DIOL LIPIDS

COMMUNICATION 17. SIMULTANEOUS GAS CHROMATOGRAPHIC DETERMINATION OF DIESTERS OF DIOLS AND TRIGLYCERIDES

V. A. Vaver, A. N. Ushakov,
M. L. Sitnikova, N. P. Kolesova,
and L. D. Bergel'son

UDC 543.544.25:547.42

Neutral lipids, the alcohol portion of which represents C_2-C_4 diols, have been found in nature comparatively recently [1-4] and are now under intensive study in a number of laboratories. In physical and chemical properties these compounds are close to the glycerides, and their content in the total lipids is comparatively small [1, 2, 4]. Therefore, the isolation of diol lipids, with rare exceptions, is a very difficult problem. The method of adsorption chromatography does not give the desired result. Only unsaturated diesters of diols can be isolated by reversed phase partition chromatography [5, 6], while the saturated diol lipids do not dissolve in the systems usually used, and as a result are not separated from the triglycerides [6].

At the present time, the method of high-temperature gas–liquid chromatography (GLC) is being successfully used for the separation of synthetic and natural triglycerides [7-11]. This method has also been used for the separation of a comparatively simple mixture of synthetic diol lipids – derivatives of ethylene glycol [12] and 1,2-propanediol [13]. Several years ago we demonstrated that diol diesters can be detected in natural "triglyceride" fractions by high-temperature GLC [4]. In this communication we describe a gas chromatographic method for the qualitative and quantitative determination of diesters of C_2-C_4 diols, developed for the determination of diol lipids in triglyceride fractions.

EXPERIMENTAL RESULTS AND DISCUSSION

Diol lipids and triglycerides were separated with a model mixture of synthetic dipalmitates and distearates of ethylene glycol, 1,2-propanediol, 1,3-propanediol, 1,2-butanediol, 1,3-butanediol, 1,4-butanediol, tripalmitin, and tristearin as an example. Esters of 2,3-butanediol were not introduced into the model mixture, since they are separated from triglycerides and esters of the above-mentioned diols during the isolation of the triglyceride fractions [3, 4].

TABLE 1. Synthetic Dipalmitates and Distearates of Diols*

Polyols	Palmitate		Stearates	
	yield, %	mp, °C	yield, %	mp, °C
Ethylene glycol	90	69,5—71,0	93	75,0—75,5
1,2-Propanediol	83	48,0—49,0	95	57,0—59,0
1,3-Propanediol	88	58,0—60,0	88	63,0—65,0
1,2-Butanediol	91	32,5—34,0	87	45,0—46,0
1,3-Butanediol	85	37,0—39,0	93	44,0—45,0
1,4-Butanediol	89	63,0—65,0	90	61,0—62,0

* In elemental analysis of the synthesized diol derivatives, satisfactory values were obtained for the content of C and H.

Diol diesters were produced by adding an ether solution of two equivalents of the corresponding acid chloride to a solution of one equivalent of the dihydric alcohol and two equivalents of pyridine in abs. ether, chilled to 0°. The reaction products were mixed for 2 h at room temperature, diluted with water, and extracted with ether. The ether extract was washed with 5% HCl, with water, dried with anhydrous $MgSO_4$, and evaporated under vacuum. The diol dipalmitates and distearates obtained in the residue were recrystallized from 96% ethanol. The yields and melting points of the compounds

Institute of the Chemistry of Natural Compounds, Academy of Sciences of the USSR. Translated from Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya, No. 5, pp. 918-922, May, 1971. Original article submitted July 23, 1969.

