CHROM. 4033

STATISTICAL ANALYSIS OF THE TECHNIQUES OF QUANTITATIVE GAS CHROMATOGRAPHY

P. BOČEK, J. NOVÁK AND J. JANÁK
Institute of Instrumental Analytical Chemistry, Czechoslovak Academy of Sciences, Brno (Czechoslovakia)

(Received March 1st, 1969)

SUMMARY

A comparison of the theoretically predicted coefficients of variation of quantitative gas chromatographic determinations, by various techniques, was made with the corresponding experimental data obtained by replicate isothermal analyses of model mixtures on a commercial analytical apparatus with a flame ionization detector. In the work with conventional recording, results were obtained with variation coefficients of about 0.5, 1.0, and 5.0% under favorable, medium and unfavorable conditions, respectively, when employing the internal normalization, internal standard, and absolute calibration techniques. The standard addition technique yielded results with about half the precision in all cases. Under the above circumstances, the main contributions to the resultant error were the errors of measuring the amount of sample, evaluating the peak size, and determining the relative molar response value; the apparatus errors were relatively insignificant.

INTRODUCTION

The problems of quantitative analysis by gas chromatography have been given relatively little attention. Most of generally oriented studies on quantitative gas chromatography are aimed at the estimation of the errors associated with the manual evaluation of the chromatogram[1] or with the automatic integration of the detector response[2,3,7,12,15]; these problems have been combined with some studies of the internal normalization technique[4,8]. Some recent papers have concerned themselves with the instrumental aspects of quantitation in gas chromatography[3,5,13], and some attention has also been given to the role of the sorption system[10,11].

KAISER, in his book[9] has discussed at length the errors of single methodical steps in quantitation as well as the errors incidental to imperfect functioning of the apparatus. However, the questions concerning the working techniques as a whole have only been considered briefly, and merely rough data have been given on the reliability of the individual working techniques.

The present paper is an objective comparison of all the currently used working techniques of quantitative gas chromatography. The study has been designed in such a way that it should be possible to take into account as many variants of each technique as possible, and to preclude the difficulties stemming from the decomposition or irreversible sorption of components, and the presence of substances not subject to chromatography or detection, etc.

THEORETICAL

The procedure in quantitative analysis by gas chromatography involves a number of steps, each of which is associated with a certain contribution to the resultant error. The basic steps in a typical GC analysis are: (i) sampling and treatment of the sample, (ii) injection and chromatography, (iii) detection and generation of an analogue signal, and (iv) conversion of the analogue to a digital value. The role played by the individual steps in the analysis depends on the nature of the problem given, on the performance of the apparatus, and on the working technique employed.

An objective answer to the questions concerning the reliability of the individual techniques can only be obtained with the aid of statistics[6,16]. As there is frequent confusion about the various concepts when using statistics, we shall consequently adhere to the terms and definitions usual in the respective literature. Precision will be expressed in the form of the coefficient of variation (I), defined by the relation $I = S/\bar{X}$ where S is the estimation of the standard deviation (briefly the standard deviation) given by the expression

$$\left[\frac{1}{n-1}\sum^{n}(X-\bar{X})^2\right]^{\frac{1}{2}}$$

and the symbols X and $\bar{X}$ designate, respectively, the result of a single determination and the mean value of n determinations. The quantity S^2 represents the estimation of the variance (briefly the variance). In the individual cases studied, the quantity X is represented by molarity (m), expressed by the number of moles of either the component under examination or the standard substance in 1 l of the solution. If molarity is expressed by a mathematical formula for some technique, it is a function of further characteristic variables (*e.g.* V, v, and others—*cf.* following); the resultant variance of molarity, S^2_m, is calculated by

$$S^2_m = \left(\frac{\partial m}{\partial V}\right)^2 S^2_V + \left(\frac{\partial m}{\partial v}\right)^2 S^2_v + \ldots$$

where S^2_V and S^2_v are independently determined variances of the quantities V and v. The respective coefficient of variation, I_m, is then determined according to the relation $I_m = (S^2_m/m^2)^{\frac{1}{2}}$. All our data on precision will always represent repeatability. The accuracy of determination is evaluated by testing the statistical significance of the absolute value of the difference $\mu - \bar{X}$ where μ is the true value. This is performed by comparing the experimental value of the Student criterion t for the 95% confidence level with the corresponding critical value.

Design of the analysis

Theoretical precision was determined by means of the independent variances

for each technique. The respective relations for the calculations were derived on the basis of the mathematical formulae of the individual techniques[14]. The predictions obtained were checked experimentally by repeatedly analysing, according to the respective procedures, samples of known composition. The following independent variances were taken into account in the predictions:

(A) The variance of the sample charge, S^2_v, was determined by weighing the doses of tetrabromoethane as measured out by a Hamilton microsyringe 701-N (10 μl) and injected into a specially adapted weighing bottle. A value of 16×10^{-4} μl^2 was found for this variance.

(B) The variance of the volumes used in mixing the sample to be analyzed with the standard, S^2_V, was determined by weighing the doses of toluene as measured out by a 5 ml pipette (toluene was used as the solvent in all experiments). In this case, the variance amounted to 36×10^{-6} ml^2.

(C) The variance of length, S^2_l, was determined by measuring standard lengths with the rule used for measuring the chromatograms. This variance was 4.0×10^{-4} cm^2.

(D) The variance of the recorder deflection, S^2_R, was determined for a Servogor RE 511 recorder on the basis of the data given by the producer. A precision of 0.15% at full scale deflection (20 cm) has been quoted, so that we obtain a value of 9.0×10^{-4} cm^2 for the S^2_R. This variance contributes to the variance of measuring the peak height, S^2_h; which is represented by $S^2_h = S^2_l + S^2_R$. In case of the variance of measuring the peak width at half height, S^2_b, it can be assumed that $S^2_b = S^2_l$. If the product hb is used for the determination of the peak area, the variance of the area, S^2_A, is given by $S^2_A = h^2S^2_b + b^2S^2_h$.

