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STUDY OF THE EFFECTS OF INSTRUMENT VARIABLES ON ACCURACY AND PRECISION IN GAS CHROMATOGRAPHY

I. CONTRIBUTION OF THE METHOD OF PEAK MEASUREMENT

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SUMMARY

The sources of error in gas chromatography have been tabulated and the precision of seven methods for the measurement of peaks has been compared. Of the methods tested the electronic integrator gives the highest precision provided the peaks are completely separated and provided stable baseline conditions apply. The use of peak height gave a comparable precision to that of the integrator and is less affected by long-term variations except where peak ratios are used. The use of manual construction for the measurement of peak area gave a low order of precision but the "peak height × width at half height" method was significantly better than triangulation methods.

INTRODUCTION

This paper is the first of a series to be prepared from the results of a three-year research project carried out for the British Standards Institution under a Department of Trade and Industry contract. The principal purpose of this project was to provide detailed information relating to accuracy and precision in a form that could be utilised in future or revised standards. BSI has recommended the publication of these results in view of their potential importance in quantitative gas chromatography.

ACCURACY AND PRECISION

The study was concerned only with the use of katharometers and flame ionization detectors as these two detectors are employed for the vast majority of gas chromatographic (GC) analyses at the present time. Both of these detectors require calibration and several different procedures are used, each having its own sources of error. Table I summarises the equations used in the three most commonly used methods, together with the relevant error sources expressed as fractional variances.

If the terms in this table were to be estimated from several chromatograms, then

TABLE I

ERRORS ASSOCIATED WITH THE MOST COMMON METHODS OF ANALYSIS

Method	*Equation*	*Contributions to error (fractional variances)*
Direct method (injection of known masses or volumes)	$F_{A2} = \dfrac{R_{A2} \times M_1 \times F_{A1}}{R_{A1} \times M_2}$	$\dfrac{\sum_0^n \Delta F_{A2}^2}{(n-1)\bar{F}_{A2}^2} = 2\sigma_r^2 + 2\sigma_m^2 + \sigma_w^2 + 2\sigma_f^2 + 2\sigma_v^2 + + \left[\dfrac{\bar{R}_{A2} \times \bar{M}_1 \times \bar{F}_{A1}}{\bar{R}_{A1} \times \bar{M}_2 \times \bar{F}_{A2}} - 1\right]^2 +$ interactions
Total summation (internal normalisation)	$F_{A2} = \dfrac{F_{A1} \times \dfrac{R_{A2}}{R_{A1}}}{\sum F_{X1} \times \dfrac{R_{X2}}{R_{X1}}}$ where X = A, B, C, etc.	$\dfrac{\sum_0^n \Delta F_{A2}^2}{(n-1)\bar{F}_{A2}^2} = 2(1+Y)\sigma_r^2 + 2(1+Y)\sigma_f^2 + + 2Y\sigma_u^2 + (1+Y)\sigma_w^2 + Y\sigma_t^2 + + Z^2 + + \left[\dfrac{F_{A1} \times \dfrac{\bar{R}_{A2}}{\bar{R}_{A1}}}{F_{A2} \times F_{X1} \times \dfrac{\bar{R}_{X2}}{\bar{R}_{X1}}} - 1\right]^2 +$ interactions
Internal standard	$F_{A2} = \dfrac{\dfrac{R_{A2}}{R_{A1}} \times F_{S2} \times F_{A1}}{\dfrac{R_{A1}}{R_{S1}} \times F_{S1}}$	$\dfrac{\sum_0^n \Delta F_{A2}^2}{(n-1)\bar{F}_{A2}^2} = 4\sigma_r^2 + 4\sigma_f^2 + 3\sigma_w^2 + 2\sigma_t^2 + 2\sigma_u^2 + + \left[\dfrac{\dfrac{\bar{R}_{A2}}{\bar{R}_{A1}} \times F_{S2} \times F_{A1}}{\dfrac{\bar{R}_{A1}}{\bar{R}_{S1}} \times F_{S1} \times F_{A2}} - 1\right]^2 +$ interactions

the errors of the means would be reduced to σ^2/n and where there is more than one source of a particular type of error, and the error is not the same for all sources, then the term represents an appropriate average.

