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Gas-Liquid Chromatography of Fatty Derivatives. IV. Quantitative Analysis of n-Alcohols[1]

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The separation of fatty alcohols has been accomplished by using a nonpolar substrate on a solid support treated with alkali to reduce adsorptivity. By this technique well-resolved symmetrical peaks are obtained, and the stability of a nonpolar substrate is retained. Relative detector sensitivity factors have been determined for the alcohols with chain lengths of C_8 through C_{18}, permitting a correction of peak areas. The response of the detector is approximately a linear function of chain length for the n-alcohols.

The accuracy and precision of the determination have been studied by a statistical comparison of the results of replicate values with values from known compositions.

FATTY ALCOHOLS have been separated by gas-liquid chromatography through the use of selected substrates, such as silicones (10), polyglycol esters (10), and polyglycols (3), all of which are relatively polar in character. If the alcohols are converted to their respective acetates (11), these may be separated on polyester substrates in respect to both chain length and degree of unsaturation.

All of the above-mentioned partitioning agents suffer from certain disadvantages. The silicones do not afford a high efficiency, and, as a result, the peaks for long-chain lengths are broad. When alcohols are separated on polyester columns, it is difficult to relate percentages to area percentages. The use of the acetates results in a more linear response but requires an additional step. The poor stability of the polyglycols and their esters, both of which contain ether linkages, precludes their use as partitioning agents at high column-temperatures for long periods of time and sometimes results in base-line drift. In addition, quantitative techniques are hampered by the asymmetry of the peaks frequently encountered.

Since tailing, a form of asymmetry commonly encountered with polar materials, is caused by adsorption by the solid support (9), numerous attempts have been made to reduce it. Dal Nogare and Bennett (13) obtained symmetrical peaks for alcohols by increasing the temperature during the analysis.

Knight (8) reduced tailing with highly polar compounds by adding a volatile material, similar in type to the sample, continuously with the carrier. A reduction in the asymmetry of peaks produced by polar compounds on a nonpolar phase was achieved by Ormerod *et al.* (14) by the use of C-22 brick dust coated with silver. Gold was said to be ineffective. Johns (6) employed small amounts of polar solvents to reduce the tailing of polar molecules on a silicone substrate. Decora and Dineen (2) used a solid support prepared from a commercial detergent for the separation of basic nitrogen compounds, including close-boiling pyridines (1).

Treatment of the solid support by base-washing has afforded some success in the chromatography of amines containing 1 to 12 carbon atoms (4,5). This procedure has been applied in our laboratory to the separation of fatty amines of chain lengths ranging from C_8 to C_{22}, using nonpolar liquid substrates on Chromosorb W solid supports treated to reduce adsorptivity (12). Preliminary investigations revealed that fatty alcohols could also be run on the nonpolar substrate under approximately the same conditions as the corresponding fatty amines, with resulting comparable symmetry.

It was the object of this work to study quantitatively the behavior of fatty alcohols on this type of column, also to take advantage of the peak symmetry afforded by the support and also the stability of the nonpolar substrate.

Experimental

Preparation of Packing. The support was prepared as previously described (12). Acid-washed Chromosorb W, 40-60 mesh, was poured into a solution of potassium hydroxide in methanol. After the methanol was removed, the support was impregnated with Apiezon L in a ratio by weight of 5:1. The solvent was removed from the solid material in each operation through evaporation from a flat tray with the aid of an infrared lamp. The packing material was screened to insure uniformity and was heated for several hours in a vacuum oven at 80°C. to remove residual solvent.

Instrumental Conditions. The gas-liquid chromatographic apparatus used in this work was also described in a previous publication (10,11). A stainless steel column (2 ft. long by ¼ in. o.d.) was packed with the above material and flushed with helium in the instrument for a few hours at 220°C.

The purified fatty alcohols used were prepared by fractional distillation of commercially available materials.[2] The individual alcohols were checked for purity by gas chromatography. With a one-millivolt recorder full-scale deflection was found for the major

[1] ADM Talk No. 186.

[2] Adols, Archer-Daniels-Midland Company.