(E) The variance of the relative molar response (RMR), S^2_{RMR}, was determined from RMR values which had been obtained by the internal standard technique. The ratio RMR_{sr}/RMR_{ir} occurs in most techniques, the subscripts i, s, and r designating the substance under determination, the standard, and a reference substance, respectively. The above ratio is obviously identical to the quantity RMR_{si}, which can be expressed by $(A'_s/m_s)/(A'_i/m_i)$ where the areas A'_s and A'_i correspond to the molarities m_s and m_i (*cf.* ref. 14). Provided the ratio of the molarities, m_i/m_s, can be determined precisely by weighing, we can say that

$$S^2_{RMR_{si}} = RMR^2_{si}\,[(S_{A_s}/A'_s)^2 + (S_{A_i}/A'_i)^2]$$

and the respective variation coefficient can be expressed in the form

$$I_{RMR_{si}} = [(S_{A_s}/A'_s)^2 + (S_{A_i}/A'_i)^2]^{\frac{1}{2}}.$$

Both the calculations and the experimental measurements were carried out for three typical situations representing favorable, medium, and unfavorable conditions; the respective characteristics are summarized in Table I. The rated values, characterizing the respective conditions, apply to all the variables occurring in a given technique, regardless of whether the values are related to the component determined or to the standard substance. Hence it follows that the characterization of the conditions quoted implies the presupposition that the values of the corresponding variables, relative to the substance determined and to the standard, are approximately equal. This also applies in the experimental estimation of precision. In the measurements proper, the above conditions were observed to within $\pm$ 10% of the rated values;

TABLE I

DATA ON THE CONDITIONS OF ANALYSIS

I = coefficients of variation determined from the rated values chosen and the corresponding independent standard deviations.

Characteristic variables	*Unfavorable conditions*		*Medium conditions*		*Favorable conditions*	
	Rated value	*I (%)*	*Rated value*	*I (%)*	*Rated value*	*I (%)*
Volume injected (μl)	1	4.0	5	0.80	10	0.40
Peak height (cm)	2	1.8	12	0.30	18	0.20[a]
Peak width (cm)	0.5	2.5	4	0.50	10	0.20
Volume mixed (ml)	0.2	3.0	2	0.30	5	0.12
RMR_{si}	—	4.3	—	0.83	—	0.40

[a] In calculations with calibration curve h = 12 cm and I_h = 0.30%.

as for the methods involving work with a calibration curve, the conditions mentioned correspond to the region around the middle of the calibration limits; this region was also used for reading out. The theoretical variation coefficients were determined from the independent variances, quoted under (A)–(E), by relating them to the rated values chosen for the individual conditions. As far as the expression of concentration is concerned, we have restricted our considerations to the determination of molarity or, if need be, mole fractions.

Prediction of the resultant error

The relations representing the mathematical formulations for the individual techniques are quoted without any detailed commentary; the derivation of these relations has been shown elsewhere (*cf.* ref. 14). In the relations mentioned the following symbols occur: the substance determined and the standard substance are again designated by i and s, respectively. In one of the variants of the standard addition technique, an auxiliary substance, designated by p, is also used. The symbol r is reserved for the reference substance used in the expression of the relative molar response. The volumes handled in the preparation of the sample (prior to injection) are denoted by V, while v is used to denote the volume introduced into the chromatograph. The height and the area of the chromatographic peak are designated by h and A, respectively. The subscripts placed in brackets indicate that the respective symbol refers to the material (sample) analysed for the content of the component distinguished by the subscript. If without brackets, the subscripts relate the symbol to pure substance indicated. The symbol N designates the number of moles. Hence, the molarity of the component i in the sample analyzed is given by $m_i = N_i/V_{(i)}$; the molarity of the standard is formulated analogously. Peak areas are determined as the product hb. Since for all symmetrical peaks the same proportionality exists between the value

of A and the corresponding product hb, the ratio A_i/A_s is equal to the ratio h_ib_i/h_sb_s. The molarities of the calibration solutions were determined by weighing, and are supposed to represent precise values.

TECHNIQUE OF ABSOLUTE CALIBRATION

Direct comparison of the peak heights of the substance determined in the chromatograms of the analyzed and calibration samples (the pure substance determined serves as the standard)

This method is characterized by the relation

$$m_i = \frac{v_{(s)}}{v_{(i)}} \frac{h_i}{h_s} m_s$$

With regard to the assumption quoted above, it holds that $v_{(i)} \doteq v_{(s)}$ and $h_i \doteq h_s$ (this situation is assumed in all the methods where it must be considered), so that the respective variation coefficient can be expressed by

$$I_m = [2(I_v^2 + I_h^2)]^{\frac{1}{2}}$$

Direct comparison of the peak areas of the substance determined in the chromatograms of the analyzed and calibration samples (the pure substance determined is used as the standard)

In this case

$$m_i = \frac{v_{(s)}}{v_{(i)}} \frac{A_i}{A_s} m_s$$

which leads to

$$I_m = [2(I_v^2 + I_A^2)]^{\frac{1}{2}}$$

Direct comparison of the peak area of the substance determined in the chromatogram of the mixture analyzed with the peak area of a standard in the chromatogram of the calibration sample (the substance analyzed and the standard are different compounds)