The penultimate term in the error equations contains the systematic errors caused by the non-linearity of the chromatograph, the detector and the recording or measuring system. This aspect was not considered separately in the investigation as it is impossible to generalise on the effects of these errors. Instead, it was assumed that GC apparatus is naturally operated in a condition of linearity arrived at by a strict

test procedure and any deviations from linearity that occur in practice are then included in the random error variances.

The equations in Table I express the variation obtained between results as derived from an infinite number of results and therefore they measure the "precision" of the procedure. In order to estimate the "accuracy" of the method, it would be necessary to know the true values of all the variables, *viz.* mass injected, mass conveyed to the column, areas of peaks, etc. In practice, however, the methods employed for the measurement of these factors are subject to bias errors arising from sample impurity, operator error, peak overlap, injection problems, electrical factors, particularly in peak measuring instruments, adverse adjustments and many other sources. However, the mean bias error from each source tends to be constant with a given apparatus when working repetitively under nominally constant conditions, particularly where only a single operator is involved. Such conditions tend to give an average result which may be either higher or lower than the "true" result. If the same analysis is then performed on a different apparatus, with a different operator, then the mean bias errors from each source would have a different magnitude. The results obtained on one apparatus would therefore show less variation than the mean results obtained on different apparatus. The variation of results obtained "within apparatus" is termed the "repeatability" of the analysis and the variations "between apparatus" are termed the "reproducibility".

Generally, GC analysis can be highly repeatable and yet show poor accuracy because of the bias errors and so the measurement of precision using a single apparatus can be misleading. A high degree of precision using different apparatus, on the other hand, does suggest that bias errors are minimal and that the method is accurate but this is by no means a foregone conclusion. Although the work reported here was carried out on a single chromatograph, it has been possible, by suitable experimental design, to study some of the important bias errors arising from the chromatograph, its injection system, and the method of peak measurement.

MAIN OBJECTIVES

The main objectives of the investigation were: (1) to estimate and compare the precisions of various methods of peak measurement, (2) to study the effects of variations in instrumental variables on the source variances σ_v^2 σ_t^2 σ_u^2 σ_f^2 and σ_m^2, (3) to estimate these variances, where possible, from known variations in the instrument variables for a given apparatus, and (4) to determine, where possible, levels of instrument variables, etc., for which the instrument variances are a minimum.

PRECISION OF PEAK MEASUREMENT

The contribution of the measurement error to the overall error depends on the particular method used, the peak shape and size and the stability of the chromatographic signal.

In principle the precisions of several different methods of measurement can only be compared by reference to a highly reproducible signal of zero variance. Although this situation cannot be realised in practice, Scott and Grant showed that a useful comparison could be made between the less precise manual methods by apply-

ing them to accurately reproduced chromatograms[1]. For the more precise methods, the variance due to the process of reproducing the chromatograms would certainly be greater than that of the method used to measure the peaks and so any significance would be lost.

In practical GC, random variations in temperatures, pressures, electrical conditions, etc., cause slight fluctuations in peak shape and size. The effect of these fluctuations on the analytical precision is critically dependent on the method of measurement. For instance, a decrease in column temperature causes an increase in retention volume, which has the effect of increasing the peak width and decreasing the peak height. Peak height measurements therefore would be expected to show greater variation than area measurements when the column temperature control is poor. Clearly then there are interactions between the variance of the signal and of the method employed for its measurement and so it is necessary to include such interactions in the comparison of precisions for the results to be realistic. This can only be achieved by using the actual signal derived from a gas chromatograph but operated under carefully controlled conditions. A gas sample repetitively analysed under nominally identical conditions gives a high degree of repeatability over short intervals of time and so this procedure was adopted for this part of the project.

