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APPLICATION OF GAS CHROMATOGRAPHY IN THE FIELD OF FATS II: QUANTITATIVE EVALUATION*

(Anwendung der Gas-Chromatographie auf dem Fettgebiet II: Quantitative Auswertung)

By Prof. Dr. h.c. H.P. Kaufmann, Prof. Dr. A. Seher and Dr. G. Mankel.

From the German Institute for Fat Research, Münster (Westfalen).

For the quantitative evaluation of gas-chromatograms, the area of the bands is generally used as measure of the quantity for the corresponding components. The size of these areas is related neither to the weight nor to the molar percentage of the productive substances in a simple mathematical dependence. Therefore an empirical standardization of the apparatus is necessary. The factors have been determined by the use of purest

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*Extracts read by A. Seher on the occasion of the DGF-lecture meeting in Hamburg on October 23, 1961; compare "Fette, Seifen, Anstrichmittel" Vol. 63, 1006 1961. "Studien auf dem Fettgebiet" (Studies in the Field of Fats), 282, Communication.

fatty acid methylesters and their influence by changes in the working conditions have been tested. The still unsatisfactory methods of measurement of area have been found to be an essential source of error during the quantitative GLC-analysis.

The gas-chromatographic separation of fatty acid methylesters belongs today in many laboratories to the routine methods of fat analysis. Recently we published[1] a general survey of the extensive bibliography, in which we treated mainly the qualitative analysis and the identification of the separated components. Now we shall report on the quantitative evaluation of GLC-fractograms. In this field, literature transmits very inhomogeneous views which are evaluated here on the basis of our own experiments.

As measure for the amount of a reported component generally serves -- independently of the kind of detector used -- the area under the pertinent bands in the fractogram. The quantitative relationship between amount of substance and area of bands is affected to such a degree by some of the experimental data, that, in spite of the uniform evaluation, comparisons of most of the results are possible only by taking into consideration the measuring devices used. Of prime importance are the manner of operation of the detector and the kind of carrier gas.

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[1] H.P. Kaufmann, G. Mankel and K. Lehmann, Fette, Seifen, Anstrichmittel 63, 1109 (1961).

I. General Survey of Literature

Because of the extensive application we shall in the following paragraphs discuss mainly the thermal conductivity detector (katharometer). The preferred carrier gases for this are helium and hydrogen. Results obtained with nitrogen are not directly transferable. There are further essential differences in the quantitative relations, depending on whether the investigated compounds belong to homologous series or not.

1. Heat-Conductivity Cell, Helium, or Hydrogen as Carrier Gas

a) Compounds of Varying Constitution (not homologous compounds)

H.H. Hausdorf[2] found a direct proportionality between area of the bands and molar percentages. According to investigations by E.A. Hinkle and S.E.J. Johnsen[3] this proportionality holds true only after a correction by the steam pressure of the components. On the other hand, L.C. Browning and J.O. Watts[4] determined that with larger differences in the heat conductivity of the components of a mixture the quotient from the area of bands and the

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[2] In: D.H. Desty, Vapour Phase Chromatography, Butterworth Sci. Publ. London 1957, p. 377.

[3] In. V.J. Coates, H.J. Noebels and I.S. Fagerson, Gas-Chromatography, Academic Press Inc. Publ., New York/ /London 1958, p. 25.

[4] Analytic Chem. 29, 24 (1957).

[5] Analytic. Chem. 31, 230 (1959).

heat conductivity is proportional to the weight percentages. The relative molar thermal conductivity developed by A.E. Messner, D.M. Rosie, and P. Argabright[5] will be discussed in detail at the end of this article.

b) Compounds of a homologous series

Numerous authors[6] found in analyses of homologous series under the working conditions treated here a direct proportionality between area of bands and weight percentages which makes ~~it~~ unnecessary a conversion with correction factors. Because of this, most of the quantitative data in literature with regard to the composition of fats and oils were obtained through a direct transmission of the area percentages into weight percentages.[1] With this kind of evaluation no consideration was given to the fact that, e.g., saturated, unsaturated, or branch-chained fatty acids are not homologous compounds. In contrast, D.M. Rosie and R.L. Grob[7] determined that homologous hydrocarbons furnish band areas which are proportional to the molar percentages only after taking into consideration the heat conductivity. C.H. Orr and J.E? Callen[8], in

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[5]Analytic. Chem. 31,230 (1959).