In this case, it is necessary to perform the calculations with corrected peak areas, *i.e.*,

$$m_i = \frac{v_{(s)}}{v_{(i)}} \frac{RMR_{sr}}{RMR_{ir}} \frac{A_i}{A_s} m_s$$

Provided the empirical determination of the respective RMR values is also involved in the performance of the technique, the RMR's so obtained represent normal variables, manifesting themselves in the resulting error in accordance with the relation quoted in the paragraph under (E). It follows from analogy with the preceding case that

$$I_m = [2(I^2_v + I^2_A + I^2_{RMR})]^{\frac{1}{2}}$$

Calibration curve method: calculation by peak heights

Work with a calibration curve is based on the relation $m_i = \bar{K}_h h_i/v_{(i)}$ where $\bar{K}_h$ is an empirical constant, determined by analyzing a series of samples, of known molarities, of the substance under determination. The results of n such analyses can be processed using the relation

$$\bar{K}_h = \frac{1}{n} \sum^{n} \frac{m_i v_{(i)}}{h_i}$$

where m_i represents precise values determined by weighing. The variance of the constant $\bar{K}_h$ is given by

$$S^2_{\bar{K}_h} = \frac{1}{n}\left[\frac{m^2_i S^2_v}{h^2_i} + \frac{m_i v^2_{(i)} S^2_h}{h^4_i}\right]$$

and the corresponding coefficient of variation is expressed by the relation

$$I_{\bar{K}_h} = \left[\frac{1}{n}(I^2_v + I^2_h)\right]^{\frac{1}{2}}$$

With respect to the relation for m_i, we can write

$$S^2_m = \frac{h^2_i}{v^2_{(i)}} S^2_{\bar{K}_h} + \frac{\bar{K}^2_h}{v^2_{(i)}} S^2_h + \frac{\bar{K}^2_h h^2_i}{v^4_{(i)}} S^2_v$$

and the corresponding coefficient of variation is given by

$$I_m = \left[\frac{1}{n}(I^2_v + I^2_h) + I^2_v + I^2_h\right]^{\frac{1}{2}}$$

This relation applies to the cases where a new calibration curve is provided for each individual determination. If a single calibration curve is used for reading out in several analyses, the relation $I_m = (I^2_h + I^2_v)^{\frac{1}{2}}$ holds, and the coefficient of variation of the calibration curve slope (I_{K_h}) will manifest itself as a fraction of the systematic error.

Calibration curve method: calculation with peak areas (calibration carried out with the pure substance determined)

The relationship between this method and the preceding one is similar to the relationship between the corresponding variants of the direct comparison methods. We can immediately write

$$m_i = \bar{K}_A A_i / v_{(i)}$$

where

$$\bar{K}_A = \frac{1}{n}\sum^{n}(m_s v_{(s)}/A_s)$$

so that

$$I_m = \left[\frac{1}{n}(I^2_v + I^2_A) + I^2_v + I^2_A\right]^{\frac{1}{2}}$$

In some analyses, when using a single calibration curve the term

$$\frac{1}{n}(I^2_v + I^2_A)$$

will again drop out.

Calibration curve method: calculation with peak areas (calibration carried out with a substance different from the substance determined)

In this case, it is again necessary to calculate with corrected areas; the procedure can be based on the relation

$$m_i = \bar{K}_A \frac{A_i}{v_{(i)}} \frac{RMR_{sr}}{RMR_{ir}}$$

where $\bar{K}_A$ is given by the same expression as in the preceding case. If separate values of RMR_{sr} and RMR_{tr} are used, determined specially for each individual reading out, the coefficient of variation is given by

$$I_m = \left[\frac{1}{n}(I^2_v + I^2_A) + I^2_v + I^2_A + 2I^2_{RMR}\right]^{\frac{1}{2}}$$

In the case where the RMR_{st} values are readily available, we can write

$$I_m = \left[\frac{1}{n}(I^2_v + I^2_A) + I^2_v + I^2_A + I^2_{RMR}\right]^{\frac{1}{2}}$$

When working with only one calibration curve, the term

$$\frac{1}{n}(I^2_v + I^2_A)$$

can be omitted.

INTERNAL STANDARD TECHNIQUE

Direct comparison of the peak areas of the component determined and of the standard added

This technique can be represented by the formula

$$m_t = \frac{V_{(s)}}{V_{(t)}} \frac{A'_t}{A'_s} \frac{RMR_{sr}}{RMR_{tr}} m_s$$

where the dashes on the A's denote that the chromatogram refers to the mixture of sample and standard. It can be derived from the relation quoted that

$$I_m = [2(I^2_V + I^2_A + I^2_{RMR})]^{\frac{1}{2}}$$

If directly determined RMR_{st} values are available, the term I^2_{RMR} is not multiplied by 2.

Calibration curve method: calculation with peak heights

This variant is based on the relation

$$m_t = \bar{K}'_h \frac{V_{(s)}}{V_{(t)}} \frac{h'_t}{h'_s} m_s$$

where the dashes have the same meaning as in the preceding case. The empirical constant $\bar{K}'_h$ is obviously given by

$$\bar{K}'_h = \frac{1}{n} \sum^{n}{}' \frac{m_t}{m_s} \frac{V_{(t)}}{V_{(s)}} \frac{h'_s}{h'_t}$$

It follows from the relations for $\bar{K}'_h$ and m_t that

$$I_{\bar{K}'_h} = \left[\frac{2}{n}(I^2_V + I^2_h)\right]^{\frac{1}{2}}$$

and

$$I_m = \left[\frac{2}{n}(I^2_V + I^2_h) + I^2_V + I^2_h\right]^{\frac{1}{2}}$$

Calibration curve method: calculation with peak areas (calibration and analysis carried out with the same two substances i and s)

In this case

$$m_i = \bar{K}'_A \frac{V_{(s)}}{V_{(i)}} \frac{A'_i}{A'_s} m_s$$

where the constant $\bar{K}'_A$, virtually representing the ratio RMR_{sr}/RMR_{ir}, is given by

$$\bar{K}'_A = \frac{1}{n} \sum^{n} \frac{m_i}{m_s} \frac{V_{(i)}}{V_{(s)}} \frac{A'_s}{A'_i}$$

The relationships quoted lead to

$$I_m = \left[\frac{2}{n}(I^2_V + I^2_A) + I^2_V + I^2_A\right]^{\frac{1}{2}}$$

Calibration curve method: calculation with peak areas (calibration and analysis carried out with different standards)