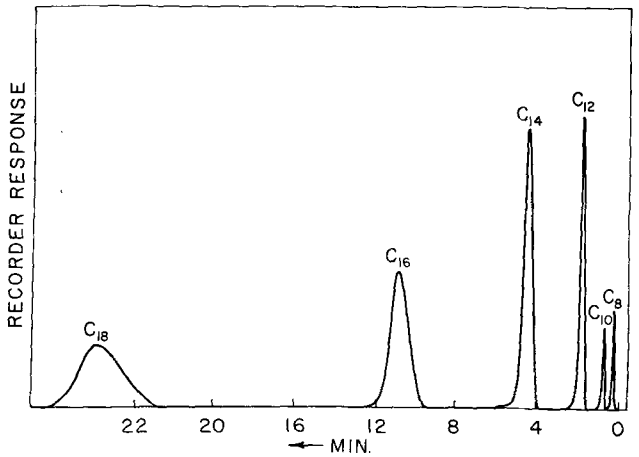

FIG. 1. Chromatogram of n-alcohol mixture A on Apiezon L, using treated Chromosorb W. GLPC conditions: column temperature, 230°C.; helium flow rate, 70 ml./min.; sample size, 0.002 ml.

component, and only trace amounts of other components were shown, a finding which indicated that all alcohol standards were better than 99% pure.

Mixtures of the pure alcohols were run under two sets of conditions of temperature and flow rate: Mixtures A and B at 230°C. and 70 ml./min. and Mixture C at 180°C. and 63 ml./min., with a sample size of 0.0015–0.004 ml. The separations of n-alcohols from C_8 through C_{18} (Mixture A) and of a commercial alcohol, Adol 15,[2] with a range C_8 through C_{16} are shown in Figures 1 and 2, respectively. Both chromatograms were run at the higher temperature. In Figure 3 a three-component mixture is shown (Mixture C), run with the same column as the second set of conditions. The two sets of conditions will permit the analysis of most commercial alcohol samples, from C_6 through C_{20}. The efficiency of the column is reflected by the number of theoretical plates obtained for C_{16}, more than 300 per foot.

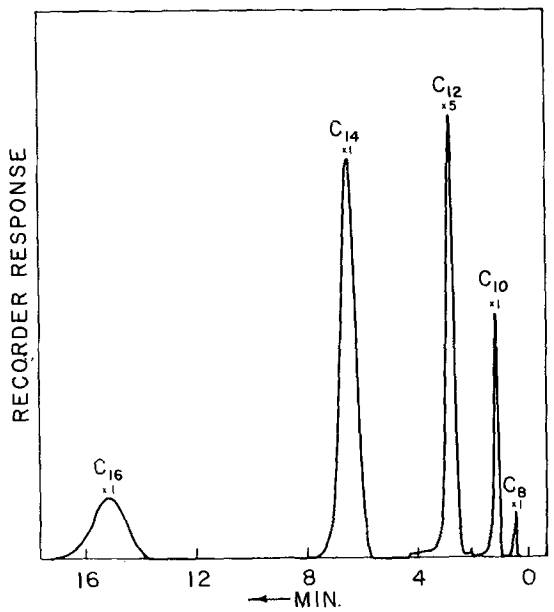

FIG. 2. Chromatogram of typical commercial alcohol, with chain lengths from C_8 through C_{16}, on Apiezon L, using treated Chromosorb W. GLPC conditions: column temperature, 230°C; helium flow rate, 70 ml./min.; sample size, 0.002 ml.

Quantitative Analysis of Alcohol Mixtures. Although it had been previously reported for the range C_8 to C_{18} that peak areas were proportional to mole percentages for fatty alcohols (3) and to weight percentages for alcohol acetates (11), it was evident from preliminary work that neither system was applicable to alcohols run on the treated support. Relative sensitivity factors were therefore determined for C_8 through C_{18} alcohols by the method used earlier for amines (12). A value of 1.000 was assigned to hexadecanol. The measured peak areas obtained for mixtures were then corrected by using the respective $K^{w/n}$ values. Table I shows the $K^{w/n}$ factors obtained

TABLE I

Relative Sensitivity Factors, $K^{w/n}$

Alcohol	$K^{w/n}$
Octanol	1.210
Decanol	1.143
Dodecanol	1.082
Tetradecanol	1.041
Hexadecanol	1.000
Octadecanol	0.970
Eicosanol	0.945 [a]

[a] Extrapolated.

from pure alcohols. The relative responses approximate a linear function of chain length among the alcohols (Figure 4) in agreement with earlier data pertaining to fatty homologous series (3,7,12).