[6]M. Dimbat et al, Analytic.Chem. 28, 290 (1956); E.M. Fredericks and F.R. Brooks, Analytic. Chem. 28, 297 (1956); E. Leibnits, R. Kaiser and C. Heft, in: H.P. Angele, Gas-Chromatographie, Akademie-Verlag, Berlin 1959, p. 59; J.L. Nunez et al, Analytic. Chem. 29, 1164 (1957); G. Schomburg, Z. analyt. Chem. 164, 147 (1958); W.E. Link, H.M. Hickman and R.A. Morrissette, J. Ameri. Oil Chemists' Soc. 36, 20, 300 (1959).

[7]Analytic Chem. 29, 1263 (1957).

[8]Ann. N.Y. Acad. Sci. 72, 658 (1959).

investigating saturated fatty acid methylesters found that the band area becomes smaller as the molecular weight increases. They explained this observation made on polyester columns by assuming an interchange of ester radicals of the specimen components with the stationary phase. It was assumed that this would preserve the "theoretical proportionality", for the reduction in the area is traced back to a reduction in the amount of the specimen which actually reaches the detector. J.A. Hudy[9] published the first objections against this interpretation when he observed analogous conditions on apiezon columns. J.V. Killheffer jr. and E. Jungermann[10] corroborated the experiments by Hudy. The authors found, in addition, a complete linearity between the reduction in area and the molecular weight of the ester. They developed a simple formula for the computational extrapolation in order to determine correction factors of long-chained fatty acid methylesters from the figures obtained on short-chained ones. After W.E. Link and R.A. Morrissette[11] found a decrease of the area of the bands with an increasing molecular weight also in the homologous series of the saturated n-alcohols on apiezon columns, the proof has been produced that substance-specific correction factors are required also in analyzing homologous compounds. This determination is further supported by data produced by R.L. Grob and collaborators[12].

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[9]Analytic. Chem. 31, 1756 (1957).

[10]J. Amer. Oil Chemists' Soc. 37, 456 (1960).

[11]J. Amer. Oil Chemists' Soc. 37, 668 (1960).

[12]R.L. Grob, D. Mercer, T. Gribben and J. Wells, J. Chromatography (Amsterdam) 3, 545 (1960).

A.J.M. Keulemans and collaborators[13] also corroborate the necessity of an experimental standardization of the detector for ascertaining these factors. L.A. Horrocks and collaborators[14] attempted to clarify the origin of these factors and discovered an agreement with the equation

$$F_1 \cdot F_2 = (M_1 \cdot M_2)^{\frac{2}{3}}$$

2. Ionization Detectors

According to articles by C.J.F. Böttcher and collaborators[15] and by M.L. Vorbeck and collaborators[16], the proportionality between areas of bands and weight percentages has not been preserved in ionization detectors either. The discovered deviation is, however, smaller than in the case of the heat-conductivity cell. The last-mentioned authors found systematic deviations of approximately 4% which may be compensated by correction factors.

This general survey of the literature shows that for a quantitative evaluation of GLC-fractograms a conversion with substance-specific correction factors is always necessary. In spite of this, in the majority

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[13]A.J.M. Keulemans, A. Kwantes, and G.W.A. Rijnders, Analytic. chim. Acta (Amsterdam) 16, 29 (1957).

[14]L.A. Horrocks, D.G. Cornwell, and J.B. Brown, J. Lipid Res. 2, 92 (1961).

[15]C.J.F. Böttcher, G.F.G. Clemens and C.M. van Gent, J. Chromatography (Amsterdam) 3, 582 (1960).

[16]M.L. Vorbeck, I.R. Mattick, F.A. Lee and C.S. Pederson, Analytic. Chem. 33, 1512 (1961).

of publications in the field of fats the view has been expressed that homologous series or chemically similar compounds require no correction of the area percentages at all for the heat-wire detector if a carrier gas with high heat-conductivity , such as hydrogen or helium, is used. Some time ago a ring analysis with methylesters of a natural fatty-acid mixture was carried out between different laboratories. The analysis furnished even values for individual components which, in part, showed scatterings of over ± 25%. In investigations on alcohols R.L. Grob and collaborators[12], when comparing the area and molar percentages, found errors up to 26.3% and in the comparison with the weight percentages errors up to 10.7%. Only through a conversion with correction factors was it possible to reduce these deviations to less than ± 1%.

