Quantitative gas chromatography

We refer to "The Standardization of Gas–Liquid Chromatography for the Analysis of Simple Hydrocarbon Mixtures" in the name of the International Conference of Benzole Producers, which appeared in the November 1963 issue of this journal.

Nine European laboratories took part in an experiment in which the two samples tested had the composition shown in Table I.

Duplicate determinations were performed and repeatability and reproducibility calculated for each sample. The authors concluded "For the first test sample... both the precision and accuracy were reasonably satisfactory. For the second sample however... the precision was appreciably worse. The reasons for the poor repeatability are not apparent".

This effect was predicted by us in a paper entitled "A Statistical Evaluation of Gas-Liquid Partition Chromatography as a Method of Quantitative Analysis" presented at the Fourth International Symposium on Gas Chromatography held under the auspices of Der Deutschen Akademie für Wissenschaften zu Berlin at Leuna, 28th–31st May, 1963, the proceedings of which will be published in due course.

We also showed that it was not meaningful to average repeatabilities over different concentrations. This is because repeatability is concentration-dependent.

Our investigation involved a $9 \times 3 \times 2$ factorial experiment covering 2, 4 and 6 component systems in which one component was varied over the range 0.25–64% by nine logarithmic steps, while, in the case of the 4 and 6 component systems the ratios of the other 3 or 5 components were maintained constant. Duplicate determinations were made and the whole 54 runs were performed in random order, over a period of two weeks by one operator.

The apparatus and conditions were as follows:

Instrument	Griffin & George D.6 (Prototype)
Column material	Stainless steel
Solid support	JJ's acid washed Celite 545 100/120 BSS mesh
Liquid phase	10% Poly(ethyleneglycol adipate)
Detector	Martin Gas Density Balance
Carrier gas	Nitrogen
Column temperature	100°, 149°, 177°
Method of injection	Griffin sampler (2 μl).

Analysis of the ranges of duplicate results led to the derivation of the following equation which accounted for 86% of the variability of the ranges:

$$\text{Range} \times 10^2 = 0.9635\,|C| - 0.009635\,|C|^2 + 0.1386\,N^2 - 0.7506;$$

where $|C|$ represents the concentration level in % w/w and N represents the total number of components.

Application of this equation to the results quoted by BENZOLE PRODUCERS for the preferred method A gives the values listed in Table I.

Thus 70% of the variability of the ranges (as measured by sum of squares) has been accounted for.

This demonstrates that the differences observed between the two samples are

TABLE I

Sample number	*Component*	*Concentration (%)*	*Average range found from all Laboratories* ($\times 10^{-3}$)	*Range predicted by equation* ($\times 10^{-3}$)
1	Benzene	98.04	6.67	3.28
	Toluene	0.73	2.11	2.17
	Ethylbenzene	0.80	2.88	2.23
	Cyclohexane	0.43	1.66	1.88
2	Toluene	90.64	25.00	10.89
	Benzene	1.82	4.00	4.44
	Ethylbenzene	2.69	8.62	5.24
	p-Xylene	3.72	14.00	6.17
	n-Nonane	1.13	2.78	3.79

due to the change in number of components and the variation in the concentration of these components.

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Paper chromatography of short chain aliphatic amides

During studies on the use of aliphatic amides by the bacterium *Pseudomonas aeruginosa* as sole source of carbon and nitrogen for growth[1–3], it became necessary to check the purity of the amides and identify very small amounts of amide remaining in the culture media after growth. The use of direct paper chromatography of amides was investigated since, although it is possible to convert amides to their hydroxamates[4] and there exist a number of suitable solvents for chromatography of hydroxamates[5,6], the procedure does not allow distinction to be made between amides such as acetamide, N-methylacetamide and N-acetylacetamide, as these amides give the same hydroxamate. A few methods for direct chromatography of amides have been published but these are for long chain amides[7] or involve treatment of the paper, *e.g.* with 5 % polycaprolactam–formic acid[8]. In the search for a suitable solvent, it was found that many of the usual chromatography solvents were unsatisfactory because the amides travelled with the solvent front, did not move at all, or in the case of *n*-butanol–acetic acid–water did not give adequate separation of the amides. The solvent finally selected was toluene–ethanol (75:25 v/v); amides were detected by conversion to hydroxamates and the hydroxamates visualised with ferric chloride[9,10].