EXPERIMENTAL

Apparatus

A Varian Model 1800-1 gas chromatograph (Varian, Palo Alto, Calif., U.S.A.) fitted with mass flow controllers and constant voltage katharometers (flow-through detector with WX filaments supplied by Gow-Mac, Madison, N.J., U.S.A.) was used in conjunction with a Servoscribe RE 512.20 recorder (Smiths Industries, Wembley Park Great Britain) fitted with Disc (Santa Ana, Calif., U.S.A.) ball and disc integrator Model 264. The recorder had a 16-cm scale, a 1-sec response time, a specified resolution of $\pm 0.2\%$, and a wide range of sensitivities and chart speeds. The column used was 2 m × 6 mm O.D. stainless steel packed with 60–85 mesh molecular sieve 5A. A Pye Series 104 gas sampling valve (Pye Unicam, Cambridge, Great Britain), manually operated and fitted with 1-ml sample loop, was employed. The electronic integrator employed was a Varian Aerograph Model 480 (Varian, Palo Alto, Calif., U.S.A.) with visual display and automatic print-outs of retention times and areas.

Methods

The following seven methods of measurement were studied (see Fig. 1): (a) electronic integrator, (b) ball and disc integrator $[100x + mo + ny - (oy/2)\{(ny - pz)/yz + (lw - mo)/wo\}]$, (c) peak height ($AC$ cm), (d) peak height × retention time ($AC \times$ retention time, electronically recorded and printed out on integrator), (e) peak height × width at half height ($AC \times EF$ cm^2), (f) peak height × peak width ($AC \times GH$ cm^2), and (g) triangulation ($AD \times GH$ cm^2).

A gas sample comprising 10% methane, 60% carbon monoxide and 30% argon was chromatographed repetitively using three different operators to obtain sets of eight consecutive chromatograms. Four such sets were obtained for three days giving a total of twelve sets of eight chromatograms. The following conditions were em-

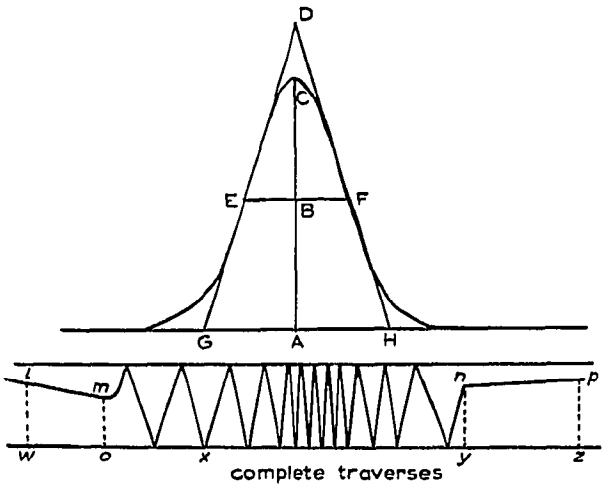

Fig. 1. Typical peak with ball and disc integrator trace showing measurements.

ployed: column temperature, 50°; carrier gas, helium; detector temperature, 108°; bridge current, 250 mA; attenuation, ×256; recorder range, 1.1 mV; chart speed, 30 mm/min; sample volume, 1 ml (nominal).

Before attempting any runs, the equipment was left switched on under these conditions for several days for stabilisation. No detectable baseline noise or drift was then obtained. Each run provided three slightly asymmetrical peaks, eluted in the order argon — methane — carbon monoxide. There was a baseline resolution between the three peaks and each run was completed in 25 min. Each peak was measured using the seven methods of measurement and so each set of chromatograms provided three estimates of the relative standard deviations of single results for each method of measurement, *viz.* within set variations with seven degrees of freedom.

RESULTS

Table II gives the estimated relative standard deviations for each method of measurement calculated for all the sets. Inspection of these values shows that for the electronic integrator there was a tendency for the precision to improve during the course of the tests whereas there is little significant variation with time for the other methods. This improvement with time for the integrator was probably due to further settling down of the integrator circuits when left on for several days.

