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COMPARISON OF DIFFERENT METHODS OF QUANTITATIVE ANALYSIS EMPLOYING GAS–LIQUID CHROMATOGRAPHY, ILLUSTRATED BY THE DETERMINATION OF WATER IN ORGANIC SOLVENTS

ADAM SHATKAY
Israel Institute for Biological Research, P.O. Box 19, Ness-Ziona (Israel)
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SUMMARY

The methods used in quantitative analysis employing gas–liquid chromatography are critically examined, and a division into three major categories is suggested. The various methods have been applied to the determination of water in organic solvents. It was found that for a moisture content of 0.1–5 wt.-% the external standard method is preferable to the internal standard method, and the method of standard additions is the least satisfactory. The "volume effect" and the "solute effect" were examined for various water–standard–solvent systems, and the errors were evaluated.

INTRODUCTION

In this paper we compare a number of analytical methods for use with gas–liquid chromatography (GLC) in order to determine water in organic liquids. The determination of water in the 0.1–5% (w/w) range is of interest in many applications[1]. However, our main objective was to utilize our results in order to examine critically the merits and limitations of the various methods currently employed in GLC.

THEORETICAL

Methods of quantitative analysis in GLC

In the literature there appears to be considerable confusion in the discussion of the various methods that can be employed for quantitative analysis in GLC. The list of such methods ranges from two in some texts[2–5], to five major methods, subdivided into ten variants, by Novák[6]. Part of the confusion is due to the lack of uniformity in nomenclature between the different authors, and part to some difficulties in distinguishing between the various methods. As an example we cite verbatim the list of Kaiser[7], who enumerates seven methods, given equal hierarchical value:

(1) Evaluation from peak area measurements. The direct method.

(2) Evaluation from peak area measurements using specific calibration factors.

(3) Evaluation of the analysis by a calibration method. External calibration of the peak heights.

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(4) External calibration of the peak areas.
(5) Internal calibration with addition of extraneous substance.
(6) Internal calibration with component already present.
(7) Evaluation by the height and area methods for constant sample quantity.

We note that the use of height or area of a peak is a criterion of a method when distinguishing between methods 3 and 4, but is not a criterion in method 7. The use of peak areas or peak heights is of considerable interest, and had been discussed extensively[3,8–10], but we doubt whether it is relevant in the present context of classification of methods. In fact, we shall refer from now on only to peak areas, but all our arguments will apply equally to instances where peak height measurement is preferred.

Similarly, some authors distinguish between methods that yield molar fractions and methods that give weight-percentages. It appears to us that such distinctions are not fundamental, and that it would be helpful to define methods using more basic differences. We suggest that the following distinction into three categories (external standard method, internal standard method and method of standard additions) might be adopted.

External standard method (ESM)

A known quantity of the analyte, ${}_1q_2$, is introduced into the column. This is the "external standard". We shall use right-hand subscripts for the different substances, reserving 1 for the solvent and 2 for the analyte. When different experiments are considered, they will be distinguished by a left-hand subscript. Thus ${}_1q_2$ denotes the quantity of the analyte injected in the first experiment. A corresponding peak of area ${}_1A_2$ is obtained. If the response is linear, a peak ${}_2A_2$ of the same analyte corresponds to the "unknown" quantity ${}_2q_2$:

$$ {}_2q_2 = {}_2A_2 \cdot \frac{{}_1q_2}{{}_1A_2} = \frac{{}_2A_2}{\text{sensitivity factor}} \qquad (1) $$

The accuracy of the ESM is directly related to the accuracy with which ${}_1q_2$ is known; we therefore have to know exactly the concentration of the standard solution and the volume injected. We can increase the accuracy of the measurement of the sensitivity factor $({}_1A_2/{}_1q_2)$ by repeating the measurement of ${}_1q_2$ many times, or by using a number of different q_2 values of the same standard, so that we obtain a "calibration graph" passing through the origin, and having a constant slope ${}_iA_2/{}_iq_2$ for all the i injections of the standard. We use quotation marks, because a graph based on a closed equation* (in our case the linear eqn. 1) is not a good calibration graph; it might be preferable to reserve this term for an empirical graph of A *versus* q, as discussed in the next paragraph. We could just as well increase the accuracy by improving the sampling apparatus (say the syringe). All such improvements do not affect the case that we are using the same ESM, and do not warrant splitting the method into sub-methods.

We have discussed up to now eqn. 1, which depended on the linearity of the response. When the response is not linear, we are obliged to construct a real experimental calibration graph, showing what ${}_iA_2$ corresponds to any ${}_iq_2$. This is done in the hope that (all conditions being equal, or at least sufficiently similar) the

* An explicit equation not involving infinite series.

area of the unknown ${}_1A_2$ will fall on the graph in the expected position, ${}_1q_2$. This complication still leaves us within the ESM.

When injecting simultaneously two or more different substances (say 1 = solvent, 2 = analyte and 3 = arbitrary substance), we obtain, for a linear response, a number of equations of the form of eqn. 1, one for each of the n substances. Thus, for the jth injection:

$$ {}_jq_i = {}_jA_i \cdot \frac{{}_1q_i}{{}_1A_i} \quad (i = 1, 2, \ldots, n) \tag{2} $$

The ratio of the weights between substances 2 and 3 is $R_q = q_2/q_3$. This does not mean that the ratio of the corresponding peak areas ($R_A = A_2/A_3$) is the same. As the detector will, in general, respond differently to each substance, the ratios R_q and R_A will be different. In fact, R_A/R_q is the "relative sensitivity factor" of substance 2 relative to substance 3, under the given conditions. When the response is not linear for both substances, the relative sensitivity factor R_A/R_q may fail to remain constant for the changing qs. Still, we find that the "relative sensitivity factors" fit well into the framework of the ESM. It is clear, therefore, that methods 1–4 in the list of Kaiser[7] all fall into this category.