Let us denote the standards used in the calibration and in the analysis by s_1 and s_2, respectively. As the general relation, quoted in connection with the method of direct comparison, holds true independently of whether s is substituted by s_1 or s_2, we can write for the calibration curve:

$$m_i = \bar{K}'_A \frac{V_{(s_1)}}{V_{(i)}} \frac{A'_i}{A'_{s_1}} m_{s_1}$$

and assume that reading is carried out for values given either by

$$\frac{A'_i}{A'_{s_2}} \frac{V_{(s_2)}}{V_{(i)}} \frac{RMR_{s_2r}}{RMR_{s_1r}} m_{s_2}$$

or by

$$\frac{A'_i}{A'_{s_2}} \frac{V_{(s_2)}}{V_{(i)}} RMR_{s_2s_1} m_{s_2}$$

The respective coefficients of variation can then be expressed by either

$$I_m = \left[\frac{2}{n}(I^2_V + I^2_A) + 2(I^2_V + I^2_A + I^2_{RMR})\right]^{\frac{1}{2}}$$

or

$$I_m = \left[\frac{2}{n}(I^2_V + I^2_A) + 2(I^2_V + I^2_A) + I^2_{RMR}\right]^{\frac{1}{2}}$$

In all the above relationships, the term multiplied by the factor $2/n$ is deleted if only one calibration curve is used for a series of determinations.

STANDARD ADDITION TECHNIQUE

In this technique, the pure substance determined is added as a standard to the analyzed sample. As there is no separation of the analyzed and standard substances, the calculation of molarity as well as the expression for the respective coefficient of variation are more complicated.

Direct measurement of the charges of the original sample and of the sample enriched by a defined addition of the substance determined (calculation by peak heights)

This technique is represented by the relation

$$m_i = \frac{V_{(s)}}{V_{(i)}} \frac{m_s}{\frac{h'_{is}}{h_i}\frac{v_{(i)}}{v'_{(i)}}\left(1 + \frac{V_{(s)}}{V_{(i)}}\right) - 1}$$

where h'_{is} and h_i are, respectively, the peak heights of the component determined in the chromatograms of the enriched and original samples, $v'_{(i)}$ and $v_{(i)}$ being the corresponding sample volumes injected. The designations $h'_{is}/h_i = \eta$; $v_{(i)}/v'_{(i)} = \varphi$, and $V_{(s)}/V_{(i)} = \psi$, are introduced and the relation for I_m can now be expressed in the form:

$$I_m = \frac{\eta\varphi}{\eta\varphi(1+\psi) - 1}\left[(1+\psi)^2 I^2_\eta + (1+\psi)^2 I^2_\varphi + \left(\frac{\eta\varphi - 1}{\psi\varphi}\right)^2 I^2_\psi\right]^{\frac{1}{2}}$$

Provided $v_{(i)} \doteq v'_{(i)}$ and $V_{(s)} \doteq V_{(i)}$, $\eta = \frac{1}{2}\left(1 + \frac{m_s}{m_i}\right)$, and I_m is given by

$$I_m = \frac{m_i + m_s}{m_s}\left[I^2_\eta + I^2_\varphi + \tfrac{1}{4}\left(\frac{m_s - m_i}{m_s + m_i}\right) I^2_\psi\right]^{\frac{1}{2}}$$

If, in addition, $m_i \doteq m_s$, we finally obtain

$$I_m = 2[2(I^2_h + I^2_v)]^{\frac{1}{2}}$$

Direct measurement of the charges (calculation by peak areas)

In this case

$$m_i = \frac{V_{(s)}}{V_{(i)}} \frac{m_s}{\frac{A'_{is}}{A_i}\frac{v_{(i)}}{v'_{(i)}}\left(1 + \frac{V_{(s)}}{V_{(i)}}\right) - 1}$$

so that it is possible, under the same presuppositions as in the preceding case, to write

$$I_m = 2[2(I^2_A + I^2_v)]^{\frac{1}{2}}$$

Comparison with an auxiliary reference substance (calculation by peak heights)

In this method, the size of the peak of an auxiliary reference substance (p) serves as a measure of the amount of sample injected. This is characterized by

$$m_i = \frac{V_{(s)}}{V_{(i)}} \frac{m_s}{\frac{h'_{is}}{h_i}\frac{h_p}{h'_p} - 1}$$

where h_p and h'_p are the peak heights of the auxiliary substance in the chromatograms of the original sample and of the sample with a known addition of the standard (substance i). The species and concentration of the auxiliary substance need not be known. Following a procedure similar to that used in the variation above ($v_{(i)} \doteq v'_{(i)}$, $V_{(s)} \doteq V_{(i)}$, and, further, $h_i \doteq h_p$), we arrive at

$$I_m = \left(2I^2_V + I^2_h\left[10\frac{m^2_i}{m^2_s} + 12\frac{m_i}{m_s} + 6\right]\right)^{\frac{1}{2}}$$

If, in addition, $m_i \doteq m_s$, we obtain

$$I_m = (2I^2_V + 28I^2_h)^{\frac{1}{2}}$$

Comparison with an auxiliary reference substance (calculation by peak areas)

The method is defined by

$$m_i = \frac{V_{(s)}}{V_{(i)}} \frac{m_s}{\frac{A'_{is}}{A_i}\frac{A_p}{A'_p} - 1}$$

so that, under the above presuppositions,

$$I_m = (2I^2_V + 28I^2_A)^{\frac{1}{2}}$$

INTERNAL NORMALIZATION TECHNIQUE

Practically, there only exists one variant of this technique, which can be characterized, for the case of calculating mole fractions (x), by the relation

$$x_i = \frac{A_i/RMR_{ir}}{(A_i/RMR_{ir}) + \Sigma (A_j/RMR_{jr})}$$

where the summation includes all the components of the mixture except component i. The respective coefficient of variation is given by

$$I_{x_i} = \frac{1}{(A_i/RMR_{ir}) + \Sigma (A_j/RMR_{jr})} \left[\left(\Sigma \frac{A_j}{RMR_{jr}}\right)^2 (I^2_{A_i} + I^2_{RMR_{ir}}) + \right.$$

$$\left. + \Sigma \frac{A^2_j}{RMR^2_{jr}} (I^2_{A_j} + I^2_{RMR_{jr}})\right]^{\frac{1}{2}}$$

Typical features of this technique are the necessity to evaluate all the components of the mixture analyzed and the interdependence of both the precision and accuracy on the individual determinations. This situation leads to a number of possible alternatives, which can occur in the range of the conditions defined above (*cf.* Table I). In order to make the task unambiguous, it is necessary to introduce further presuppositions. We shall consider two typical alternatives in this study:

(i) The concentration of the component under determination is considerably higher than the concentrations of the other components, the total number of components (k) is small, the concentrations of the minor components are mutually comparable and the RMR values of the individual components do not differ appreciably from each other.