Each set of twelve values was combined to give estimates of the relative standard deviation of each method with 84 degrees of freedom. The combined estimates and their 95% confidence limits are given in Table III. The size of the chromatographic contribution to the variance can be estimated if one assumes that the variations from this source are zero during the course of a chromatogram. Table IV shows calculated values of the "within set" variance of the ratio of the areas of the two major components compared with the added variances of the two areas. For the two most precise methods, peak height and electronic integration, the ratio of these areas would be expected to be virtually unaffected by response changes between runs and so the variance of the ratio should reflect the true variance of measurement. The added

TABLE II

PERCENTAGE RELATIVE STANDARD DEVIATION ESTIMATES OF SETS OF EIGHT MEASUREMENTS

Peak	*Day No.*	*Set No.*	*Method*						
			a	*b*	*c*	*d*	*e*	*f*	*g*
Peak 1—argon	1	1	0.16	0.83	0.15	0.47	0.88	2.93	2.49
		2	0.14	0.53	0.12	0.32	2.15	3.62	2.61
		3	0.14	0.78	0.11	0.52	1.11	1.81	1.57
		4	0.12	0.41	0.11	0.49	1.61	4.73	3.67
	2	5	0.11	0.61	0.23	0.23	1.30	1.58	2.64
		6	0.14	0.60	0.18	0.18	2.02	1.31	2.13
		7	0.12	0.38	0.20	0.20	1.34	3.52	2.44
		8	0.06	0.64	0.13	0.13	1.72	1.73	2.36
	3	9	0.20	0.80	0.31	0.30	1.70	2.93	3.14
		10	0.05	0.55	0.12	0.12	1.13	2.75	2.72
		11	0.03	0.11	0.37	0.57	1.72	3.40	3.09
		12	0.07	0.48	0.26	0.59	1.42	2.42	3.24
Peak 2—methane	1	1	0.57	1.64	1.05	1.09	2.50	2.01	2.90
		2	0.31	2.22	1.22	1.14	3.17	3.55	2.92
		3	0.40	4.59	0.82	0.82	1.25	1.90	1.36
		4	0.23	3.58	0.62	0.60	1.72	1.97	1.59
	2	5	0.19	4.70	1.11	1.16	1.87	1.97	1.70
		6	0.31	3.83	1.07	1.19	2.15	2.31	2.50
		7	0.31	3.14	0.68	0.64	1.33	1.96	1.51
		8	0.25	1.67	0.88	0.89	2.03	1.48	1.68
	3	9	0.33	1.01	0.55	0.62	1.29	1.18	1.94
		10	0.37	1.72	0.94	0.94	2.71	1.98	1.27
		11	0.18	2.42	0.84	0.84	1.31	2.48	1.94
		12	0.19	2.29	0.86	0.98	1.90	2.29	3.18
Peak 3—carbon monoxide	1	1	0.18	0.68	0.21	0.48	0.88	1.16	1.29
		2	0.22	0.83	0.45	0.48	0.36	1.20	0.95
		3	0.15	0.84	0.25	0.22	0.93	0.84	0.62
		4	0.13	1.22	0.24	0.11	0.59	1.06	0.92
	2	5	0.13	1.14	0.23	0.25	0.64	1.01	1.18
		6	0.08	0.54	0.72	0.22	1.03	1.27	1.01
		7	0.14	1.01	0.22	0.33	0.75	0.67	1.02
		8	0.09	0.45	0.21	0.27	1.13	1.56	0.73
	3	9	0.32	0.81	0.31	0.36	0.81	1.04	1.01
		10	0.03	0.54	0.19	0.23	0.45	1.00	1.06
		11	0.05	0.79	0.20	0.27	0.83	1.05	0.63
		12	0.09	0.30	0.38	0.51	0.63	1.40	1.54

variances of the two peak areas, on the other hand, will include errors both from signal response and the method of measurement. It is seen that for these two methods only the integrator gives a significantly smaller variance for the ratio. Thus, with the exception of the electronic integrator, the "within set" precisions are close to the true precisions of the method because variations in chromatographic response within the time taken to complete a set are much smaller than the error of measurement.

