefficients of those regression lines (0.9984 headspace, 0.9929 direct injection), the standard errors of the slope (0.013 headspace, 0.018 direct injection), and the standard errors of the intercept (27.4 headspace, 37.6 direct injection). Also, C.V.s of internal standard peak height for direct injection were approximately double those of the headspace analysis. The regression equation for headspace analysis (y) *versus* direct injection (x) was $y = 0.93\ x + 5$ ($r = 0.9925$).

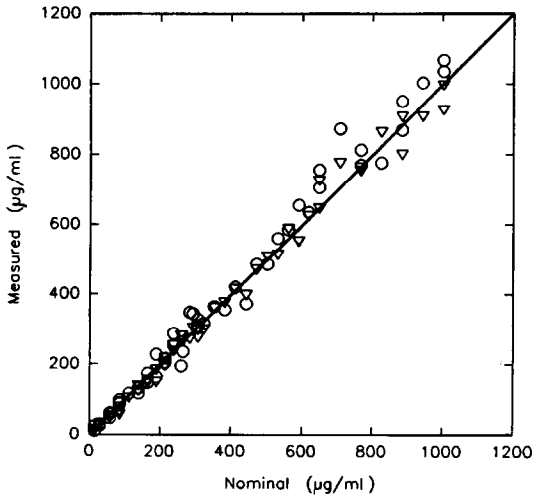

Fig. 5. Correlation between headspace analysis and direct injection with whole blood specimens. Specimens ($n = 51$) were spiked with various amounts of SF and analyzed by both methods. (○) Measured direct injection and (△) measured headspace injection *versus* nominal concentration. The diagonal line represents ideal agreement with nominal concentration.

The assay has been applied to the analysis of SF blood levels in malignant hyperthermia-susceptible and normal pigs during SF anesthesia [14]. Blood samples were collected from each pig prior to dosing with SF and did not have any interfering GC peaks. These drug-free pre-dose samples were used to prepare working standards for each pig. After dosing the pigs with SF, blood samples exhibited a single chromatographic peak identical in retention time to that of the SF standard. Concentrations ranged from 78 to 143 μg/ml for pigs dosed for 2 h with 2.0–3.5% SF.

DISCUSSION

The use of saturated aqueous solutions of SF and HT greatly enhanced the precision of internal and external standardization. Reproducible stock standards were easily prepared without precise gravimetric or volumetric manipulations. The cannula system further simplified standardization and the preparation of samples. Stable frozen quality control specimens were easily prepared and aliquoted thereby providing an independent check on daily standardization. The assay proved linear and precise over a wide range of concentrations, demonstrating that standardization using a single-concentration working standard (in duplicate) is adequate. Headspace analysis is favored by the low liquid/vapor partition coefficients and the large injection volume. This improves precision and decreases interfering peaks compared with direct injection. The use of a large vapor/liquid volume ratio in the headspace tube, the low liquid/vapor partition coefficients, and the similarity of SF and HT minimize the influence of headspace equilibration temperature — peak heights were only slightly higher at higher temperatures and peak-height ratios at 25 and 37°C were indistinguishable.

The assay also proved robust with regard to repeat injections from the same headspace tube. Over short periods, peak-height ratios remained constant with reinjection. And in spite of the slow increase in peak-height ratio with time due to differential absorption of the volatiles to the headspace tube or stopper, calculated concentrations

of unknowns remained constant over long periods since standards and unknowns displayed similar changes with time.

The fact that the nature of the liquid phase affects partitioning into the headspace vapor requires that standardization be performed in the liquid phase of interest. The recovery studies demonstrated that peak heights of both SF and HT were considerably lower and that the SF/HT peak-height ratio was higher in biological specimens compared with water. These differences could not be removed with salting-out techniques frequently used in the analysis of blood alcohol. The use of aqueous working standards will therefore yield inaccurate results on such specimens. Pooled blood specimens can serve as standards (as demonstrated by the data in Fig. 5 and the low C.V. for inter-specimen recovery ratio), but for the greatest accuracy we suggest the preparation of a working standard with a pre-dose specimen from the particular individual. This entirely eliminates the variables of specimen type and lipid concentration. In fact in so doing, any factor affecting volatile partitioning, which remains constant over the dosing interval, will have no influence on the analytical determination. And because stock standards are prepared in water, only a small volume of blood is required for the accurate preparation of working standards. Finally, for purposes of quality control, we also recommend the routine preparation of an aqueous working standard for use in calculating the concentration of a stable quality control material.

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