We have mentioned that in any application of the ESM it is assumed that the conditions under which the calibration is made correspond in all the relevant features to the conditions under which the "unknown" sample is examined. This appears self-evident when dealing with parameters such as column temperature or the velocity of the carrier gas, but can be forgotten when considering the "matrix effect". Thus, under identical conditions of the instrument, an unknown sample containing ${}_1q_2$ of the analyte can yield a peak ${}_1A_2$, while the calibration sample, containing the same amount, ${}_1q_2$, can yield a very different ${}_2A_2$. This remark applies also to the internal standard method, but not to the method of standard additions (MSA). We shall elaborate this statement when discussing the MSA.

Internal standard method (ISM)

This method has been discussed at length previously[11,12], so here we shall deal with it only briefly. We note that whereas in the ESM the term "standard" referred to the analyte (*e.g.*, when determining water in benzene, water itself is the standard), in the ISM the term "standard" refers to some third substance, such as butanol, added for the purpose of analysis to the mixture of water and benzene. The calibration is made using the area ratio $R_A = A_{\text{water}}/A_{\text{butanol}}$, corresponding to the known weight ratio $R_q = q_{\text{water}}/q_{\text{butanol}}$. For the determination of water in the "unknown" sample, a known quantity of butanol would be added to the sample, and from the experimental R_A the R_q would be calculated, thus yielding finally q_{water}.

For an instrument in which all the A_is are linear functions of the q_is, the distinction between the ESM and the ISM would be fundamental: in the first instance an exactly known amount q_i must be introduced into the column, to obtain the corresponding A_i. In the second instance the amount of the mixture introduced is immaterial; so long as R_q is known precisely, the ratio R_A will give us the exact result. We have shown, however, that a slight non-linearity is sufficient to vitiate this fundamental distinction[11]. For a non-linear response, the quantity introduced (q_i) can affect the R_A even at constant R_q. Nevertheless, the effect can be negligible. Even when

the "volume effect" is very pronounced, as in the case of the system dimethyl methylphosphonate–dodecane described earlier[11], an error of 30% in q_i would cause an error of 30% in an ESM analysis, while causing an error of less than 10% in the results obtained by ISM analysis. Thus we feel justified in maintaining that the two methods are clearly distinguishable.

A variation of the ISM may deserve special mention. Usually one assumes that the internal standard is a substance introduced into the sample. However, the solvent itself can be considered as an "internal standard". Thus the measurements would yield $R_A = A_{\text{analyte}}/A_{\text{solvent}}$, and the quantities corresponding to these areas would be $R_q = q_{\text{analyte}}/q_{\text{solvent}}$. It is evident from the form of these expressions that this method belongs to the ISM category. While papers using the above technique appear occasionally[13], we are not aware of any treatment concerning the peculiarities, merits or limitations of this approach. We shall therefore devote some attention to this subject while dealing with our experimental results.

At this point it might be pertinent to deal with the so-called "internal normalization method". This is often listed not only as an independent method, but also as the leading one. For instance, Kaiser and Debbrecht[14] listed three methods of quantitative analysis in GLC, in the following order: (1) internal normalization; (2) external standardization; and (3) internal standardization. Exactly the same classification was adopted by Umbreit[5]. We shall not describe internal normalization in detail here, as the subject was treated extensively by Kaiser and Debbrecht[14]. However, it appears to us that this "method" is merely an application of the ISM, as it relies entirely on the measured relative areas of the components when the relative weights of the components are given. It also assumes that the relative sensitivity factors are constant, so that it forms a particular and limited case of the ISM. Thus it suffers from all the limitations of the ISM, in addition to some approximations peculiar to itself.

Method of standard additions (MSA)

While this method is occasionally employed, it is very rarely referred to explicitly. For instance, of three papers in which this method has been employed to determine water[15–17], only one[17] refers to the method by its name. In texts on chromatography it is often completely ignored. Of 11 extensive reviews[2–7,14,18–21], only two[6,19] include it among the available analytical methods. This is regrettable, as MSA can be employed not only in GLC, but also in other analytical disciplines, such as photometry[22] and electrochemistry[23,24], so that it is of general interest. Nevertheless, although the use of this method is common, we have failed to find a satisfactory description of the basic principles that characterize MSA and differentiate it from ESM and ISM.

