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Systematic errors with the use of internal standard calibration in gas chromatographic headspace analysis

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ABSTRACT

The specificity of the use of internal standard calibration in headspace analysis was studied. An equation was derived that differed from the conventional equation by a factor $(V_g + K_s V_l)/(V_g + K_i V_l)$, where V_g and V_l are the volumes of the gaseous and the liquid phases, respectively, and K_i and K_s are the distribution constants between the phases of the analyte i and the internal standard s, respectively. This factor is generally not equal to unity. Its magnitude, and hence that of the systematic error, is demonstrated by using literature values and experimental data obtained by analyses of model blood and water samples. It is shown that the use of the internal standard technique does not generally eliminate the matrix effect which is a serious problem in quantitative headspace analysis.

INTRODUCTION

Nowadays headspace analysis is widely used for the determination of volatile substances in condensed materials [1–3]. In this method of sample treatment, gas (air) in contact with the material to be analysed is injected into the chromatograph rather than the material itself.

The effect of the matrix composition has long been regarded as a crucial problem in quantitative headspace analysis [4–6]. The composition of the liquid (and/or condensed) sample being analysed influences, sometimes very strongly, the concentration of analyte in the gaseous phase, and thereby the accuracy of the results (the so-called "matrix effect"). In order to compensate for this effect, and to obtain accurate results, analysts often use the internal standard technique for calibration [7–11]. This technique consists in adding to the sample a known amount of a substance (internal standard) different from that being analysed, and relating the peak area of the analyte to that of the internal standard. A calibration graph can be constructed and used for evaluation of this procedure, *viz.*, the ratio of the masses of the analyte and internal standard is plotted against the ratio of the corresponding peak areas. This technique is generally widely used to compensate for imprecisions in injected sample volume measurements and other experimental variables. As far as headspace analysis is concerned, it must be realized that both the analyte and the internal standard are

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distributed between the condensed and gaseous phases and these distributions are influenced by the composition of the condensed phase in different ways. The proportions of analytes and the internal standard in the gaseous phase are therefore generally different from those in the original condensed sample, and might also be different from those in reference calibration. Hence the calibration graph measured for a matrix of a certain composition should be used for headspace analysis of the same matrix, otherwise the results obtained can suffer from systematic errors.

This aspect seems often to be overlooked in this field, even though it has already been discussed in the literature [5], and methods that generally eliminate the matrix effect have been proposed, *e.g.*, the method of standard addition [4–6] and the method of multiple gas extraction [12,13], and also a practical approach to the decision as to whether these methods are necessary in a particular case has been described [14]. Hence, it is felt that a paper defining and demonstrating the problem clearly is lacking.

In this paper the relationships that describe the use of the internal standard technique in headspace analysis and allow an estimate of the magnitude of the systematic error are derived; the estimation was demonstrated using literature values and comparing them with experimental data.

THEORETICAL

Let us consider liquid material enclosed in a vessel to be subjected to headspace analysis, the volatile analyte i being distributed between the liquid and the gaseous phases of this two-phase system. At equilibrium, the mass balance of the distribution can be expressed as

$$m_{io} = m_{ig} + m_{il} \tag{1}$$

and described by the distribution constant, K_i:

$$K_i = \frac{c_{il}}{c_{ig}} = \frac{m_{il}/V_l}{m_{ig}/V_g} \tag{2}$$

where m_{io}, m_{ig} and m_{il} are the mass of the analyte i originally present in a liquid sample and the equilibrium mass of analyte i present in the gaseous and the liquid phases, respectively; c_{ig} and c_{il} are equilibrium concentrations of the analyte i in the gaseous and the liquid phases, respectively and formally V_g and V_l are the volumes of the gaseous and the liquid phases, respectively. By combining the above two equations we obtain

$$m_{io} = c_{ig}(V_g + K_i V_l) = \frac{m_{ig}}{V_g}(V_g + K_i V_l) \tag{3}$$

a well known equation which is the basis of quantitative evaluation in headspace analysis. The gaseous phase sample, of volume v_g, containing the mass of the volatile analyte m_i, is subjected to analysis. To this mass of analyte the peak area of the analyte in the chromatogram, A_i, corrected by a mass response factor, f_i^m, is proportional.