These results corroborate the fact that the nonuniform conception encountered in literature causes a corresponding uncertainty in practice. We, therefore, considered it necessary to study the behaviour of purest fatty acid methylesters in gas-chromatographs, especially with regard to the quantitative evaluation of the fractograms. At the same time we intended to examine the influences of different working conditions on the relation of the area of the bands to the amount of substance, in order to recognize possible sources of error for the quantitative evaluation.

II. Methods for the Determination of the Area of the Bands

As basis for the quantitative evaluation of the fractograms serves the area under the recorder curve. The exact determination of this magnitude is a prerequisite for all further mathematical operations. Since greatly varying shapes of curves must be measured, this provides substantial possibilities of error. For the determination of areas the following methods are being used:

1. Through integration with the aid of electronic, electromechanical or mechanical apparatus;
2. through planimetering with ordinary mechanical planimeters;
3. through the quadrature of the curve with the aid of the product from height and width at half height;
4. through superimposition of a triangle, the sides of which are formed by the base of the area (base line) and the turning tangents;
5. through the product from height and retention time;
6. through cutting out and weighing the areas.

In individual analyses instead of the area, only

7. the area of the bands is chosen as reference magnitude.

Table 1

Accuracy of area determination according to different methods

Method of measuring	average quadratic error for areas of			
	0-50 mm^2	51-100 mm^2	101-300 mm^2	301-1000 mm^2
Planimetering[a]	10.0 mm^2	6.84 mm^2	10.0 mm^2	35.0 mm^2
	40 %	9.1 %	5.0 %	5.8 %
five-fold planimetering	5.57 mm^2	3.84 mm^2	4.61 mm^2	15.6 mm^2
	23.0 %	5.1 %	2.3 %	2.6 %
weighing of out-out area	4.37 mm^2	7.83 mm^2	5.50 mm^2	15.2 mm^2
	17.5 %	10.0 %	2.8 %	2.5 %
triangle of tangents	4.18 mm^2	7.14 mm^2	6.80 mm^2	5.60 mm^2
	16.7 %	9.5 %	3.4 %	0.9 %
height times width at half height	4.80 mm^2	9.38 mm^2	8.52 mm^2	14.60 mm^2
	19.2 %	12.5 %	4.3 %	2.4 %
height times retention time[b]	5.45 mm^2	3.85 mm^2	5.35 mm^2	10.30 mm^2
	21.8 %	5.1 %	2.7 %	1.7 %
mechanical integrator (1 imp/5 mm^2)	9.05 mm^2	8.17 mm^2	4.80 mm^2	12.00 mm^2
	36.2 %	10.8 %	2.4 %	2.0 %
	5.85 mm^2[d]	--	--	--
	23.4 %	--	--	--
electromechanical integrator (1 imp/2 mm^2)	2.50 mm^2	2.25 mm^2	5.40 mm^2	7.80 mm^2
	10.0 %	3.0 %	2.7 %	1.3 %
full electronic integrator (inertialess) (8 imp/1 mm^2)	0.55 mm^2	1.28 mm^2	1.80 mm^2	7.80 mm^2
	2.2 %	1.7 %	0.9 %	1.3 %

[a] The numerical values depend on the size of the planimeter. Optimum of used apparatus: 100-125 mm^2

[b] Converted with a factor which reduces the value of the area to mm^2.

[c] For flat bands

[d] For steep narrow bands.

In all methods a good zero constancy of the detector and the flawless separation of the components are a prerequisite for reliable results. Beyond that every one of the enumerated area determinations shows an error peculiar to it, the magnitude of which depends on the shape and size of the area that is to be measured. A detailed investigation in this respect was published by J. Janak[17] who ascertained the statistical errors for the individual methods enumerated in Table 1.

We were able to corroborate this observation through our own comparative measurements. Table 1 shows that all at present customary processes of area measurements contain errors the magnitude of which depends on shape and size of area of bands and may be quite substantial. From this it follows that all quantitative measurements, because of the nonoptimum determination of areas, are pre-charged with an error which can be restricted only through a large number of comparative measurements under the most favourable evaluation conditions possible.