From the results in Table III the electronic integrator clearly gives the best

TABLE III

BEST ESTIMATES OF THE RELATIVE STANDARD DEVIATIONS OBTAINED WITH THE SEVEN METHODS OF MEASUREMENT AND THEIR CONFIDENCE LIMITS

Method	*Argon*		*Methane*		*Carbon monoxide*	
	% standard deviation	*95% confidence limits*	*% standard deviation*	*95% confidence limits*	*% standard deviation*	*95% confidence limits*
Electronic integrator	0.125	0.109 0.148	0.321	0.376	1.157	0.136 0.185
Ball and disc integrator	0.658	0.572 0.776	2.952	2.568 3.483	0.806	0.701 0.951
Peak height	0.230	0.200 0.271	0.906	0.788 1.069	0.270	0.235 0.319
Peak height × retention time	0.386	0.336 0.455	0.932	0.811 1.100	0.334	0.291 0.394
Peak height × peak width at half height	1.553	1.351 1.833	2.026	1.763 2.391	0.784	0.682 0.925
Peak height × peak width	2.910	2.532 3.434	2.161	1.880 2.550	1.126	0.980 1.329
Triangulation	2.732	2.380 3.224	2.140	1.862 2.525	1.026	0.893 1.211

precision of the seven methods tested, for all three peaks. This reflects the greater precision that can be obtained when both recorder and human errors are eliminated. The poorer precision for methane is due to the small peak size and this applies to all the methods except for the triangulation procedure and the peak height × peak width method.

The conclusion that electronic integrators give the most precise results must be treated with considerable caution. There are many types of integrator now available commercially and the attainment of high precision depends upon careful and correct adjustment of the integrator and on good chromatographic conditions. Because of its simple logic the precision of the integrator falls off rapidly when the conditions are

TABLE IV

BEST ESTIMATES OF THE RELATIVE STANDARD DEVIATIONS OF MEASUREMENT RATIOS COMPARED WITH THE COMBINED INDEPENDENT ERROR OF TWO PEAKS

Method	*Carbon monoxide to argon peak area ratio*		
	Percent relative standard deviation of ratio	*95% confidence limits*	*Percent relative standard deviation of sum*
Electronic integrator	0.106	0.092–0.138	0.201
Peak height	0.339	0.295–0.443	0.355

less ideal than in the test conditions used here, particularly where there is baseline instability or interference between peaks. Electronic integrators can also give significant bias errors which do not affect the "within set" precision when peaks of almost constant size are being measured, but they can have a critical effect when the peaks vary in size, as is usual in analysis.

The ball and disc integrator gave a better precision for argon and carbon monoxide than the three manual methods of area measurement (methods e, f, g); thus, although human errors are largely eliminated, recorder errors are still included. The very poor precision for the methane peak using this integrator must again be attributed to the small peak size. This indicates that considerable caution should be exercised when using this type of integrator to measure small peaks.

As mentioned before, peak height is affected by different parameters to peak area in GC. For a concentration detector such as the katharometer, peak height is critically dependent on column oven temperature and injection function but is relatively independent of flow-rate. If all these factors are well controlled, the measurement of peak height would be expected to be the most accurate manual method of measuring peak size. In fact the results indicate clearly that peak height measurements are comparable in precision to that of the electronic integrator and one may speculate that a direct electrical measurement of maximum detector response using, say, a digital voltmeter, may give a better precision than an electronic integrator.

This conclusion agrees with earlier work on this topic. Krejčí and Janák found that peak height gave the best precision when compared with peak height × retention time, weight of cut-out area, triangulation and planimetry[2]. A similar conclusion was reached by the International Conference of Benzole Producers during collaborative tests on the analysis of aromatic hydrocarbons[3]. Multiplication of the peak height by the retention time, as in method d, is seen to have a slightly adverse effect. Since the retention time is a very precise measurement, this effect suggests that the increased variance that resulted for this method was due to flow changes rather than temperature fluctuations.

The three manual methods of area measurement (methods e, f and g) give a much lower level of precision than the use of peak height or electronic integrator, although there is an indication that these methods improve for the broader peaks. Method e, *i.e.* peak height × width at half height, is significantly better than the other two manual methods and this agrees with the earlier work of Scott and Grant.