We note that the term "standard" in MSA again refers to the analyte (as in ESM) and not to some other substance (as in ISM). It seems to us that the characteristic feature of MSA is its dependence on a closed function of the response *versus* the quantity of the analyte. To illustrate the above statement, let us assume that on the introduction of the quantity q of the analyte into an instrument we obtain a measurement M, and that it is well established that in our experiments

$$M(q) = a\,q^b \tag{3}$$

where a and b are constants for this particular set of experiments. Eqn. 3 is feasible; it could even describe the non-linear function of the peak area ($M = A_t$) obtained on the injection of q_t, as suggested earlier[11]. Eqn. 3 contains two unknown parameters. On introduction of a sample containing ${}_xq$ of the analyte, we obtain

$$ {}_xM = a\,{}_xq^b \tag{4}$$

To solve eqn. 4 for the three unknowns a, b and ${}_xq$, we need two more measurements; in the first we add ${}_1q$ grams of pure analyte to the sample, and in the second ${}_2q$ grams. We obtain the corresponding measurements ${}_1M$ and ${}_2M$:

$$ {}_1M = a\,({}_xq + {}_1q)^b \tag{5}$$

$$ {}_2M = a\,({}_xq + {}_2q)^b \tag{6}$$

Eqns. 4–6 enable us, in principle, to calculate a, b and ${}_xq$. In practice, three experiments are liable to give poor accuracy. Therefore, either the experiments should be repeated using the same ${}_xq$, ${}_1q$ and ${}_2q$ a number of times, or a longer sequence of ${}_3q$, ${}_4q$, . . . , ${}_nq$ might be tried, with a, b, and ${}_xq$ evaluated from "best fit" to eqn. 3.

In general, any correct function $M(q)$ containing n parameters could be utilized by conducting $n + 1$ experiments, whether M is a response of a potentiometer on the use of ion-specific electrodes (following the Nernst equation), or a response of a photometer in spectrophotometry (following the Beer–Lambert law). The character of MSA is thus shown to be fundamentally different from ESM and ISM, where the function $M(q)$ need not have a closed form.

We have stated that the parameters (say a and b in eqns. 3–6) remain constant on the addition of the analyte. Thus any matrix effect is irrelevant: for a sample having a different matrix we shall, in the worst case, obtain a different set of parameters, which does not affect our ability to evaluate ${}_xq$. We conclude, therefore, that of the three methods considered only MSA can overcome the "matrix effect". This is one more fundamental difference between the methods considered.

Often it is assumed that $M(q)$ is linear in q. Thus, $M = aq$ and experiments using MSA resemble those conducted while using a linear case of ESM (*cf.*, eqn. 1 in the discussion of ESM). However, the resemblance is fortuitous as the principles of the two methods are different. An example of such a case is illustrated by Fig. 3, and is discussed in the accompanying text.

EXPERIMENTAL

Materials

The solvents and the standards (diethyl ether, isopropanol, *sec.*-butanol, *tert.*-butanol) were of analytical reagent grade (Merck, Darmstadt, G.F.R.) and were used without any special treatment.

Instruments

Two sets of instruments were used:

(1) F & M Model 500 gas chromatograph with thermal conductivity detector

(TCD) and a Honeywell disc and ball integrator; column, copper, 130 × 0.5 cm I.D.; stationary phase, Porapak QS (50–80 mesh); carrier gas, helium, flow-rate 50 ml/min; inlet temperature 180°C; column temperature 110°C; detector temperature 240°C.

(2) Packard 7400 gas chromatograph Model 804, oven Model 873, temperature control Model 886 TCD and Spectra-Physics Autolab Minigrator integrator; column, glass, 200 × 0.5 cm I.D.; stationary phase, Porapak Q (100–120 mesh); carrier gas, hydrogen, flow-rate, 20 ml/min; inlet temperature, 170°C; column temperature, 110°C; detector temperature, 210°C.

RESULTS AND DISCUSSION

Water has been determined many times by GLC, using the ESM[1,25–27], the ISM[13,28–31] and the MSA[15–17]. However, each worker presented results relating to his own instrument, conditions and sample, so that comparison of the different methods is difficult. A notable exception is a paper by Smith[32], who determined water in a mixture of acetone (11 wt.-%) and water (89 wt.-%), using "internal normalization" (actually ISM with the water, or the acetone, serving as standard), ISM and "fixed volume" (which is our ESM, with emphasis on using exact and identical volumes in all injections). Smith's results were almost equally good in the three instances. The system considered by Smith is of limited interest, however, and does not illustrate clearly the difference between the various methods. The present results can be considered as an extension of Smith's investigation: we shall follow the framework presented in the theoretical section, and deal with water contents in the range of 0.1–5% (w/w).

External standard method

Standard solutions of water in diethyl ether and in isopropanol were prepared, and measured in the F & M chromatograph. Some typical peaks are presented in Fig. 1.

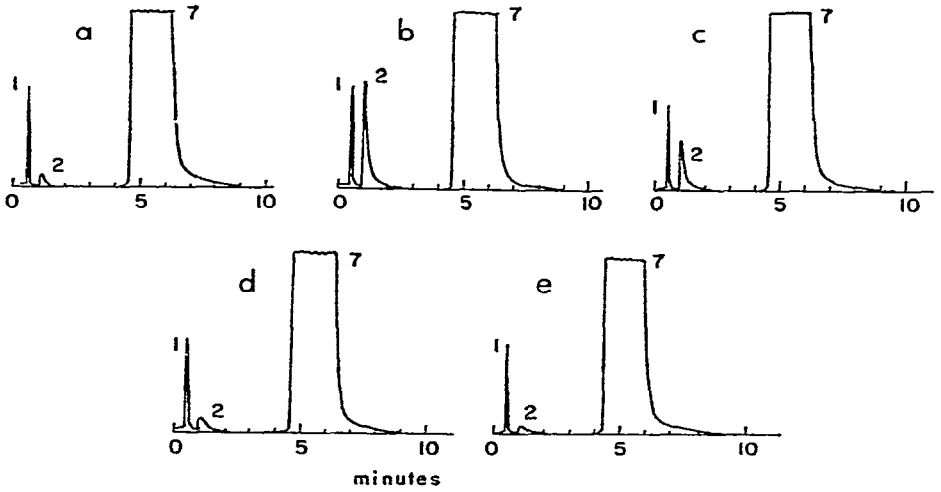


Fig. 1. Chromatograms of some mixtures of water and isopropanol. Peaks: 1 = air; 2 = water; 7 = isopropanol.