III. Determination of the Substance-Specific Correction Factors

The examination of the quantitative relation between the amount of substance and the pertinent area of the bands represents in principle a standardization of the used gas-chromatograph under certain, previously fixed working conditions. We worked with two different

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[17] J. Chromatography (Amsterdam) 3, 308 (1960).

apparatus^ which were both equipped with heat-conductivity detectors. In addition to testing the linearity of the detector and of the recorder, we took into consideration the fact that, according to E. Cremer and R. Müller[18], useful values can be obtained only if the adsorption material has been in contact with the substances that are to be measured prior to the carrying out of the analysis so that all centres on which an irreversible adsorption could possibly take place are already saturated. To solve the problem that presented itself here the following mode of operation seemed especially suited:

Standardized mixtures of an exactly known composition were prepared from fatty acid methylesters with a gas-chromatographically tested purity of more than 99.9%. All mixtures contained myristic-acid or palmitic-acid methylester, in order to be able to refer to it as a standard in the case of a change of working conditions. The analysis of these mixtures took place under exactly defined conditions which were carefully kept constant. The areas of the bands were ascertained with an electro-mechanical integrator or by means of planimetering. According to the relationship method (internal normalization) we found via the sum of the areas the area percentages

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^Used were the "Chromatotest I" of the firm Destillationstechnik Stage KG, Cologne-Niehl, and the fractometer 116 H of the firm Perkin-Elmer & Co., Überlingen/Bodensee.

[18] Z. Elektrochem. 55, 217 (1951).

for the individual compositions of the standard mixture:

$$\text{area-\%} = \frac{100 \cdot F_1}{F_1 + F_2 \cdots F_n}$$

The known weight percentages are then divided by the discovered area percentages[19]; the obtained quotients represent the standard factors looked for:

$$f = \frac{\text{weight-\%}}{\text{area-\%}}$$

For a better comparability of the various investigation series with one another, the thus obtained factors were converted to myristic acid methylester or palmitic acid methylester = 1.000.

In Fig. 1 the standard factors obtained in accordance to the above experiments for saturated and unsaturated n-fatty acid methylesters are plotted graphically against the chain length of the appertaining fatty acids. One notices distinctly that even when using helium as carrier gas within the homologous series of the saturated fatty acids the additional growth of the factors indicates a reduction of the areas of the bands with an increasing chain length. From equal amounts of substances there results for stearic acid methylester a by 58.7% smaller area than for capric acid methylester.

Fig. 1. Standard factors of saturated and unsaturated methylesters in dependence on the chain length of the fatty acids.

I. Simple unsaturated fatty acid methylesters

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[19]Cf. W. Neudert and J. Huber, Arch. Pharmaz. Ber. dtsch. pharmaz. Ges. 295, 67 (1962).

II. Saturated fatty acid methylesters

Working conditions:

Temperature: 220° C

Gas flow: 6.6 litres helium per hour

Separating column: resoflex on celite.

Eichfaktoren -- standard (or calibration) factors

Kettenlänge der Fettsäuren (C-Atome) -- chain length of fatty acids (C-atoms).

Fig. 1 shows further that the simple unsaturated fatty acid methylesters furnish a series of factors of their own the connections of which form a straight line parallel to the first line.

Fig. 2 shows the dependence of the discovered standard factors of the methylesters of the various C_{18}-methylesters on the number of double linkages.

Fig. 2. Dependence of the standard factors on the degree of saturation in C_{18}-methylesters.

Working conditions:

Temperature: 220° C

Gas flow: 4 litres helium per hour;

Detector current: 160 mA

Stationary phase: reoplex 400 on celite.

cis-Doppelbindungen (isoliert) -- cis-double linkages (isolated)

Striking is the large increase of the factors with an

increasing number of the isolated double linkages. Further measurements also showed that the size of the standard factors is affected also by the spacial shape of the molecule. Oleic acid methylester requires a larger conversion factor than the elaidic acid ester. In Fig. 3 two fractograms are copied one on top of the other.

Fig. 3. Fractograms of ester mixtures containing oleic or elaidic acid methylesters.

