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COMPARISON OF THE ABSOLUTE CALIBRATION METHOD WITH THE METHOD OF STANDARD ADDITION FOR THE DETERMINATION OF HALOTHANE IN BLOOD BY GAS CHROMATOGRAPHIC HEADSPACE ANALYSIS

PAVEL KOUPIL

Department of Clinical Biochemistry, Hospital, 792 01 Bruntál (Czechoslovakia)

and

JOSEF NOVÁK* and JOSEF DROZD*

Institute of Analytical Chemistry, Czechoslovak Academy of Sciences, 611 42 Brno (Czechoslovakia)

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SUMMARY

As long-term exposure to exhaled halothane can be a cause of hepatitis and/or damage to the liver, the determination of halothane in blood is important in clinical practice. Gas chromatographic headspace analysis appears to be the most successful method. In the present study, two methods of quantitative evaluation of the analysis were compared by statistical treatment and direct comparison. The absolute calibration method was found to be unsuitable since it yields entirely unreliable results. This is due to different contents of lipoid and other components in the blood of the normal population, and this influences the vapour phase concentration of halothane and results in different values for the slopes of calibration curves for different blood samples. The standard addition method gives reliable results.

INTRODUCTION

The problem of halothane determination in clinical practice is attracting great attention, especially in studies of its effects on liver cells, e.g. the livers of anaesthetists in operating theatres and the livers of patients exposed to longer-term anaesthesia performed with halothane.

Aliphatic halocarbons have diverse narcotic effects. Halothane (2-chloro-2-bromo-1,1,1-trifluoroethane) was first synthesized in the 1950s, its application

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and effects on experimental animals were reported, and Johnstone [1] published the first results obtained with its clinical use. Halothane is suitable for all the types of inhalation anaesthesia. A great deal of attention has been devoted to halothane for several years and its behaviour is relatively well documented. It is degraded, as are many other pharmaceuticals, in livers where the enzymic activity on microsomal structures of endoplasmic reticulum is highest. Cases of hepatitis in anaesthetists caused by long-term exposure to exhaled halothane vapour were reported in the 1970s [2,3]. On the basis of experimental studies, the authors concluded that what is concerned is probably a sensitization process in which halothane or its intermediate products are active as haptene. In the opinion of other authors [4–6], halothane stimulates degradation of liver enzymes, of cytochrome P 450 in particular.

Virtually since the 1960s, reports on liver damage caused by anaesthesia performed with halothane have been appearing in the literature [7,8]. On the other hand, in 1963 the eventual hepatotoxicity of halothane was investigated on a large set of 856 500 anaesthetic treatments from 34 hospitals. The resulting finding was that in nine cases only halothane could be considered responsible for liver necrosis. On the basis of this research halothane was recommended for further clinical applications [9]. Not even its extreme stability protects halothane from biotransformation, as Van Dyke and other authors [10–12] found in 1965. Halothane metabolism was studied both in vitro and in vivo with the aid of isotopes [13,14].

Gas chromatography (GC) is considered to be the most successful method for halothane determination in blood [15–19], and it can also be used to detect intermediate products from halothane metabolism [20–24]. The determination of halothane in blood by GC headspace analysis with absolute calibration [25] was described. The blood of the normal population, however, contains various amounts of lipoid components (the range of normal values), depending on many factors. These lipoid components retain halothane to various degrees depending on their content in blood. In headspace analysis this fact should result in considerable differences in the distribution constants of halothane and thus also in the slopes of the calibration curves of the systems with varying contents of lipoid components.

In the present paper the results of halothane determination by GC headspace analysis obtained by the method of absolute calibration and by the method of standard addition are evaluated. The reliability of both methods is examined by both statistical analysis and direct comparison.

EXPERIMENTAL

Chemicals

Narcotan Spofa (150 g of halothane plus 15 mg of thymol, Spofa, Prague, Czechoslovakia), ethanol (96%), analytical-grade Na_2EDTA (Lachema, Brno, Czechoslovakia) and Porapak P (Waters Assoc., Milford, MA, U.S.A.) were used.

Gas chromatography

A Chrom 5 chromatograph (Laboratory Instruments, Prague, Czechoslovakia) with a flame ionization detector and a stainless-steel column (2.5 m×3 mm

I.D.) packed with Porapak P (80–100 mesh) was used. The operating conditions were: column temperature, 150 °C; detector temperature, 240 °C; nitrogen carrier gas flow-rate, 35 ml/min. The analyses were performed at 1/32 of the full electrometer sensitivity.

Preparation of standard solutions

A standard halothane solution in ethanol at a concentration of 1.87 g/l was prepared by dissolving 10 μl of Narcotan Spofa (Hamilton 701N microsyringe) in 10 ml of ethanol. This solution was freshly prepared daily.