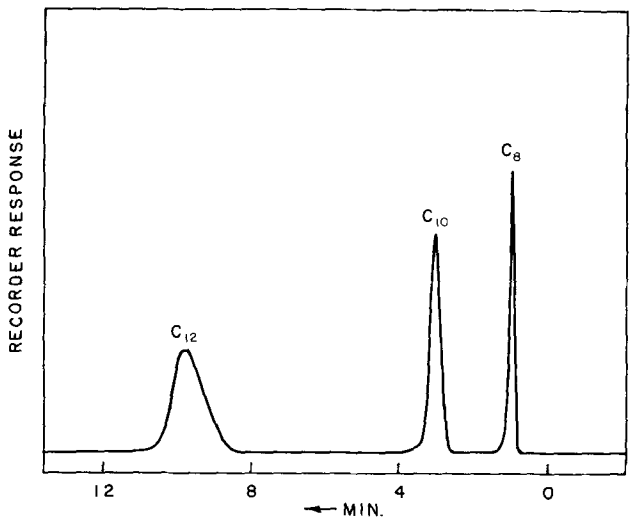

FIG. 3. Chromatogram of n-alcohol mixture C on Apiezon L, using treated Chromosorb W. GLPC conditions: column temperature, 180°C.; helium flow rate, 63 ml./min.; sample size, 0.004 ml.

To provide sufficient data for a statistical evaluation, the three mixtures of known composition, A, B and C, were run on two similar instruments, using different columns made to the same specifications, and operated under identical conditions. The accumulated data were tabulated for each mixture as shown in Tables II, III, and IV.

Discussion and Results

In the tables indicated the known sample compositions may be compared by inspection with corrected area percentages. Average values and standard de-

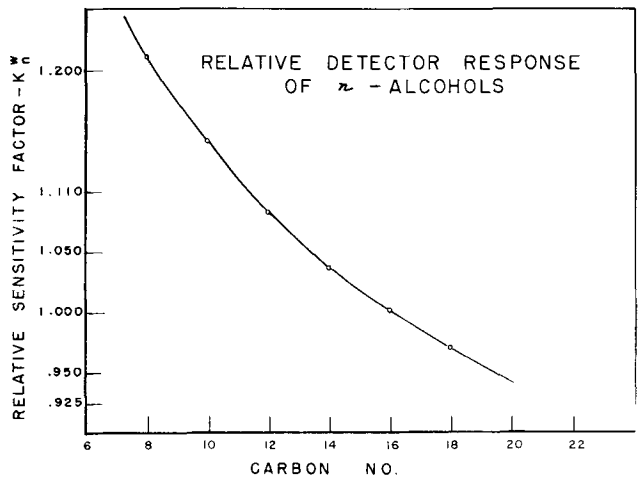

FIG. 4. Detector response of n-alcohols related to chain length (n-hexadecanal = 1.000).

viations have been calculated for each component within each mixture. The agreement between average values and known sample compositions is good. The standard deviations are those of the replicates for each component within each mixture. The standard deviations for Mixture A increase, in general, from C_8 to C_{18}; this not true for Mixtures B and C. It is believed that this increase is caused by two factors, the inability to measure with a planimeter small areas with the same accuracy as for larger areas; secondly, the relative error of the determination, which is greater for a small value than for a large one. Substantiation was obtained for this by checking the differences between the standard deviations by means of the F test. In Mixture A approximately half of the standard deviations show statistically significant differences from each other. Only chain lengths of C_8, C_{10}, and C_{18} are involved where significant differences are shown. The other mixtures show no significant differences. In addition, the actual values obtained, except in one case, C_{16} in Mixture B, show no statistical differences from the known sample compositions.