Working conditions as in Fig 2.

Ölsäure -- oleic acid

Luft -- air

The lower, fainter, fractogram was obtained from a mixture of methylesters of myristic, palmitic and oleic acids. The upper fractogram was furnished by an exactly equal amount of an ester mixture in which merely the oleic acid had been replaced by elaidic acid. The mixing ratios are shown in Table 2.

Table 2

Composition of test mixture with oleic and elaidic acid

		(%)
Mixture 1:	myristic acid methylester	21.2
	palmitic acid "	34.5
	oleic acid "	44.3
Mixture 2:	myristic acid methylester	21.3
	palmitic acid "	33.7
	elaidic acid "	44.4
	stearic acid "	0.6

The areas of the bands for myristic acid methylesters have practically the same coverage in the two gas chromatograms. In the areas for the palmitic acid ester one recognizes the enlargement caused by the difference in the amount of 0.8% in the lower chromatogram. The comparison of the bands for oleic and elaidic acid methylesters shows at a mere 0.1% weight difference an enlargement of the area by 1.5% for elaidic acid. The ascertained factors are: oleic acid methylester = 1.166 and elaidic acid methylester = 1.152.

These measurements show already the extent of the calibration analyses required for an exact and complete standardization of a gas chromatograph. The large differences in the discovered factors emphasize clearly the necessity of a careful standardization as prerequisite for exact quantitative measurements. The corrections of the gas-chromatographic analyses results made possible through the standard factors become the more conspicuous, the more the chain length and the degree of saturation of the fatty acids vary in an ester mixture.

IV. Effect of Correction Factors on Quantitative GLC-analyses

In Tables 3 to 7 the area percentages and the corrected results of the evaluation of several fractograms are compared with the given weight percentages of ester mixtures, such as were used for the standardization.

Table 3

Evaluation of a fractogram from two saturated fatty acid methylesters

Fatty acid	Area of bands (Imp.)	Area-%	Weight-% given	corrected area-%
C_{10}	8640	55.9	36.7	36.7
C_{16}	6810	44.1	63.3	63.3

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Table 4

Evaluation of a fractogram from numerous saturated fatty acid methylesters

Fatty acid	Area of bands (Imp.)	Area-%	Weight-% given	Corrected area-%
C_6	115	1.1	1.1	1.1
C_8	255	2.4	2.2	2.2
C_{10}	375	3.6	3.3	3.2
C_{11}	455	4.3	4.2	4.1
C_{12}	620	5.9	5.5	5.4
C_{13}	585	5.6	5.6	5.5
C_{14}	1195	11.4	10.9	10.8
C_{16}	1375	13.1	13.0	13.0
C_{17}	1490	14.2	14.6	14.8
C_{18}	1860	17.8	17.8	18.1
C_{19}	2145	20.5	21.8	21.8

If in Tables 3 and 5 one compares the columns 4 and 5, one notices especially clearly the large deviation between the area percentages (internal normalization) and the given weight percentages. Through multiplication with the appertaining standard factors these differences are, as shown by column 6, equalized. Table 4 shows that there are examples in which the occurring errors are accidentally approximately equalized.

Table 5

Evaluation of a fractogram from saturated and unsaturated fatty acid methylesters

Fatty acid	Area of bands (Imp.)	Area-%	Weight-% given	Corrected area-%
C_{16}	1246	7.1	5.5	5.5
C_{18}	450	2.6	2.1	2.2
Oleic acid	3792	21.6	18.7	18.6
Linoleic acid	2866	16.3	14.8	14.8
Linolenic acid	9230	52.4	58.9	58.9

The numerical data of Tables 3 to 5 underline once more the above-described importance of the standardization. It must, however, emphatically pointed out that in mixtures with very high portions of short-chained and only low contents of very long-chained fatty acids the evaluation conditions are more unfavourable. In such

cases errors up to ± 10% may occur in the individual components. As an example of this the results of a corresponding analysis have been compiled in Table 6.