Fig. 1a refers to a newly opened batch of isopropanol from Merck ("zur analyse"). The peak was measured at maximum sensitivity (attenuation 1, out of a range 1–1024). The area of water is only 3 scale units, which corresponds (*cf.*, Fig. 2) to $1 \cdot 10^{-6}$ g of water in the 3 μl injected, *i.e.*, to *ca.* 0.05%(w/w) of water, which is well within the limits specified by the manufacturer. Fig. 1b relates to shelved isopropanol. Here the area (again on attenuation 1) is 29 scale units, corresponding to $1 \cdot 10^{-5}$ g of water, *i.e.*, to *ca.* 0.5% (w/w). Fig. 1c relates to isopropanol stored over Drierite. The water content was found to be *ca.* 0.2% (w/w). Fig. 1d refers to a random sample of isopropanol, the water content of which was *ca.* 0.03% (w/w). Fig. 1e shows the results for isopropanol dried over molecular sieve[28]; the peak area of 2 scale units corresponds to $6 \cdot 10^{-7}$ g (in 3 μl), *i.e.*, *ca.* 0.03% (w/w).

A calibration graph for the F & M instrument, based on similar chromatograms, is presented in Fig. 2.

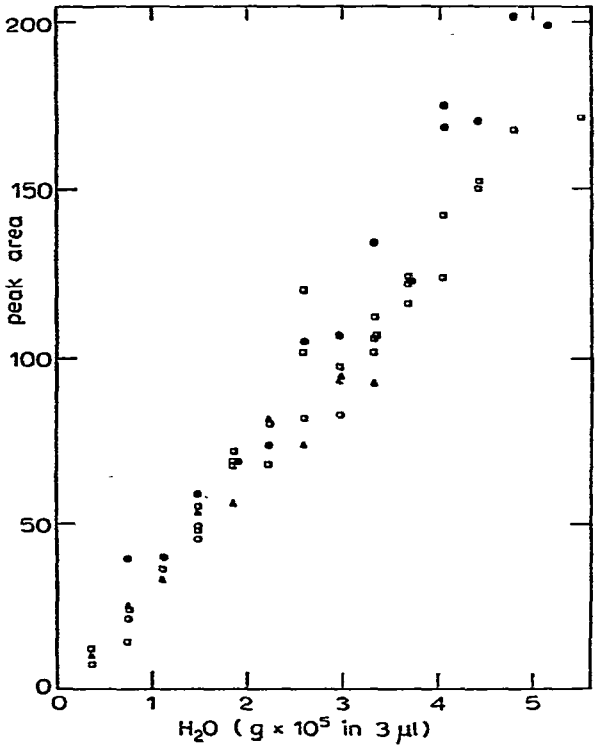


Fig. 2. Calibration graph of peak area *versus* mass of water, obtained with the F & M chromatograph. For all injections $v = 3$ μl. ○, First day; △, after 14 days; □, after 70 days; ●, after 10 months.

It can be seen that the column and the instrument were fairly stable over a period of 10 months. The reproducibility of the readings is, however, not very satisfactory. All of the results presented in Fig. 2 had to be obtained using the maximum sensitivity of the instrument (attenuation 1), so that quantities of water below $5 \cdot 10^{-7}$ g (in 3 μl) could not be measured.

Similar results obtained with the Packard instrument are presented in Fig. 3.

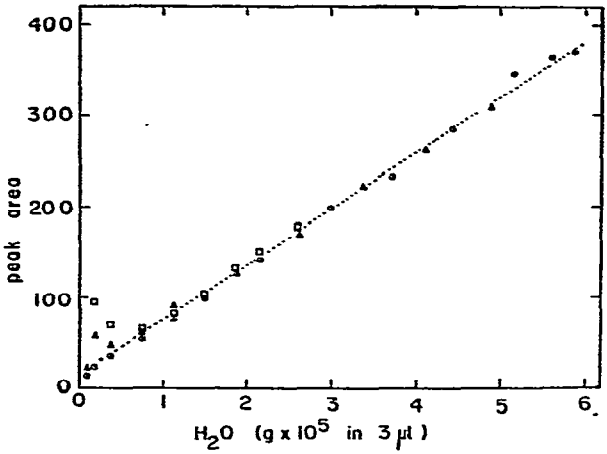


Fig. 3. Calibration graph of peak area *versus* mass of water, obtained with the Packard chromatograph. For all injections $v = 3\,\mu l$. ○, First day; △, after 3 days; □, after 5 days.

The attenuation used for the data in Fig. 3 was 20–50 mV, while the available scale is 1 mV–1 V. It can be seen that for quantities of water above $1 \cdot 10^{-5}$ g (in 3 μl) the reproducibility is very satisfactory. However, below this level (*i.e.* below 0.5%, w/w) the results are not reliable. Thus the Packard instrument gives better accuracy at water contents above 0.5% (w/w), but does not improve the limit of detection.