Headspace technique

Blood samples were taken into disposable syringes containing an anticoagulation agent, 18 g/l Na_2EDTA (1 ml of this agent is used per 9 ml of blood). Then 4 ml of blood were transferred into a 20-ml bottle, and standard halothane solution (ca. 1–10 μl) was added. The bottles were immediately closed with rubber stoppers and placed in a water-bath heated to 56 °C and thermostatted for at least 30 min.

Construction of the calibration curve

After equilibrium had been reached 1-ml samples were taken by gas-tight syringe (Hamilton 1001 TLL) from the gas phase in the bottles and injected into the gas chromatograph. The retention time of halothane was ca. 220 s under the given conditions. Quantitative evaluation was performed by measuring the peak heights.

Standard addition method

The sample was divided into two aliquot portions and transferred into the bottles as described above. A defined amount of the standard halothane solution was added to one of the aliquots. After equilibrium had been reached, the samples were taken from the gas phase and the results were calculated according to the relationship

$$m_i = \frac{m_s}{A_2/A_1 - 1}$$

where m_i is the mass of halothane contained in the original blood sample, m_s is the mass of halothane added as a standard addition, and A_1 and A_2 are surface areas (or the heights) of the halothane peaks in the original and the enriched samples, respectively. The relationship is valid on condition that in both steps the same volume of the gas phase is taken for analysis, and a linear relationship exists between the signal (A_2) and the amount of halothane added (m_s) [26].

RESULTS AND DISCUSSION

Fig. 1 shows the calibration curve plotted for the range of halothane concentrations from 0 to 478.3 μl in blood containing 6.2 mmol/l cholesterol and 2.74 mmol/l triglycerides. Each point represents the average of five measurements.

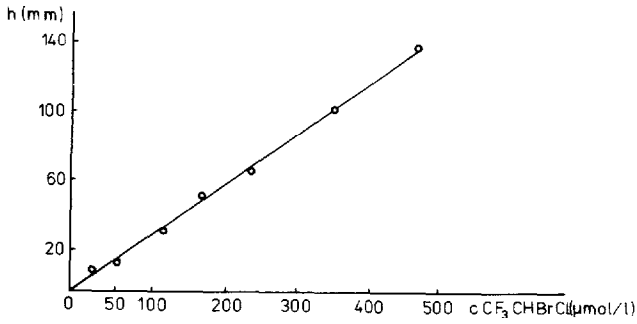

Fig. 1. Calibration curve for the determination of halothane by headspace analysis of blood containing 6.2 mmol/l cholesterol and 2.74 mmol/l triglycerides.

TABLE I

RESULTS OF THE DETERMINATION OF HALOTHANE IN BLOOD BY HEADSPACE ANALYSIS WITH EVALUATION BY ABSOLUTE CALIBRATION AND BY STANDARD ADDITION

Blood sample No.	Halothane (μmol/l)		Cholesterol (mmol/l)	Triglycerides (mmol/l)	Relative error (%)
	Absolute calibration method	Method of standard addition			
1	232.3	211.9	5.2	2.44	9.62
2	498.5	183.4	5.5	1.74	172.19
3	769.6	104.7	5.2	1.41	633.24
4	1256.0	164.2	3.4	1.12	665.73
5	591.7	172.3	5.9	1.29	243.84
6	123.0	181.2	5.2	3.61	−32.15
7	298.8	142.4	5.2	2.01	110.14
8	191.6	149.8	6.5	1.53	27.87
9	100.8	102.1	7.1	1.98	−1.27
10	132.4	113.4	5.8	2.30	16.81
11	397.9	174.4	6.1	1.45	128.45
12	985.6	371.3	5.7	2.05	165.59
13	511.8	108.6	3.6	1.63	369.91
14	1844.0	122.4	4.0	0.61	1411.15
15	1159.4	218.3	5.2	1.06	431.70
16	65.3	34.2	4.5	1.42	91.47
17	234.3	292.1	6.1	3.11	−19.79
18	420.0	219.5	5.6	1.36	91.14
19	371.9	162.8	4.3	1.31	128.28
20	413.4	136.8	4.5	1.64	201.90
21	658.4	254.9	4.2	1.20	158.24
22	252.4	377.5	4.9	3.57	−33.09
23	174.6	95.6	4.7	1.44	82.29
24	1487.4	125.0	3.9	0.76	1089.92
25	150.6	107.9	5.9	2.36	39.54
26	559.0	120.8	4.1	1.06	362.20

TABLE II

HEADSPACE ANALYSIS OF MODEL SAMPLES OF HALOTHANE IN BLOOD BY ABSOLUTE CALIBRATION AND BY STANDARD ADDITION

Concentration of halothane added to model blood samples, 58.5 μmol/l.