Table 6

Evaluation of a fractogram with high portions of short-chained fatty acid methylesters

Fatty acid	Area of bands (mm^2 x sensitivity)	Area-%	Weight-%	Corrected area-%
C_8	148·32	42.3	35.2	34.9
C_{10}	112·32	24.3	25.1	23.5
C_{12}	280·8	15.1	14.9	16.8
C_{14}	298·4	8.0	9.7	10.0
C_{16}	420·2	7.5	10.5	10.4
C_{18}	312·1	2.8	4.6	4.4

The substantial errors reported in Table 6 are due largely to difficulties in the planimetric measurement of the area. Added to this is the multiplication of the error by the measuring-range factor of the recorder (sensitivity). In mixtures which can be registered with the same, or approximately the same, measuring range the deviations of the corrected results are smaller. The scatterings are then additionally affected by the absolute dimension of the area as well as by the shape of the bands and the error resulting therefrom according to Table 1. As an example the results of 3 analyses ~~are~~ are reported in Table 7.

The comparison of the results given in Tables 3 to 7 indicates that the errors in the quantitative results are caused by inexactitudes in the determination of areas. The used electromechanical integrator produced better values than the planimetering.

Because of the evaluation of the GLC-fractograms via the sum of areas, deviations in individual components have an effect on all component parts of a mixture.

Table 7

Dependence of results of analyses on the size of the area of bands

Fatty acid	Area of bands (mm^2 x sensitivity)	Weight-% given	Weight-% found	relative error
C_8	130·2	2.1	2.2	+ 4.8
C_{10}	360·2	3.4	3.2	- 6.3
C_{12}	325	3.4	3.4	± 0
C_{13}	487	5.1	5.2	+ 2.0
C_{14}	655	7.6	7.6	± 0
C_{15}	819	9.7	9.8	+ 1.0
C_{16}	1132	15.1	14.7	- 2.7
C_{17}	978	13.6	13.3	- 2.3
C_{18}	1482	20.9	21.0	+ 0.5
C_{19}	1320	19.1	19.8	+ 3.7
C_{16}	1150	24.3	24.5	+ 0.8
C_{17}	1530	31.7	32.0	+ 0.9
C_{19}	1770	44.0	43.5	- 1.1
C_{17}	2290	48.3	48.2	- 0.2
C_{19}	2340	51.7	51.8	+ 0.2

V. Influence of Working Conditions on the Correction Factors

The standard straight lines represented in Fig. 1 are valid only for the used heat-wire detector and the working conditions chosen during the setting up. According to the experiments to be described farther down we must draw still a further limitation, namely, that the kind of separating column used for the magnitude of the standard factors is also co-determinant. To get an impression of the precision with which the individual conditions must be kept constant in quantitative investigations the working conditions were systematically varied.

The greatest effect on the level of the standard factors results from changing the bridge current. Fig. 4 shows the standard straight lines for saturated n-fatty acid methylesters at varying heat currents in the detector bridge.

Fig. 4. Effect of the cell-current on the standard factors.

Working conditions:

Temperature: 220° C

Gas flow: 4.8 litre helium per hour

Separating column: reoplex 400 on celite.

Prozentualle Flächenabnahme---percentage reduction in area

Kettenlänge der Fettsäure -- chain length of fat acid.

With an increasing current intensity in the heating wire the differences between the standard factors of the neighbouring esters also increase. A lower current intensity makes the standard straight lines flatter. From this, however, no conclusion as to an increasing accuracy may be drawn since the lessening of the current intensity is connected also with a reduction in the indicator sensitivity. Table 8 gives the relative size of the areas for three different current intensities.

Table 8

Dependence of the relative area of bands on the detector current

Detector current	relative area of bands*
130	41.0
150	64.3
170	100.0

*The reported values were measured at 220° C and a gas velocity of 4.8 litres of helium per hour.

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The next-largest influence is exerted on the calibration curve in a few detectors by the change in the through-flow amount of carrier gas.

The extent of the influence depends in this case to an especially large degree on the construction of the detector block, in particular on the kind of gas conduction. In other tested detectors the changes were smaller.

Changes in the working temperature also affect the size of the calibration factors, depending on the construction characteristics of the detector. Fig. 6 reproduces the results obtained on a katharometer.

Fig. 5. Influence of through-flow amount of carrier gas on the calibration factors.