The experimental points in Fig. 3 lie on a straight line, intercepting the ordinate at 15 peak area units. This enables us to consider the experiments summarized in Fig. 3 as a case of the standard additions method (*cf.*, the theoretical section). Calculation yields 0.1% (w/w) of water in the "unknown" (isopropanol). This agrees with the results obtained from Fig. 1.

TABLE I

DETERMINATION OF WATER IN ISOPROPANOL USING F & M CHROMATOGRAPH AND EXTERNAL STANDARD METHOD

Sample	*Water added (%, w/w)*	*Water found (%, w/w)*			
1	0.4	0.4	0.4	0.3	—
2	0.6	0.6	1.0	0.6	1.2
3	0.7	0.8	0.6	—	0.5
4	0.9	1.1	0.9	—	1.0
5	0.9	0.9	0.8	0.7	—
6	1.1	1.3	1.2	1.3	1.3
7	1.1	1.1	1.0	—	1.0
8	1.4	—	1.4	1.2	1.7
9	1.4	1.5	1.2	1.2	—
10	1.5	1.7	1.6	1.6	1.9
11	1.6	1.9	1.6	—	1.5
12	1.8	—	—	1.7	—
13	2.1	3.3	2.3	—	2.2
14	2.1	2.4	2.1	1.8	—
15	2.3	2.6	2.3	—	2.5
16	2.5	2.4	2.6	2.4	—
17	2.8	3.4	2.6	—	3.0

To test the various methods for the determination of water using GLC, we obtained from an independent source 17 "unknown" samples, containing 0.4–2.8% (w/w) of water. The results obtained by using the ESM on the F & M instrument are given in Table I. The results in the four columns for water found are for four sets of determinations, each set executed by different workers on different days. Each result is based on an average of 4–6 injections.

Fig. 4 is a graphical comparison of the "real" (expected) values with the "found" values for a set of 11 samples. Each symbol represents a different group out of the four groups of determinations carried out for this set of samples.

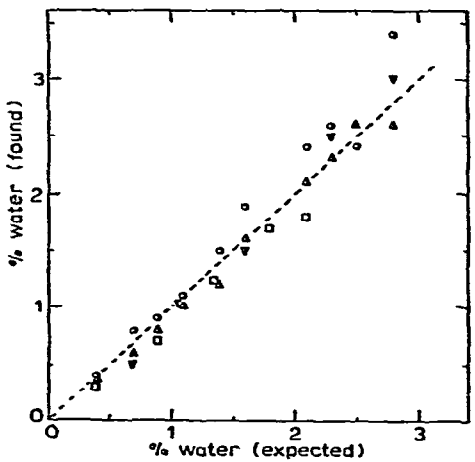


Fig. 4. Comparison of "expected" water content with experimental results, obtained using ESM and the F & M chromatograph. Each symbol relates to one of the four groups examined.

The deviations of the analyses from the "true" values are about ±0.1% (w/w) of water, *i.e.*, the coefficient of variation is *ca.* 5%, reflecting the precision of the calibration graph in Fig. 2.

Method of standard additions

We have seen that this method requires the knowledge of the closed function $A(q)$. All workers who use MSA in GLC assume that this function is linear, and that it passes through the origin, *i.e.*, it is of the very simple form of $A_i = aq_i$. On such an assumption we could calculate ${}_xq_{water}$ in isopropanol, as we did when discussing Fig. 3. Unfortunately, it has been shown[11,12] that A_i is often a complicated function of q_i, and that linearity can be a poor approximation. This is true even when q_i tends to zero.

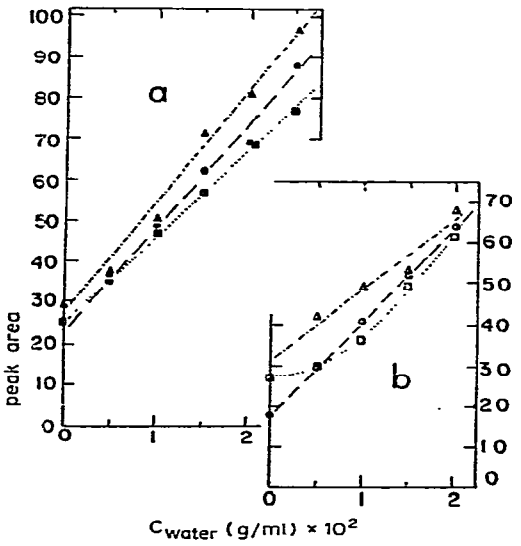
When the "unknown" samples described in the previous section were analysed using MSA, two types of results were observed. The first type is illustrated in Fig. 5a.

The three sets of points (circles, triangles and squares) represent three determinations carried out on different dates on the same sample. It can be seen that in each instance the area varies linearly with q_{water}, although the slope differs for each instance. We have considered this possibility in the theoretical discussion, and found that it need not affect the results. Indeed, the calculations yield 1.1% (w/w) of water for the circles, 1.5% for the squares and 1.1% (w/w) for the triangles. The "true"

value is 1.4% (w/w). Had the method been perfect, we would expect the three straight lines to intersect at the same point on the negative sector of the abscissa.

Fig. 5b illustrates very different behaviour. While both the circles and the triangles lie on straight lines, they do not converge to q_{water} on the abscissa. The circles indicate that the sample contains 1.3% (w/w) of water, whereas the triangles indicate 3 wt.-%. The "true" value is 0.9% (w/w). It is interesting that the squares do not fit a linear relation.