Table V shows the results of an analysis of variance for "between sets" and "between days" effects, using the two most precise methods, *viz.* methods a and b. These effects, where significant, can be attributed to changes in chromatographic response and also to changes in integrator response or sensitivity. There are significant changes between sets and between days for all three peaks, particularly for the integrator measurements. The much larger effects for the integrator compared to peak height measurements are almost certainly due to a variable bias error arising from drift in the peak detection circuit.

By contrast, the results for the ratios of two peaks show less variation with time (*viz.* between sets and days) using the integrator than by peak height. In this case the bias errors would tend to cancel out within each chromatogram and changes in chromatographic response, particularly those initiated by column temperature changes, would then have a larger effect on peak height ratios than area ratios.

TABLE V

ANALYSES OF VARIANCE

Method of measurement	*Source of variation*	S^2	S_{rel}^2	*Degrees of freedom*	*F*	*Significance level (%)*
Argon peak						
Electronic integrator	within sets	0.0019	0.0156	84	—	—
	between sets, within days	—	0.015	9	7.68	99
	between days	—	0.340	2	179	99
Peak height	within sets	0.0066	0.053	84	—	—
	between sets, within days		0.0086	9	1.30	n.s.
	between days		0.0185	2	2.80	99
Methane peak						
Electronic integrator	within sets	0.0128	0.103	84	—	—
	between sets, within days		0.055	9	4.31	99
	between days		0.116	2	9.03	99
Peak height	within sets	0.103	0.821	84	—	—
	between sets, within days		0	9	—	n.s.
	between days		0.023	2	0.23	n.s.
Carbon monoxide peak						
Electronic integrator	within sets	0.003	0.025	84	—	—
	between sets, within days		0.020	9	6.8	99
	between days		0.285	2	95.1	99
Peak height	within sets	0.009	0.073	84	—	—
	between sets, within days		0.049	9	5.42	99
	between days		0.059	2	6.48	99
Ratio argon to carbon monoxide						
Electronic integrator	within sets	0.0014	0.0112	84	—	—
	between sets, within days		0	9	—	n.s.
	between days		0.0012	2	1.43	n.s.
Peak height	within sets	0.0143	0.1149	84	—	—
	between sets, within days		0.0310	9	2.17	95
	between days		0.0130	2	0.91	n.s.

LIST OF SYMBOLS

n = number of determinations
F_{A1} = fraction of component A in calibration sample
F_{A2} = fraction of component A in unknown sample
F_{S1} = fraction of standard in calibration sample
F_{S2} = fraction of standard added to unknown sample
Y = ΣF_{x2}, where x = A, B, C, etc.
R_{A1} = response to component A in calibration sample
R_{A2} = response to component A in unknown sample

R_{S1} = response to standard in calibration sample
R_{S2} = response to standard in unknown sample
M_1 = mass of calibration sample chromatographed
M_2 = mass of unknown sample chromatographed
Z = fraction of components assumed to have zero concentration
$\bar{R}, \bar{M}, \bar{F}$ = mean values from a very large number of determinations
ΔF_{A2} = $F_{A2} - \bar{F}_{A2}$
σ = relative standard deviation of:
subscript r = the measurements of an identical response
subscript m = the masses of sample injected in different injections of the same prepared sample
subscript w = the fractions of a component in separately prepared samples
subscript f = the fraction of a component injected in different injections of the same prepared sample
subscript v = the response of the equipment to identical injected masses of a component in different chromatograms
subscript t = the relative responses of the equipment to identical injected masses of two components in different chromatograms
subscript u = the difference between the actual response and the response that would have resulted if the component had been eluted under the same conditions as the standard within the chromatogram
$\bar{S}^2$ = estimate of variance of mean values = S_{rel}^2/n
S_{rel} = estimate of relative standard deviation
F = significance test = $\dfrac{\text{estimated variance from source under test}}{\text{estimated variance of mean } (= S_{rel}^2/n)}$

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REFERENCES

1 R. P. W. Scott and D. W. Grant, *Analyst (London)*, 89 (1964) 179.
2 M. Krejčí and J. Janák, *Chemie (Prague)*, 10 (1958) 264.
3 International Conference of Benzole Producers, *J. Chromatogr.*, 12 (1963) 293.