Working conditions:

Temperature: 220° C

Stationary phase: resoflex on celite

Amount of gas: I = 5.8 litres of helium per hour

II = 6.3 litres of helium per hour

III = 6.6 litres of helium per hour

(or standard)

Eichfaktoren -- calibration factors

Kettenlänge der Fettsäuren (C-Atome) -- chain length of fatty acids (C-atoms)

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Fig. 6. Effect of measuring temperature on the calibration factors.

Working conditions:

Temperature: I = 190° C

II = 210° C

III = 220° C

Flow of gas: 6.6 litres of helium per hour

Separating column: resoflex on celite.

Even the replacement of a separating column by a similarly filled other column of the same dimension and of similar separating properties exerted an influence on

the size of the calibration factors (Fig. 7).

In spite of the retention of the stationary phase, distinct changes in the calibration factors thus do occur. These are especially pronounced if the retention times experience a shifting. It is, therefore, self-evident that a change of the stationary phase brings about also a change in the calibration factors.

In Fig. 8 the calibration factors obtained as stationary phase on a separation column with silicon fat are represented graphically alongside those obtained on reoplex.

Fig. 7. Effect of the exchange of similarly filled separation columns on the calibration factors.

Working conditions:

Temperature: 220° C

Flow of gas: 6.6 litres of helium per hour

Separation column: resoflex on celite

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Fig. 8. Calibration factors for n-fatty acid methylesters in different stationary phases.

I = reoplex

II = silicon fat

Working conditions:

Temperature: 210° C

Detector current: 160 mA

Flow of gas: 4 litres of helium per hour.

VI. Retention Times and Correction Factors when Using Polar and Nonpolar Stationary Phases

As is known, on nonpolar columns the unsaturated fatty acid methylesters emerge before the saturated ones. In spite of this, on the silicon column, too, the unsaturated fatty acid methylesters show higher calibration factors than the saturated ones with equal chain length. However, under the influence of the changed retention time shifts in the size do occur. This observation may be demonstrated especially clearly on the example of oleic acid and elaidic acid methylesters. With the same mixtures as were used in the investigation on the reoplex a repetion of the separation in a column, with silicon fat as stationary phase, took place. In this case the oleic acid shows a shorter retention time and forms a larger area of bands than the elaidic acid methylester emerging later. The discovered numerical values are compared with one another in Table 9.

Table 9

Comparison of the retention times and calibration factors of oleic acid and elaidic acid methylesters.

	Reoplex column		Silicon column	
	Oleic acid methyl-ester	elaidic acid methyl ester	Oleic acid methyl-ester	elaidic acid methyl ester
Relative retention time^	3.75	3.67	3.71	3.83
Calibration factor	1.166	1.152	1.106	1.112

^Myristic acid methylester = 1.00;

Temperature: 210° C;

Detector current: 160 mA;

Flow of gas: 4 litres of helium per hour.

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The change in the relative retention times through the interchange of the stationary phase at otherwise equal working conditions is shown in Fig. 9.

Fig. 9. Dependence of the relative retention time on the length of the chain.

Working conditions:

Temperature: 210° C

Detector current: 160 mA

Gas flow: 4 litres of helium per hour.

Relative Retentionszeit -- relative retention time

Gesättigte Fettsäurenmathylester -- saturated fatty acid methylester

Arbeitstemperatur -- working temperature

Silikonfett -- silicon fat

Kettenlänge der Fettsäure (C-Atome) -- chain length of the fatty acids (C-atoms)

VII. Relative Molar Heat-Conductivity Values

From a comparison of the reported measurement results it is evident that the relation between area of bands and the amount of substance producing same does not depend only on the kind and structure of the compounds that are to be determined, but that many kinds of apparative influences must also be taken into consideration. At the same time the results underline the already previously established demand for a careful constancy of the working conditions.

The numerous measuring data that affect the size of calibration and correction factors have caused various investigators to look for substance-specific basic magnitudes from which the appertaining correction factors can be calculated independently of the respective working conditions. E.A. Messner, D.M. Rosie, and P. Argabright[5] determined in investigations on numerous low-boiling compounds that from the relationship between area of the bands and amounts of substance proportional numbers could be derived which they called "relative molar heat-conductivity values" (rmW). The/related all values to benzene, the rmW of which they established at 100. According to the conception of the authors the relative molar heat-conductivity values are independent:

1. of the kind of the heat-conduction cell (thermistor, heat-wire, partial current, full current, or diffusion cell),

2. of the temperature,

3. of the flow of the amount of gas, and

4. of the sensitivity of the measuring cell.