Five of the "unknown" solutions described in the previous section were also analysed by the MSA. A summary of three series of analyses for each of the five samples is given in Fig. 6.



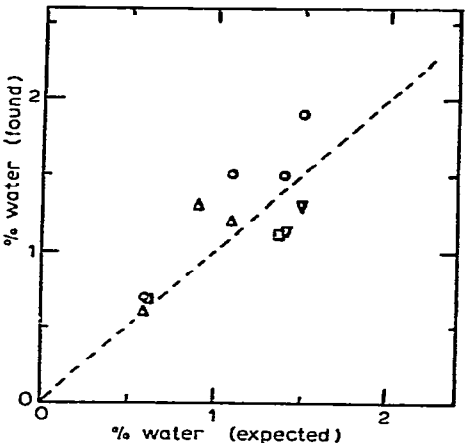


Fig. 5. Method of standard additions. For details and symbols, see text.

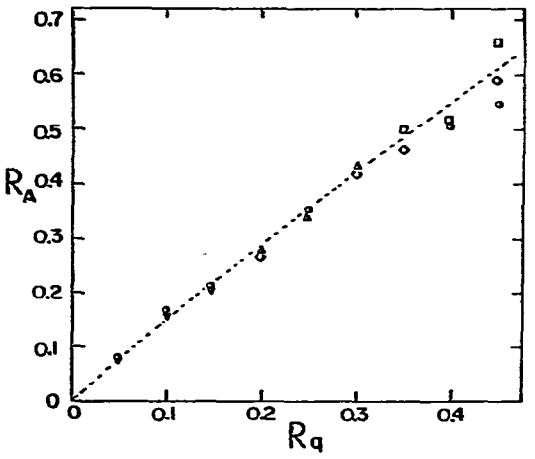
Fig. 6. Comparison of "expected" water content with experimental results, obtained using MSA and the F & M chromatograph.

Each of the four symbols represents one series of analyses. For each analysis the sample was dosed with five increments of water (as shown in Fig. 5), and each of the six resulting solutions was injected at least three times to obtain the average peak area. Compared with Fig. 4, the MSA appears to yield less reproducible results than the ESM; the average scatter is about ±0.2 % (w/w), and the coefficient of variation is *ca.* 10%.

Internal standard method

A number of experiments were carried out, in which *tert.*-butanol was employed as the internal standard. When the concentration of *tert.*-butanol was 0.077 g/ml, and volumes of 3 μl were injected, the R_A *versus* R_q calibration for the F & M instrument was as shown in Fig. 7.

We have stressed the importance of a preliminary examination of the "volume effect" and the "solute effect"[12] before the employment of such a calibration graph.



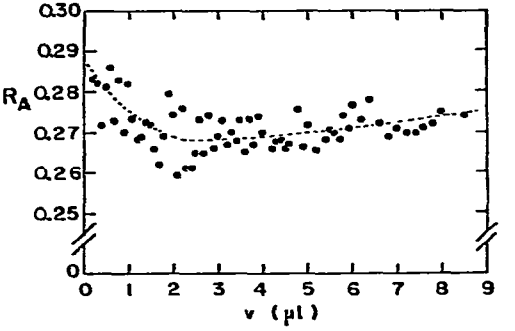


Fig. 7. Calibration graph for ISM, obtained using the F & M chromatograph. Each of the five symbols relates to a different series of experiments. Analyte, water; standard, *tert.*-butanol.

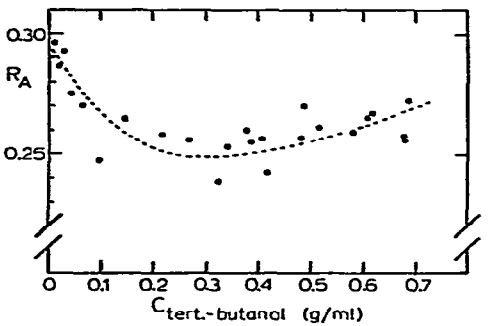
Fig. 8. "Volume effect" for a *tert.*-butanol concentration of 0.077 g/ml at constant $R_q = 0.19$.

We found that both of the above effects were present in the system of analyte–standard–instrument used here.

Fig. 8 presents the results relating to the "volume effect". It can be seen that the effect is not very significant, especially when the injection volume is 3 µl; for injections of 1–2 µl it can cause a 5% error.

The "solute effect" is even more pronounced, although less troublesome. As shown in Fig. 9, at *tert.*-butanol concentrations of about 0.1 g/ml an error of 10% in concentration will cause only a 1% error in R_A.

When the "unknown" samples referred to above were analysed with the aid of Fig. 7, the results summarized in Fig. 10 were obtained.