Thus we can write

$$A_i f_i^m = k m_i \tag{4}$$

where k is a proportionality constant characteristic for an apparatus employed [15].

The mass of the analyte in the gaseous phase, m_{ig}, can then be expressed as

$$m_{ig} = \frac{s_i f_i^m}{k} \cdot \frac{V_g}{v_g} \tag{5}$$

By rearranging this expression and substituting for m_{ig} from eqn. 3, we obtain

$$A_i = \frac{k m_{ig} v_g}{f_i^m V_g} = \frac{m_{io}}{V_g + K_i V_l} \cdot \frac{k v_g}{f_i^m} \tag{6}$$

The same situation occurs with the internal standard and a corresponding relationship can also be written:

$$A_s = \frac{m_{so}}{V_g + K_s V_l} \cdot \frac{k v_g}{f_s^m} \tag{7}$$

Division of eqns. 6 and 7 results in

$$\frac{A_i}{A_s} = \frac{m_{io} f_s}{m_{so} f_i^m} \left(\frac{V_g + K_s V_l}{V_g + K_i V_l} \right) \tag{8}$$

This equation describes the use of an internal standard in headspace analysis and differs from that used for this technique in a conventional manner by containing the expression in parentheses. This expression describes the effect of matrix material on the ratios of the concentrations in the gaseous phase of the analyte and the internal standard. In other words, when constructing the calibration graph in a conventional way by plotting various ratios of masses of the analyte and the internal standard against ratios of the corresponding peak areas, we have to multiply the slope of the curve by the magnitude of the factor in parentheses so that we can use this calibration for headspace analysis. It is obvious that the magnitude of this factor is generally not equal to unity and in most practical instances such an operation cannot be made owing to a lack of knowledge of the distribution constants, but the magnitude of the factor corresponds to that of systematic errors from which the results of headspace analysis suffer if the internal standard technique is employed. In further work we calculated values of the slopes of the calibration graphs for different matrix materials from the literature and measured values of the distribution constants with the use of a flame ionization detector, and demonstrated the occurrence of systematic errors in the determination of volatile substances in water and blood on models samples.

EXPERIMENTAL

The measurements of distribution constants were based on the mass balance expressed by eqn. 3. For blood, a known amount of analyte (1–5 μl of a particular substance) was added to 1.5 ml of blood in an 8.2-ml vial, which was closed tightly with a septum. After equilibration for 30 min at 52°C, 0.2 ml of the gaseous phase was withdrawn with a 1-ml gas-tight syringe (Hamilton, Bonaduz, Switzerland) preheated to about 60°C to suppress condensation and adsorption. The gas chromatographic (GC) analyses were performed on a Chrom-5 instrument (Laboratory Instruments, Prague, Czechoslovakia) equipped with a flame ionization detector and a glass column (2.5 m × 3 mm I.D.) packed with Porapak P (80–100 mesh) (Waters Assoc., Milford, MA, U.S.A.), with nitrogen as the carrier gas at a flow-rate of 35 ml/min. The column temperature was 160°C and the injection port and detector temperatures were 180 and 240°C, respectively.

With water and aqueous solutions, a known amount of the analyte was added to 50 ml of water or aqueous phase in a 100-ml bottle fitted with a septum. The bottle was thermostated at 40°C for 20 min and 1 ml of the gaseous phase was withdrawn for analysis as above with a gas-tight syringe preheated to about 60°C. The GC analyses were carried out on a Shimadzu GC4A instrument (Shimadzu Seisakusho, Kyoto, Japan) with a flame ionization detector and a stainless-steel column (1.5 m × 3 mm I.D.) packed with 15% (w/w) Carbowax 20M on Chromosorb G (100–120 mesh) (Carlo Erba) with nitrogen as the carrier gas at a flow-rate of 35 ml/min. The column temperature was 70°C and the injection port and detector temperatures were 150°C, respectively. The peak areas were measured with a CI-100 electronic integrator (Laboratory Instruments) in all instances.