Blood sample No.	Method	Found (mean ± S.D., $n=6$) (μmol/l)	Relative error* (%)	Student's coefficient t
1	Absolute calibration	12.86 ± 2.78	78.0	36.74
	Standard addition	52.47 ± 10.53	10.3	1.280
2	Absolute calibration	6.20 ± 2.77	89.4	42.11
	Standard addition	51.19 ± 9.40	12.5	1.739

* Relative difference of the observed value with reference to the true halothane concentration.

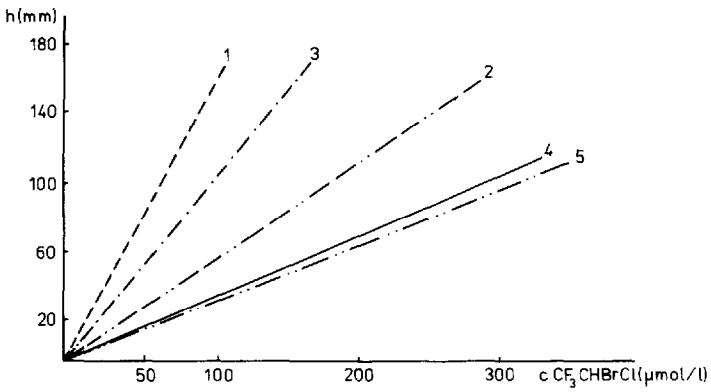

Fig. 2. Calibration curves for the determination of halothane by headspace analysis in blood with various contents of lipoid components: 1 = 3.6 mmol/l cholesterol, 1.63 mmol/l triglycerides; 2 = 4.5 and 1.42; 3 = 5.9 and 1.29; 4 = 5.2 and 2.44; 5 = 5.8 and 2.30.

The reproducibility of the halothane determination according to this calibration curve was verified to be 5.2% for fifteen samples of the same blood.

Of blood lipoid components that can affect the halothane distribution in the headspace technique, the influence of cholesterol and triglycerides was studied. Together with the content of these substances, the halothane content was determined for 26 different samples of blood by the absolute calibration method and by the method of standard addition. The data obtained are summarized in Table I. The last column of this table gives the relative difference between the results of the two methods expressed as percentages.

The results were evaluated statistically by correlation analysis. It was found that there is no correlation between the two methods (correlation coefficient 0.077; $P > 0.05$).

The accuracy of the results of both methods was further examined by analysing a set of two different blood samples with different contents of cholesterol and triglycerides, which were adjusted to a model mixture with a known amount of

ethanolic halothane solution. The results are presented in Table II. Blood sample No. 1 contained 3.70 mmol/l cholesterol and 1.60 mmol/l triglycerides, and sample No. 2 contained 4.50 mmol/l cholesterol and 1.60 mmol/l triglycerides. The amounts in the column headed "Found" are averages of six determinations. A *t*-test was used to see whether this mean differed significantly from the value 58.5. For both samples it was found that the mean result obtained with the absolute calibration method differed significantly from 58.5 (*t*-values: 36.74 and 42.11; $P > 0.05$), but the mean results obtained with the standard addition method did not differ significantly from 58.5 (*t*-values: 1.280 and 1.739; $P > 0.05$).

It follows unambiguously from the above results that the absolute calibration method is by no means suitable for the evaluation of headspace analysis since it yields entirely unreliable results. As mentioned earlier, this is due to the different contents of lipoid and other components. This fact is illustrated in Fig. 2, which shows calibration curves for the determination of halothane by headspace analysis similar to Fig. 1, but for five different blood samples with various contents of cholesterol and triglycerides. If we simply sum the contents of both the lipoid components investigated, the halothane concentration is highest, as expected, in the vapour of the blood sample with the lowest content of these components (see line 1, the sum of both the values being 5.23 mmol/l) and, vice versa, it is lowest in the sample with the highest content of lipoids (see line 5, the sum being 8.10 mmol/l). The slopes of lines 2 and 3, which are not in agreement with the preceding consideration, suggest that the halothane content in the vapour phase is not a simple function of the content of lipoid components under investigation, but that it is also affected by other substances present at various concentrations in the blood of the normal population. It is therefore not possible to propose correction factors by which the results obtained with the aid of one calibration curve could be corrected for the content of lipoid components in order to obtain accurate values. With complicated matrices, such as blood, similar cumbersome and mostly incorrect procedures can be easily avoided by using the method of standard addition.

Finally, it should be emphasized that these conclusions must be respected even in the cases of the analysis of other analytes in complex matrices such as blood, e.g. in the analysis of ethanol, acetone and other toxic volatiles by means of GC headspace analysis.

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