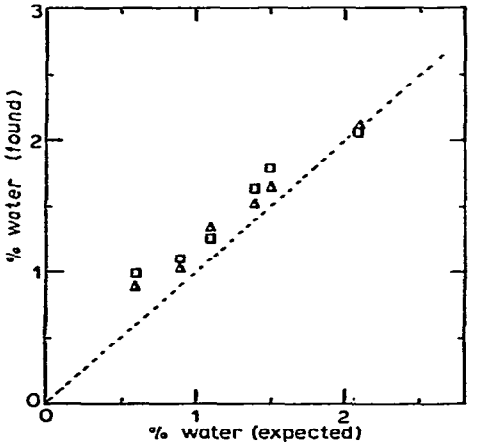
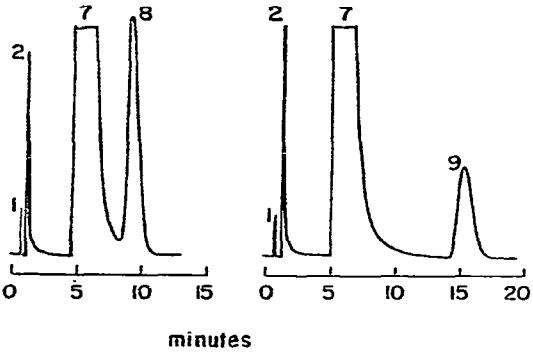


Fig. 9. "Solute effect": change of R_A with concentration of *tert.*-butanol at constant $R_q = 0.19$.

Fig. 10. Comparison of "expected" water content with experimental results, obtained using ISM (with *tert.*-butanol as standard) and the F & M chromatograph. The two symbols represent two different series of measurements.



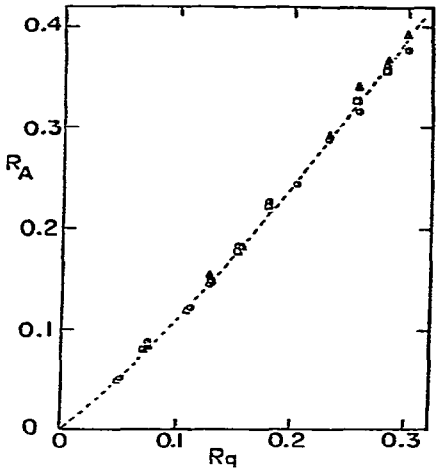


Fig. 11. Resolution obtained with two isomers of butanol (left, *tert.*-butanol; right, *sec.*-butanol). Peaks: 1 = air; 2 = water; 7 = isopropanol; 8 = *tert.*-butanol; 9 = *sec.*-butanol.

Fig. 12. Calibration graph for ISM obtained using the F & M chromatograph. Analyte, water; standard, *sec.*-butanol. The three symbols represent three different series of measurements.

It can be seen that there is a tendency for all the results to be high. A possible reason could be inefficient resolution between the peaks of the *tert.*-butanol and the peaks of the isopropanol. We therefore prepared a new calibration graph, using *sec.*-butanol as the internal standard. The resolution obtained with the use of the two isomers of butanol is shown in Fig. 11. While the time of measurement increases by about 50%, the resolution appears to improve.

The calibration graph based on *sec.*-butanol is shown in Fig. 12.

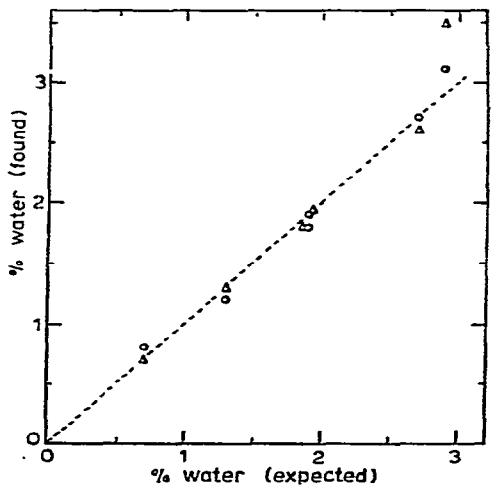


Fig. 13. Comparison of "expected" water content with experimental results, obtained using ISM with *sec.*-butanol as standard, and the F & M chromatograph. The two symbols represent two different series of measurements.

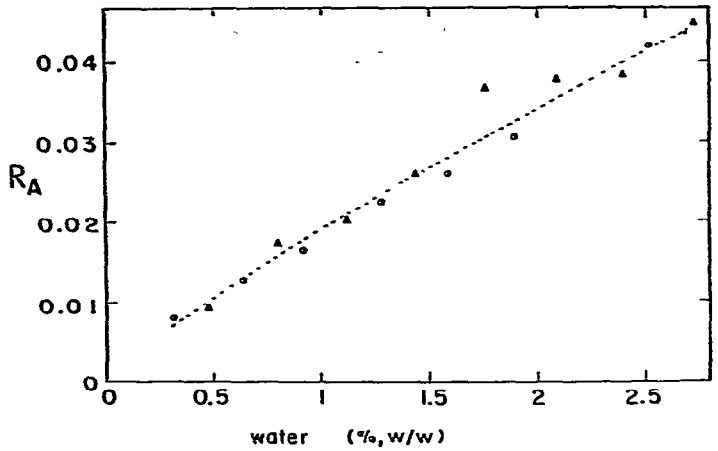


Fig. 14. Calibration graph for ISM, with water as analyte and isopropanol serving as solvent and standard, obtained using the Packard chromatograph. The two symbols represent two different series of measurements.

When the "unknown" samples were analysed using the new internal standard, the results were satisfactory, as shown in Fig. 13.

In the theoretical section we discussed the interesting variation of the ISM in which the solvent serves as the internal standard. A graph corresponding to Figs. 7 and 12 should be prepared. However, as R_q would not have a very direct meaning in this context, it is more convenient to plot R_A directly against the % (w/w) of water. It should be noted that that the values of R_A are small (when the peaks of water and isopropanol are converted to the same scale). The calibration, carried out on the Packard instrument, takes the form shown in Fig. 14.