TABLE II

Analysis of Known Alcohols
Mixture A

Carbon No.	C_8	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}
Theo. %	1.5	2.0	12.3	24.6	28.3	31.3
Actual % (1)	1.3	2.1	12.3	24.7	28.4	31.2
(2)	1.3	2.2	12.4	24.7	29.2	30.2
(3)	1.6	2.1	12.0	24.5	28.4	31.4
(4)	1.3	1.9	12.7	25.0	28.2	30.9
(5)	1.2	1.9	12.7	24.6	28.1	31.5
(6)	1.3	2.0	12.2	24.8	29.1	30.6
(7)	1.3	1.9	12.2	25.2	28.3	31.1
Aver.	1.33	2.01	12.36	24.79	28.53	30.99
S.D.	.125	.122	.264	.24	.44	.46
Aver. S.D.			.306			

In Table V are compared the data obtained when a known mixture is run on different columns. When the alcohol itself is run on a conventional polyester column (butanediol 1, 4 succinate), the differences between known compositions and actual values (measured as area percentages) are pronounced, especially for C_{12} and C_{18}. If this same mixture is first converted to the acetates and run under the same conditions, the differences are less obvious, but significantly the actual value for C_{12} is high and that for C_{18} is low. This phenomenon has been observed by other investigators (11,12). The trend is also exhibited when the mixture is run on the alkali-treated column.

TABLE III

Analysis of Known Alcohols
Mixture B

Carbon No.	C_{12}	C_{14}	C_{16}	C_{18}
Theo. %	9.2	17.9	42.4	30.5
Actual % (1)	9.6	18.2	42.2	30.0
(2)	9.6	17.9	42.7	39.8
(3)	9.8	17.7	42.2	30.3
(4)	9.3	17.4	42.6	30.7
(5)	9.5	18.0	42.6	29.9
(6)	9.1	18.1	42.8	30.0
(7)	9.0	17.9	43.0	30.1
Aver.	9.41	17.89	42.59	30.11
S.D.	.29	.267	.297	.302
Aver. S.D.		.29		

TABLE IV

Analysis of Known Alcohols
Mixture C

Carbon No.	C_8	C_{10}	C_{12}
Theo. %	18.6	32.8	48.6
Actual % (1)	18.9	33.1	48.0
(2)	19.3	32.3	48.4
(3)	18.9	32.2	48.9
(4)	18.5	32.8	48.7
(5)	18.6	33.1	48.3
(6)	18.2	33.4	48.4
(7)	19.1	32.6	48.3
(8)	18.0	33.2	48.8
Aver.	18.69	32.84	48.48
S.D.	.445	.437	.301
Aver. S.D.		.40	

TABLE V

Known Mixture on Various Columns

Carbon No. Mixture	C_{12}	C_{14}	C_{16}	C_{18}
Theo. %	34.1	27.6	20.2	18.1
Area % A [a]	37.4	29.7	19.0	13.9
B [b]	34.6	28.1	19.6	17.7
C [c]	35.6	27.4	19.9	17.1
	34.0 [d]	27.2 [d]	20.6 [d]	18.2 [d]

[a] Run as alcohols on polyester.
[b] Run as acetates on polyester.
[c] Run as alcohols on the treated column, uncorrected.
[d] Calculated from C, using $K^{w/n}$ factors.

The data, A, B, and C of Table V, do not substantiate the strict relationships between peak area percentages and weight percentages or between area percentages and mole percentages that have previously been assumed for thermal-conductivity detectors.

When the areas representing the data in Group C of Table V are corrected, using relative sensitivity factors, the results are in better agreement with the known composition, and the familiar high results for short-chain length components and low results for long-chain length components are avoided.

Acknowledgment

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Color Index for Cottonseed Oils[1]

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The color index determined by the area under the absorption curve in the region of 400–500 millimicrons is preferred over the A.O.C.S. photometric method in research on cottonseed oil color because the color index gives the more accurate measure of the relative chromogen concentration in cottonseed oils. The evidence that the color index method is more reliable includes: (a) a demonstration that the area under the absorption curve may be used in place of absorbance in the Beer-Lambert equation; b) a panel score for cottonseed oil color intensity that agrees with the color index better than it does with the photometric color; and c) sources of error in the photometric method that do not occur in the color index method, including those contributed by the high emphasis on absorption at 550 and 670 millimicrons.