The advantages offered by such numerical values for the quantitative GLC-analysis are quite evident. Unfortunately, R. Kaiser[20] already considered it necessary to make considerable restrictions for the general applicability of these numbers. However, he traces them chiefly back to the still unsatisfactory accuracy in the determination of these values.

The relative molar heat-conductivity values were defined by E.A. Messner and collaborators by the equation

$$rmW_i = A_R + B_R \cdot M_i$$

where

rmW_i = relative molar heat-conductivity value of substance i;

A_R and B_R = constants, independent of the homologous series R to which substance i belongs;

M_i = molecular weight of substance i.

With the experimentally determined conversion factors (area into weight percentages) the rmW is connected through the equation

$$sWf_i = \frac{100 \cdot M_i}{M_{st} \cdot rmW_i}$$

where

sWf_i = conversion factor for substance i;

M_i = molecular weight of substance i;

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[20] Chromatographie in der Gasphase, Bibliographisches Institut, Mannheim 1961, Vol. III, p. 116 f.

M_{st} = molecular weight of the standard substance

rmW_i = relative molar heat-conductivity value of i.

With the aid of the last equation the relative molar heat-conductivity values were calculated from 7 definite series of conversion factors -- with 4 different heating-wire detectors at varying temperatures and 3 different stationary phases -- for saturated n-fatty acid methylesters. The resulting figures are plotted in Fig. 10 against the respective molecular weights.

Fig. 10. Relative molecular heat-conductivity values for saturated n-fatty acid methylesters.

Kettenlänge der Fettsäuren -- chain length of fatty acids
Relative molare Wärmeleitwerte -- relative molar heat-conductivity values
Mol. Gew. der Methylester -- mol. weight of methylesters
n. Literatur -- according to literature

For the analysis of the methylesters of higher fatty acids benzene is useless as standard substance. J.W. Killheffer and E. Jungermann[10] calculated the rmW for caproic to stearic acid methylesters, whereby they had to choose methyl acetate as reference point for the scale established by E.A. Messner and collaborators. In order to have for fatty acid methylesters a directly determinable reference point, myristic acid methylester was fixed at 100 in the following calculations. In the series of E.A. Messner and collaborators it has a value of 208.9.

Fig. 11. Stadard straight lines whose values were used for setting up Fig. 10.

Fig. 10 illustrates the following:

1. The numerical values obtained with the detectors of the firm Stage KG are similar to those reported by J.W. Killheffer and E. Jungermann.

2. The detectors of the firm Perkin-Elmer provide greatly deviating, and more variable from one another, curves.

3. The connecting lines in all measuring series are noticeably to distinctly curved.

These determinations show that there are in the fatty acid methylesters conditions which greatly deviate in part from the previous observations. The cause for this cannot be discussed as yet.

The previous investigations on the rmW have been carried out mainly at working temperatures which lie at least 100° C below those required for the analysis of higher fatty acids. From data by R. Kaiser the rmW for prim. n-alcohols were calculated from octonol to eicosanol through extrapolation according to the equation

$$rmW = 34.9 + 0.808 \cdot M$$

from the data from propanol to heptanol. For the same alcohols W.E. Link and R.A. Morrissette[11] have reported calibration factors from which it was also possible to

calculate the rmW. Both series of figures have been plotted in Fig. 12 against the mol. weights of the alcohols. One notices that here, too, deviations occur such as were found in the fatty acids.

Fig. 12. Relative molar heat-conductivity values for prim. n-alcohols.

I = calculated according to data by R. Kaiser
II = calculated from measuring values by W.E. Link and R.A. Morrissette

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From these findings the conclusion must be drawn that the suppositions made when setting up the relative molar heat-conductivity values have not been completely fulfilled. With a growing mol. weight and a rising working temperature the existing deviations appear more and more distinctly.

The described investigations show that quantitative statements can be made from GLC-fractograms only after a preceding careful standardization of the apparatus under the working conditions prevailing in each case. The accuracy of the achieved results depends essentially on the precision of the measurement of the areas in the fractograms.

We wish to thank the German Research Association for their material aid in carrying out these investigations.