Hence, the peak areas of the minor components as well as the respective RMR values are determined with approximately equal relative errors under these circumstances; and for this alternative $A_i/RMR_{ir} \gg \Sigma (A_j/RMR_{jr})$ and $\Sigma A_j/RMR_{jr}) \doteq (k-1)(A_j/RMR_{jr})$ which makes it possible to write

$$I_{x_i} = \frac{A_j}{A_i} [(k-1)^2(I^2_{A_i} + I^2_{RMR_{ir}}) + (k-1)(I^2_{A_j} + I^2_{RMR_{jr}})]^{\frac{1}{2}}$$

Furthermore, it follows from the above presuppositions that

$$I^2_{A_j} \gg I^2_{A_i} \text{ and } I^2_{RMR_{ir}} \doteq I^2_{RMR_{jr}} \doteq I^2_{RMR}$$

so that we obtain, for the case of a binary mixture ($k = 2$),

$$I_{x_i} = (A_j/A_i)\ (I^2_{A_j} + 2I^2_{RMR})^{\frac{1}{2}}$$

In case of a greater number of minor components, keeping the other presuppositions unchanged, we obtain

$$I_{x_i} = (k-1)\ (A_j/A_i)\ (I^2_{A_i} + I^2_{RMR})^{\frac{1}{2}}$$

The consequences resulting from $I^2_{A_j}$ being much greater than $I^2_{A_i}$ are obviously outweighed by the fact that

$$(k-1)^2 I^2_{A_i} \gg (k-1)\ I^2_{A_j} \text{ and } (k-1)^2 I^2_{RMR_{ir}} \gg (k-1)\ I^2_{RMR_{jr}}$$

Thus, under the circumstances quoted

$$x_i = \frac{A_i/RMR_{ir}}{(A_i/RMR_{ir}) + (k-1)\ (A_j/RMR_{jr})}$$

so that

$$I_{x_i} = \frac{1 - x_i}{x_i}\ (I^2_{A_i} + I^2_{RMR})^{\frac{1}{2}}$$

It follows from the above relations that the significance of the relative error of the peak areas of the minor components decreases to such an extent on increasing the number of the components that the error of the peak area of the main component prevails.

(ii) The component determined represents a small fraction of the mixture analyzed. The presuppositions introduced in the first alternative are again applicable.

In this case again $A_i/RMR_{ir} \gg \Sigma\ (A_j/RMR_{jr})$, and if the components of the main part are present in mutually comparable concentrations, it can be written that $\Sigma\ (A_j/RMR_{jr}) \doteq (k-1)\ (A_j/RMR_{jr})$. In this case, the above mentioned general relation for I_{x_i} will acquire the form

$$I_{x_i} = \left(I^2_{A_i} + I^2_{RMR_{ir}} + \frac{1}{k-1}\ (I^2_{A_j} + I^2_{RMR_{jr}})\right)^{\frac{1}{2}}$$

For small k values, as in the limiting case of a binary mixture, we obtain

$$I_{x_i} = (I^2_{A_i} + 2I^2_{RMR})^{\frac{1}{2}}$$

as

$$I^2_{A_j} \ll I^2_{A_i}$$

For large k values

$$I_{x_i} = (I^2_{A_i} + I^2_{RMR})^{\frac{1}{2}}$$

The theoretical variation coefficients are compared with the corresponding experimental data in Tables II, III, and IV.

TABLE II

PRECISION DATA: UNFAVORABLE CONDITIONS

i = substance under determination; s = standard substance; $s1$ and $s2$ = different standard substances; h = calculation by peak heights; A = calculation by peak areas; I_{RMR} = coefficient of variation of the RMR value employed; I_m = coefficient of variation of the determination of molarity; all I values are expressed in percentages ($100\,S_m/\bar{m}$).

Technique	*Method*			*Model compounds*		I_{RMR}		I_m	
				i	*s*	*Theoretical*	*Experimental*	*Theoretical*	*Experimental*
Absolute calibration	Direct comparison	$s = i$	*h*	*p*-Xylene				5.8	4.4
			A	Chloroform				6.7	6.3
		$s \neq i$	*A*	Chloroform	*p*-Xylene	4.3	3.7	7.7	7.0
	Calibration curve	$s = i$	*h*	Chloroform				4.3	4.3
			A	Chloroform				5.1	4.9
		$s \neq i$	*A*	*p*-Xylene	Chloroform	4.3	3.7	6.4	5.0
Internal standard	Direct comparison	$s \neq i$	*A*	Benzene	Isooctane	4.3	3.7	5.9	4.8
	Calibration curve	$s1 = s2$	*h*	Isooctane	Benzene			3.7	3.4
			A	Isooctane	Benzene			4.4	4.3
		$s1 \neq s2$	*A*	Chloroform	Benzene Isooctane	4.3	3.7	6.9	6.0
Standard addition	Measurement of charges	$s = i$	*h*	Chloroform				11	9.6
			A	Chloroform				13	10
	Auxiliary substance	$s = i$	*h*	Chloroform	Isooctane			6.8	5.6
			A	Chloroform	Isooctane			15	10
Internal normalization	i = major component		*A*	Chloroform		4.3	3.7	1.2	0.53
	i = minor component		*A*	Benzene		4.3	3.7	4.8	2.7

EXPERIMENTAL

Apparatus

All measurements were carried out on a Becker Multigraph F – Model 410 (Becker Delft N.V., Delft, Holland) furnished with a Servogor recorder – RE 511. Columns, 1 m × 4 mm, were packed with 4 g of 20 wt.% squalane-on-Celite 545 (30–60 mesh) and used under isothermal conditions at 60°. The flow rates of the carrier gas N_2, H_2, and air were 0.80, 1.25, and 10 ml/sec, respectively, as measured at the detector outlet under atmospheric conditions (24°, 746 mm Hg); the overpressure at the column inlet was 0.2 atm. The injection port was kept at 140°. Samples were introduced by a 10 μl Hamilton microsyringe 701-N (Hamilton Co., Whittier, U.S.A.).