Similarly, the calibration graphs were measured and constructed for several analyte–internal standard pairs, the range of concentrations being from tens to hundreds of μg/ml in distilled water, in 0.34 g/ml aqueous sodium chloride solution and in blood.

All chemicals were of analytical-reagent grade from various suppliers.

RESULTS AND DISCUSSION

To obtain an idea of how serious the systematic error in headspace analysis with internal standard calibration could be, we calculated the values of the slopes of calibration graphs obtained with eqn. 8. For this purpose we used the K values published by Sato and Nakajima [16], who measured the distribution constants in two-phase systems with the gaseous phase being air and the other phase water, blood and/or oil. The mass response factors for the flame ionization detector were taken from ref. 17. The magnitudes of the slopes were calculated for the pairs of various substances, one being considered as the analyte and the other as the internal standard. The results are illustrated in Table I for a ratio of the volumes of the phases of $V_g/V_l = 4$.

It is obvious from Table I that for different liquid phases the value of the slope is not the same for a particular pair of compounds, *i.e.*, that the effect of the matrix composition is not eliminated by an internal standard technique. The ratio of the slopes gives an idea of the systematic error due to the evaluation of the analysis of one

TABLE I

SLOPES OF THE CALIBRATION GRAPHS CALCULATED WITH K VALUES FROM REF. 5

Analyte (i)	Internal standard (s)	Distribution constant		Slope	Liquid phase[a]
		K_i	K_s		
Toluene	Ethylbenzene	2.23	1.69	0.998	w
		15.60	28.40	0.591	b
		1471	3791	0.384	o
Toluene	Benzene	2.23	2.78	0.865	w
		15.60	7.80	1.611	b
		1471	492	3.003	o
m-Xylene	*p*-Xylene	1.66	1.57	0.932	w
		26.4	37.6	0.722	b
		3842	3694	1.04	o
Diethyl ketone	Methyl ethyl ketone	181	254	0.641	w
		168	202	0.746	b
		808	263	2.719	o

[a] w = Water, b = blood, o = oil.

matrix material by calibration with another condensed phase. For instance, if we determine toluene in blood with benzene as an internal standard and the calibration graph is constructed with distilled water, we obtain systematically results that are roughly twice as high. The differences are not very significant only with compounds that are chemically very similar, such as *m*- and *p*-xylene.

To show influence on the results of very small differences in the composition of the matrix of the same character, we measured the distribution constants of acetone and propanol in a blood–air system as described under Experimental. The three blood samples differed in the content of lipophilic substances, *viz.*, blood sample 1 having 3.7 nmol/l of cholesterol and 1.13 nmol/l of triglycerides, sample 2 having 4.2 and 1.28 nmol/l and sample 3 4.5 and 1.5 nmol/l, respectively. The results are given in Table II; the relative standard deviations of the K values are *ca.* 5%. From the magnitude of the slopes calculated according to eqn. 8, again for the phase volume ratio $V_g/V_l = 4$, one can estimate that even with a matrix material of such a similar composition the systematic error can reach up to 20%. One should be aware that in our particular instance such an accuracy might be tolerable, but in other instances the error might be

TABLE II

SLOPES OF CALIBRATION GRAPHS FOR DIFFERENT BLOOD SAMPLES

Analyte, acetone; internal standard, *n*-propanol.

Distribution constant		Slope	Liquid phase
K_i	K_s		
205	935	0.269	Blood sample 1
261	1053	0.303	Blood sample 2
297	1115	0.323	Blood sample 3

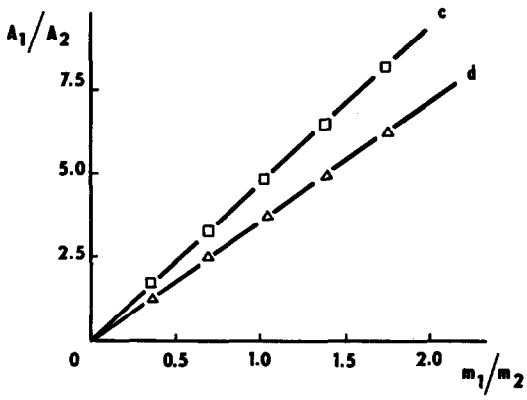

Fig. 1. Example of calibration graphs for the determination of acetone (subscript 1) in blood in headspace analysis with *n*-propanol (subscript 2) as the internal standard. Line c, blood sample 4; line d, blood sample 5.

even higher. For example, the internal standard technique used in headspace analysis for the determination of ethanol in blood has been intensively studied and used [18,19] with an accuracy that was found to be acceptable.