It should be noted that despite the good precision of the instrument (*cf.*, the discussion of Figs. 2 and 3), the reproducibility in Fig. 14 compared with Fig. 12 is poor. This is due to the 100-fold difference between the quantities and the peaks of the analyte and the standard.

It remains for the "volume effect" and the "solute effect" in the present system to be investigated. However, "solute effect" was defined by us[12] as the change in R_A when the quantity of the standard was changed at constant R_q. In our case such

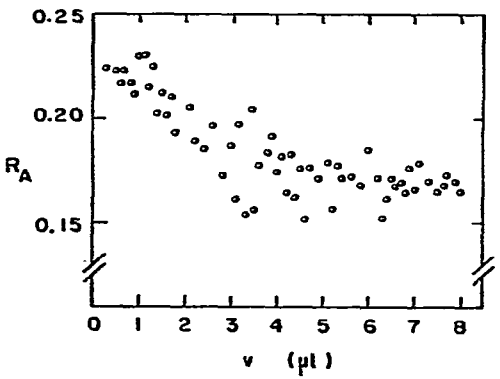


Fig. 15. "Volume effect" in the system water–isopropanol.

an experiment is meaningless. There remains, therefore, only the "volume effect". Fig. 15 shows that the effect exists, and that an error of 1 μl corresponds to an error of 5–10% in R_A.

The experiments reported in Figs. 14 and 15 allow us to illustrate some points from our previous theoretical argument.

Firstly, on measuring R_A we are actually measuring A_{water} and $A_{isopropanol}$, peaks given by exactly known quantities of water in exactly known quantities of isopropanol solvent. Thus, from our measurements a graph can be constructed similar to that required for the ESM (*cf.*, Figs. 2 and 3). Such a graph is represented by the triangles in Fig. 16. For the triangles, the quantity of water on the abscissa relates always to an injection of 3 μl.

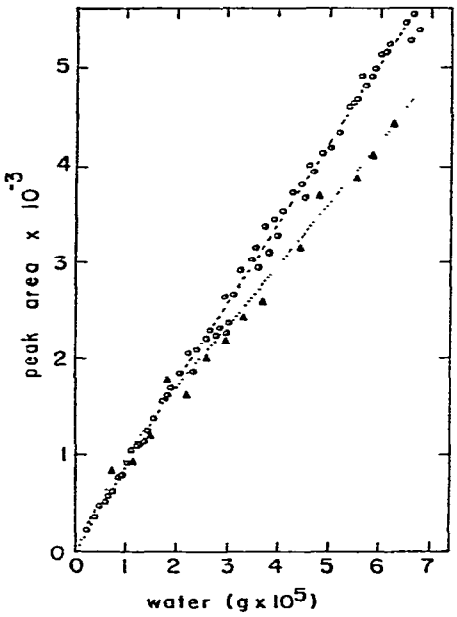


Fig. 16. Calibration of peak area *versus* mass of water. ▲, At constant volume; ○, at constant concentration.

However, the graph could be constructed only because the q values were known accurately. We recall that in the ISM the ratio of the analyte to the standard has to be exact; in the ESM the ratio of the analyte to the solvent has to be exact; in the present case the two requirements coincide. Still, the method itself is more characteristic of ISM than of ESM, because when we actually come to analyse the sample, it is more convenient to inject an approximate volume and use Fig. 14, than to inject exactly 3 μl and use the "triangle" graph in Fig. 16.

Another point of interest arises on comparison of the A_t values obtained on the injection of constant volumes (as in Fig. 14) with the A_t values obtained at constant concentration of the analyte (as in Fig. 15). The latter results are presented as circles in Fig. 16. We have pointed out that in such a case the two graphs appearing in Fig. 16 need not be identical. This conclusion has also been drawn by Boček and Novák[33],

who even attempted to illustrate it by experimental results in their Fig. 5. However, it appears that their results are questionable. Firstly, they obtained two straight lines, which we shall immediately show to be incompatible with the theory; and secondly, one of their graphs does not pass through the origin for $q_t = 0$. The last difficulty has been explained by Novák[34] to be due to the volume of the needle, which contained *ca.* $3 \cdot 10^{-7}$ moles of the analyte, and which should be added to each ordinate of their curves.

Some consideration of the function $A(q)_v$ (at constant volume) and $A(q)_c$ (at constant concentration) shows that these graphs intersect at least at two points. The first point is the trivial one at the origin: when $q = 0$, A must be 0, irrespective whether we inject 0 ml of concentrated solution, or v ml of pure solvent, unless A is an artifact. Next, we note that in one series of experiments we deal with a constant volume of injections (v_{constant}) in which the concentration of the analyte increases from zero; in the second series the concentration of the analyte remains constant (c_{constant}) while the volume of the injection increases from zero. There must be one solution in the first series where the concentration reaches the value c_{constant}, and there must be one solution in the second series where the volume reaches the value v_{constant}. In both of these solutions $q = c_{\text{constant}} \cdot v_{\text{constant}}$, and the two solutions are identical. Therefore the A values obtained in both instances will be identical, so that the two curves will intersect at this q.

If both graphs are linear, it is obvious that, having two points in common, they coincide. If at least one graph is not linear, they may differ, but as they are approximately linear they will be close together, and considerable experimental effort will be required to establish the difference between them. The above argument is well illustrated in Fig. 16.

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