A QUANTITATIVE relationship between the red color intensity of darkly-colored, bleached cottonseed oils and the concentration of the chromogens in these oils is basic to research on the genesis, the identity, and the elimination of the red-colored bodies that create a color problem for about one-fourth of the cottonseed oil produced in the United States. The methods currently used for measurement of color do not satisfy the research requirements for determining the chromogen concentration in off-colored cottonseed oils. The A.O.C.S. Wesson method (1), using Lovibond glasses, is subjective, and it is an adaptation of the Lovibond method, which was developed originally for use in measuring the color of beer. The photometric A.O.C.S. method (1) was devised to give color values from spectrophotometric data that would match those obtained by use of Lovibond glasses.

It has long been established that the percentage of incident light that is transmitted through most transparent solutions is an exponential function of the concentration of the solution. This principle is known as the Beer-Lambert law, and it forms the basis of colorimetric procedures that find wide use in analytical chemistry. Normally, where the system is defined and where the absorption characteristics of the solute are known, the determination of the percentage of the incident light that is transmitted through the solution is made in a narrow wavelength band. It is obvious however that, when the system is poorly defined and where several unidentified solutes of unknown absorption characteristics are present, the use of a narrow wavelength band may not be relied upon for an accurate measure of the solute concentration.

The effort is made here to use the area under the absorption curve,[3] where the absorbance is plotted against the wavelength, as a measure of the relative chromogen concentration of the unidentified pigments in cottonseed oil. Thus, if the Beer-Lambert law describes the absorption behavior of the solutes in cottonseed oil, the relationship

$$C_t = \frac{R \sum_{\lambda=400}^{\lambda=550} \left(\log \frac{I}{Io}\right)}{b \sum_{\lambda=400}^{\lambda=550} \left(\sum_{p=1}^{p=n} k_p\right)}$$

where C_t is the total concentration of the absorbing solutes, R is a proportionality constant, b is the length of the light path, λ is the wavelength in millimicrons, I and Io are light intensities, p is the number of solute components, and k_p is the absorptivity for the $_p$th component at wavelength λ, can be used to estimate the total concentration of solutes in the oils. The summation

$$\sum_{\lambda=400}^{\lambda=550} \left(\log \frac{I}{Io}\right)$$

is essentially the area under the absorption curve in the 400–500 millimicrons region in which the problem pigments absorb.

The denominator of the equation should remain constant in any dilution experiment with bleached

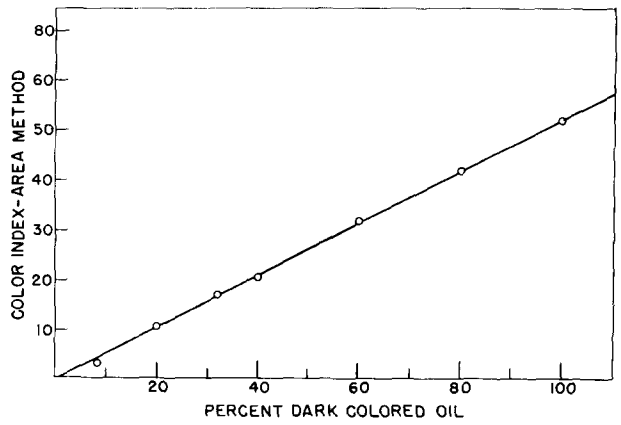

FIG. 1. Color index (area method) *vs.* percentage of dark-colored, bleached cottonseed oil mixed with a lightly-colored, bleached cottonseed oil.

[1] Presented at the 51st Annual Meeting, American Oil Chemists' Society, Dallas, Tex., April 4–6, 1960.
[2] One of the laboratories of the Southern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.
[3] This principle was proposed by Jacini and Carola (3) as a color index for olive oil and more recently was used by Frampton *et al.* (2) as a measure of egg yolk discoloration.