Materials and working procedure

Model mixtures were prepared containing chloroform, benzene, isooctane, and

BLE III

CISION DATA: MEDIUM CONDITIONS

hnique	*Method*			*Model compounds*		I_{RMR}		I_m	
				i	*s*	*Theo-retical*	*Exper-imen-tal*	*Theo-retical*	*Exper-imen-tal*
olute alibration	Direct comparison	$s = i$	*h*	Isooctane				1.2	1.4
			A	Isooctane				1.4	1.4
		$s \neq i$	*A*	Isooctane	Benzene	0.83	1.0	1.6	2.0
	Calibration curve	$s = i$	*h*	Isooctane				0.83	0.57
			A	Isooctane				0.97	0.92
		$s \neq i$	*A*	Benzene	Isooctane	0.83	1.0	1.3	1.2
ernal standard	Direct comparison	$s \neq i$	*A*	Isooctane	Benzene	0.83	1.0	1.1	1.2
	Calibration curve	$s1 = s2$	*h*	Isooctane	Benzene			0.46	0.55
			A	Isooctane	Benzene			0.70	0.71
		$s1 \neq s2$	*A*	Chloroform	Benzene Isooctane	0.83	1.0	1.1	1.1
ndard addition	Measurement of charges	$s = i$	*h*	Benzene				2.4	2.6
			A	Benzene				2.7	2.8
	Auxiliary substance	$s = i$	*h*	Benzene	Isooctane			1.6	1.9
			A	Benzene	Isooctane			3.0	2.6
ernal ormalization	i = major component		*A*	*p*-Xylene		0.83	0.40	0.26	0.32
	i = minor component		*A*	Benzene		0.83	0.40	0.92	1.2

p-xylene in an excess of toluene as the solvent; in the internal normalization technique, the toluene content in the mixture was comparable with the contents of the other components. All the substances were chromatographically pure. The molarities of the individual components were determined by weighing on an analytical balance (Type A3/100, Meopta, N. E., Czechoslovakia); the precision of weighing was better than to 10^{-2}% of the value weighed out. The compositions of the model mixtures were chosen with a view to the possibility of studying the effect of the *RMR* value on the precision and accuracy of determination ($CHCl_3$ has much lower *RMR* in flame ionization detection than the other compounds). The concentrations of the components studied as well as the other working parameters were adjusted in such a way that it might be possible to realize the chosen conditions (*cf.* Table I).

The molarities of the components determined, detector sensitivity attenuation factors, sample charges, and recorder chart drives were varied within 10^{-5}–10^{-3} mole/ml (0.1–1 wt.%); 10^3–10^4; 1–10 μl; and 1–12 cm/min; respectively. The column temperature and the flow rates of the gases were kept constant throughout the experiments.

Under the given conditions, the retention times of methane (nonsorbed com-

TABLE IV

PRECISION DATA: FAVORABLE CONDITIONS

Technique	*Method*			*Model compounds*		I_{RMR}		I_m	
				i	*s*	*Theoretical*	*Experimental*	*Theoretical*	*Experimental*
Absolute calibration	Direct comparison	$s = i$	*h*	Benzene				0.65	0.74
			A	Isooctane				0.70	0.90
		$s \neq i$	*A*	Benzene	Isooctane	0.40	0.40	0.80	0.99
	Calibration curve	$s = i$	*h*	Benzene				0.50	0.40
			A	Benzene				0.54	0.50
		$s \neq i$	*A*	Isooctane	Benzene	0.40	0.40	0.66	0.57
Internal standard	Direct comparison	$s \neq i$	*A*	Isooctane	Chloroform	0.40	0.33	0.57	0.50
	Calibration curve	$s1 = s2$	*h*	Isooctane	Chloroform			0.32	0.30
			A	Isooctane	Chloroform			0.39	0.38
		$s1 \neq s2$	*A*	Benzene	Isooctane Chloroform	0.40	0.40	0.54	0.53
Standard addition	Measurement of charges	$s = i$	*h*	Isooctane				1.3	1.4
			A	Isooctane				1.4	1.4
	Auxiliary substance	$s = i$	*h*	Chloroform	Isooctane			1.1	1.4
			A	Chloroform	Isooctane			1.7	2.0
Internal normalization	i = major component		*A*	Chloroform		0.40	0.33	0.12	0.11
	i = minor component		*A*	Isooctane		0.40	0.33	0.48	0.58

ponent), chloroform, benzene, isooctane, and *p*-xylene were, respectively, 13, 96, 138, 196, 342, and 828 sec, and the time intervals between the beginning and end of the elution of the respective zones were 2, 23, 29, 40, 112, and 146 sec.

The statistical analysis was always performed by processing 15 determinations carried out by each method under the favorable, medium, and unfavorable conditions. In the variants with the calibration curve, the latter was constructed from 15 experimental points, and the read-out was carried out from about the middle of the region covered by calibration.