The magnitude of the error is illustrated graphically in Fig. 1, where calibration graphs are plotted for the determination of acetone (subscript 1) in blood by headspace analysis with *n*-propanol as the internal standard (subscript 2). Under the conditions described under Experimental, line c was measured for blood sample 4 containing 7.9 nmol/l of cholesterol and 2.1 nmol/l of triglycerides and line d was measured for blood sample 5 containing 3.8 nmol/l of cholesterol and 1.6 nmol/l of triglycerides. Fig. 1 demonstrates the error made if blood with a composition that is accidentally close to that of sample 5 is analysed and if the evaluation is carried out according to line c.

In another model situation we measured the calibration graphs for pairs of compounds in distilled water and in 0.34 g/ml aqueous sodium chloride solution. The resulting slopes for six chosen pairs are given in Table III, and the calibration graph for acetone with isopropanol as an internal standard is shown in Fig. 2. It is obvious that only very similar compounds such as *n*-propanol and isopropanol are influenced to the

TABLE III

SLOPES OF CALIBRATION GRAPHS OF THE INTERNAL STANDARD TECHNIQUE FOR VARIOUS PAIRS OF SUBSTANCES IN WATER AND IN 0.34 g/ml SODIUM CHLORIDE SOLUTION

Analyte	Internal standard	Slope of calibration graph		Ratio of slopes
		In water	In NaCl solution	
n-Propanol	Isopropanol	0.644	0.649	0.992
Acetone	Isopropanol	3.754	2.771	1.350
Acetone	*n*-Propanol	4.041	3.010	1.340
Ethanol	*n*-Propanol	0.482	0.385	1.260
Acetone	Methyl ethyl ketone	0.456	0.326	1.410

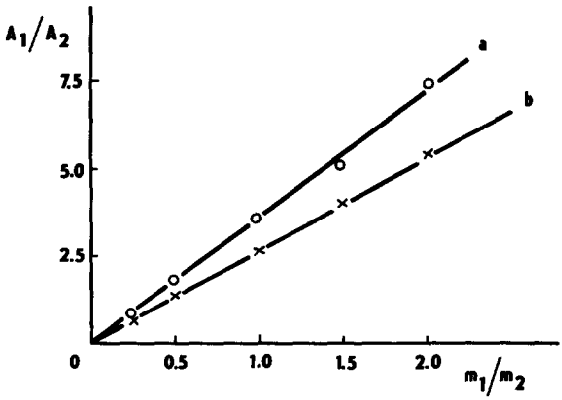

Fig. 2. Example of calibration graphs for the determination of acetone (subscript 1) by headspace analysis with isopropanol (subscript 2) as the internal standard. Line a, in water; line b, in 0.34 g/ml sodium chloride solution.

same extent by a change in the liquid phase composition, so that the calibration graphs are almost identical. With other pairs the relative error reaches 40%.

However, one also has to take account of the influence of adsorption that may possibly occur during the procedure, namely in the syringe used. Although the syringe was kept at an elevated temperature, adsorption may occur in it. If the effect of adsorption varies for different analytes, the result could be similar to that observed in this study or may, at least, contribute to it. According to our previous experience, these effects are not very pronounced and it is also difficult to imagine glass being a selective adsorbent towards the analytes that we studied. As the final consequence for the analytical results seemed to be the same, we did not carry out any special experiments to distinguish the extents of various fine particular effects.

CONCLUSIONS

When using internal standard calibration with headspace analysis, it should be realized that the use of the internal standard technique does not generally eliminate the matrix effect in headspace analysis and systematic errors may occur; the calibration graph for the internal standard technique should be applied to the evaluation of headspace analysis of the same matrix material for which that graph was measured; and one can only expect similar values of the slopes of calibration graphs for very closely chemically related compounds (*e.g.*, isomers).

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