RESULTS AND DISCUSSION

The experimental values of the variation coefficients found for the individual methods and conditions are compared with the corresponding theoretical values in Tables II, III, and IV. Relatively good agreement between both sorts of results indicates that there are no other factors which contribute appreciably to the resultant error except those which have already been presumed. This is significant considering that the analyses were performed on a common commercial apparatus without any

TABLE V

ACCURACY DATA: UNFAVORABLE CONDITIONS

m = molarity determined by weighing (true value); $\bar{m}$ = molarity determined by analysis (mean value of 15 determinations); S = standard deviation; $t_{exper.}$ = experimental value of the Student factor, given by $\sqrt{n}(|\bar{X} - \mu|)/S$ where n is the number of determinations.

Technique	*Method*			*Substance determined*	m (μ)	$\bar{m}$ ($\bar{X}$)	S	$t_{exper.}$[a]
Absolute calibration	Direct comparison	$s = i$	h	*p*-Xylene	0.04504	0.04420	0.0019	1.7
			A	Chloroform	0.03726	0.03600	0.0023	2.0
		$s \neq i$	A	Chloroform	0.04014	0.04076	0.0029	0.69
	Calibration curve	$s = i$	h	Chloroform	0.03726	0.03730	0.0016	1.0
			A	Chloroform	0.03726	0.03700	0.0018	0.56
		$s \neq i$	A	*p*-Xylene	0.03673	0.03630	0.0018	0.89
Internal standard	Direct comparison	$s \neq i$	A	Benzene	0.90500	0.91010	0.043	0.45
	Calibration curve	$s1 = s2$	h	Isooctane	0.39000	0.38900	0.013	0.30
			A	Isooctane	0.39000	0.39020	0.016	0.05
		$s1 \neq s2$	A	Chloroform	0.69700	0.70700	0.042	1.9
Standard addition	Measurement of charges	$s = i$	h	Chloroform	0.23840	0.23160	0.023	0.81
			A	Benzene	0.42900	0.42500	0.040	0.39
	Auxiliary substance	$s = i$	h	Chloroform	0.23840	0.24100	0.013	1.8
			A	Chloroform	0.23840	0.23800	0.024	0.01
Internal normalization	i = major component		A	Chloroform	0.83300	0.83480	0.0044	1.6
	i = minor component		A	Benzene	0.16700	0.16520	0.0044	1.6

[a] $t_{0.05}(14) = 2.14$.

additional refinements, equipped with a conventional recorder, the evaluation of the chromatograms being carried out manually.

In this respect, our findings are at variance with the statement that a precision of 1–2% at best is attainable in quantitative GC analysis when using a conventional recorder (*cf.* ref. 9, p. 9). Both theoretical predictions and experiment demonstrate that it is possible to obtain results with a coefficient of variation considerably less than 1% with the above equipment, even under not very favorable conditions (*cf.* Table I). Under virtually favorable conditions, the theoretical and experimental coefficients of variation approach a value of 0.4% in some cases. Such a value has been declared to be attainable only by automatic processing of the detector response, *i.e.*, without employing the recorder; the precision of the results obtained by the internal normalization technique (0.1%) is even comparable with the precision attained when employing a precisely adjusted gas chromatograph with automatic evaluation of the detector response (*cf.* ref. 4). On the other hand, however, one of the variants of the standard addition technique yields, under equally favorable conditions, results with a coefficient of variation of about 2%.

TABLE VI

ACCURACY DATA: MEDIUM CONDITIONS

Technique	*Method*			*Substance determined*	m (μ)	$\bar{m}$ ($\bar{X}$)	S	$t_{exper.}$[a]
Absolute calibration	Direct comparison	$s = i$	h	Isooctane	0.11190	0.11220	0.0016	0.74
			A	Isooctane	0.11190	0.11110	0.0016	2.0
		$s \neq i$	A	Isooctane	0.10080	0.09990	0.0020	1.7
	Calibration curve	$s = i$	h	Isooctane	0.08959	0.08950	0.00050	0.72
			A	Isooctane	0.10080	0.10120	0.00093	1.8
		$s \neq i$	A	Benzene	0.10215	0.10230	0.0012	0.50
Internal standard	Direct comparison	$s \neq i$	A	Isooctane	0.39000	0.38900	0.0046	0.83
	Calibration curve	$s1 = s2$	h	Isooctane	0.39000	0.39010	0.0021	0.50
			A	Isooctane	0.39000	0.39050	0.0028	0.70
		$s1 \neq s2$	A	Chloroform	0.39000	0.39100	0.0044	0.89
Standard addition	Measurement of charges	$s = i$	h	Benzene	0.06645	0.06640	0.0019	0.10
			A	Benzene	0.06645	0.06640	0.0019	0.10
	Auxiliary substance	$s = i$	h	Benzene	0.06645	0.06620	0.0013	0.77
			A	Benzene	0.06645	0.06580	0.0017	1.4
Internal normalization	i = major component		A	p-Xylene	0.79570	0.79600	0.0025	0.48
	i = minor component		A	Benzene	0.20430	0.20400	0.0025	0.48

[a] $t_{0.05}(14) = 2.14$.

In our analysis, the precision (repeatability) of a determination by the individual techniques decreases in the sequence: internal normalization, internal standard technique, absolute calibration, standard addition technique. However, it is necessary to point out that this situation has only been substantiated for a case of isothermal chromatography with flame ionization detection. It follows from the nature of the problem that both the above sequence and the very data on the precision of the individual techniques may be altered appreciably when a different detector and different working regime (*e.g.* temperature programming) are employed; the composition of the mixture analyzed and the column packing may also play an important role. The present analysis should therefore not be taken as a definitive evaluation of quantitative GC techniques, but as a pattern applicable to various situations. Hence it is necessary for objective characterization of the precision of a quantitative GC determination not only to state the technique used, but to specify the whole problem; the specification of the criteria of precision is of no less importance. Otherwise the significance of the data on precision is considerably limited.

The data in Tables II, III, and IV apply to the case where manual evaluation of the chromatographic record was used. If a different method is used for processing the detector response (*e.g.* the use of analogue or digital integrators), it is necessary to introduce into the relationships for calculating the resultant coefficients of variation

TABLE VII

ACCURACY DATA: FAVORABLE CONDITIONS

Technique	*Method*			*Substance determined*	m (μ)	$\bar{m}$ ($\bar{X}$)	S	t_{exper} [a]
Absolute calibration	Direct comparison	$s = i$	h	Benzene	0.05105	0.05084	0.00038	2.1
			A	Isooctane	0.05554	0.05556	0.00050	0.16
		$s \neq i$	A	Benzene	0.05105	0.05095	0.00047	0.85
	Calibration curve	$s = i$	h	Benzene	0.05105	0.05090	0.00020	1.9
			A	Benzene	0.05105	0.5110	0.00025	0.80
		$s \neq i$	A	Isooctane	0.05554	0.05562	0.00031	1.0
Internal standard	Direct comparison	$s \neq i$	A	Isooctane	0.19570	0.19570	0.00098	0.0
	Calibration curve	$s1 = s2$	h	Isooctane	0.19570	0.19560	0.00059	0.66
			A	Isooctane	0.19570	0.19570	0.00075	0.0
		$s1 \neq s2$	A	Benzene	0.19880	0.19900	0.0010	0.78
Standard addition	Measurement of charges	$s = i$	h	Benzene	0.06645	0.06645	0.00093	0.0
			A	Benzene	0.06645	0.06645	0.00098	0.0
	Auxiliary substance	$s = i$	h	Benzene	0.06645	0.06644	0.00098	0.50
			A	Benzene	0.06645	0.06644	0.0013	0.30
Internal normalization	i = major component		A	Chloroform	0.83600	0.83570	0.00095	1.1
	i = minor component		A	Isooctane	0.16400	0.16430	0.00095	1.1

[a] $t_{0.05}(14) = 2.14$.

the corresponding values of the coefficients of variation of the respective integrals (*cf.* ref. 8) instead of the I_A values ($I_A = (I^2_h + I^2_b)^{\frac{1}{2}}$). The experimental coefficients of variation should also be recalculated to take into account this effect.

The present analysis also makes it possible to appreciate the accuracy of the determination. The respective data are summarized in Tables V, VI, and VII. In all cases, the $t_{exper.}$ coefficients, calculated from $t_{exper.} = \sqrt{n}\,(|\bar{X} - \mu|)/S$, are less than the corresponding critical value ($t_{0.05}(14) = 2.14$). The practical meaning of this test consists in the statement that, if there is any difference between the mean value of n determinations ($\bar{X}$) and the corresponding true value (μ), we can be 95% certain that the absolute value of this difference is less than the respective value given by the expression $[t_{0.05}(14)]S/\sqrt{n}$.

CONCLUSIONS

In the work with a good commercial analytical gas chromatograph, equipped with a conventional recorder, the precision of the determination can be defined practically by the precision of: (i) measuring the volume (in processing the sample prior to chromatography and in injecting the sample charge); (ii) measuring the size of the chromatographic peak; and (iii) determining the *RMR*. The role of the individu-

al factors depends on the technique used. Under isothermal conditions, the contribution of other factors to the resultant error is insignificant.

When employing the internal normalization, internal standard, and absolute calibration techniques, it is possible with the above equipment to obtain, under medium conditions, results with a coefficient of variation of less than 1%, provided that a component determined is in a mixture whose components all yield well separated symmetrical peaks, and that there are no reasons for irreversible sorption, decomposition, or indetectability of some component. Under favorable conditions, a precision of less than 0.5% is attainable, while the results obtained under unfavorable conditions have a coefficient of variation of about 5%. In all cases, the precision of the standard addition technique is about a half that of the other techniques.

With all common techniques, the deviations of the analytical results from the true values were, under the above quoted circumstances, statistically insignificant at the 95% confidence level.

ACKNOWLEDGEMENTS

The work has been proceeded in connection with the UNESCO Longterm Postgradual Course of Modern Analytical Methods (UNALCO II) held in the Institute of Instrumental Analytical Chemistry of the Czechoslovak Academy of Sciences, Brno.

The authors wish to express their thanks to the Directors of the Becker Delft N.V., Delft, The Netherlands, for a loan of gas chromatographic instruments for this purpose.

REFERENCES

1 D. L. Ball, W. E. Harris and H. W. Habgood, *J. Gas Chromatog.*, 5 (1967) 631.
2 J. C. Bartlet and D. M. Smith, *Can. J. Chem.*, 38 (1960) 2057.
3 F. Bauman and F. Tao, *J. Gas Chromatog.*, 5 (1967) 621.
4 F. Bauman, F. Tao and J. M. Gill, paper presented at the ACS meeting, New York, September 1966.
5 C. A. Cramers, *Thesis*, Technische Hogeschool Eindhoven, 1967, p. 39.
6 W. J. Dixon and F. J. Massey, *Introduction to Statistical Analysis*, McGraw-Hill, New York, 1957.
7 E. M. Emery, *J. Gas Chromatog.*, 5 (1967) 596.
8 J. Janák, *J. Chromatog.*, 3 (1960) 308.
9 R. Kaiser, *Chromatographie in der Gasphase*, Vol. IV, Bibliographisches Institut, Mannheim, 1965.
10 M. Krejčí and K. Hána, in C. L. A. Harbourn and R. Stock (Editors), *Gas Chromatography 1968*, Institute of Petroleum, London, 1968, preprints.
11 V. Kusý, *Anal. Chem.*, 37 (1965) 1748.
12 V. Kusý, *Anal. Chem.*, 37 (1965) 1748.
13 L. Mikkelsen, *J. Gas Chromatog.*, 5 (1967) 601.
14 J. Novák, in J. C. Giddings and R. A. Keller (Editors), *Advances in Chromatography*, Marcel Dekker, New York, in press.
15 J. T. Shank and H. E. Persinger, *J. Gas Chromatog.*, 5 (1967) 631.
16 W. J. Youden, *Statistical Methods for Chemists*, Wiley, New York, 1951.