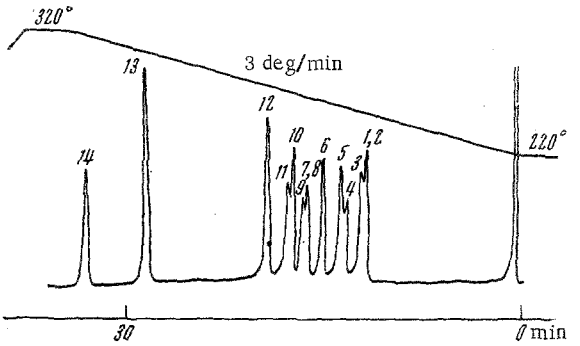

Fig. 1. Chromatogram of a mixture of dipalmitates and distearates of diols, tripalmitin, and tristearin. (Column 1000 × 4 mm with 3% QF-1 on a "Gas-chrom Q," 85-100 mesh, argon consumption 75 ml/min.) 1, 2) Dipalmitates of ethylene glycol and 1,2-propanediol; 3) 1,2-butanediol dipalmitate; 4) 1,3-butanediol dipalmitate; 5) 1,3-propanediol dipalmitate; 6) 1,4-butanediol dipalmitate; 7) distearates of ethylene glycol and 1,2-propanediol; 9) 1,2-butanediol distearate; 10) 1,3-butanediol distearate; 11) 1,3-propanediol distearate; 12) 1,4-butanediol distearate; 13) tripalmitin; 14) tristearin.

obtained are cited in Table 1. Tripalmitin and tristearin were synthesized according to the method of [14] with yields of 91 and 85%, respectively.

Qualitative Analysis. GLC of a mixture of diol lipids and triglycerides was conducted on columns 1000 × 4 mm with 3% fluorinated silicone QF-1 on a silanized carrier "Gas-chrom Q," 85-100 mesh. The instrument was a Pye chromatograph, series 104, model 24, with a differential flame-ionization detector. Before use, the columns with silicone were heated for five days in a stream of argon at 320°. The mixture of lipids was introduced with a microsyringe in the form of a 10% solution in chloroform or CCl_4. In this case, for reproducible evaporation of the sample it was necessary that the end of the needle of the microsyringe be in direct contact with the packing of the column. From the moment of introduction of the sample, the temperature of the thermostat was raised from 220 to 320° at a rate of 3 deg/min. The consumption of the carrier gas at the initial temperature of analysis was 75 ml/min, size of the sample 1-2 μl.

Under the indicated conditions, dipalmitates and distearates of diols are eluted in the interval 244-271°; tripalmitin and tristearin are eluted at 302 and 317°, respectively, and do not interfere with the determination of the diol lipids (Fig. 1, Table 2). As can be seen from the chromatogram, dipalmitates and distearates emerge from the column in approximately the same order as the corresponding diacetates [15] or free alcohols [16]. However, the quality of separation is appreciably poorer: esters of ethylene glycol and 1,2-propanediol emerge in a single peak and are only partially separated from the esters of 1,2-butanediol. Diesters of 1,3-propanediol and 1,3-butanediol are also incompletely resolved. Attempts might be made to improve the separation by lengthening the column. However, the use of longer columns involves an increase in the temperature of analysis, rapid wear of the packing, and appreciable losses of triglycerides. Therefore, we conducted the separation of model mixtures and natural lipids exclusively on short (1000 mm) columns.

Quantitative Analysis. For a quantitative estimate of the content of diol lipids in the triglyceride fractions it is necessary to determine the empirical correction factors. These coefficients, reflecting the summary error of the analysis, are obtained by comparing the true ratio of the components of the model mixtures (according to the sample weight) and the ratio found by integration of the areas of the peaks on the chromatograms. In high-temperature chromatography, a great influence on the empirical correction factors is exerted by the nonuniformity of evaporation of the sample [9-11]. Moreover, it has been shown that under conditions of high-temperature GLC, the correction factors depend on the degree of stabilization of the columns and are not always the same for each pair of columns [9].

TABLE 2. Separation of a Model Mixture of Dipalmitates and Distearates of Diols and Triglycerides*

Polyols	Palmitates		Stearates	
	abs. temperature of retention, °C	rel. temperature of retention	abs. temperature of retention, °C	rel. temperature of retention
Ethylene glycol	244	0,938	260	1,000
1,2-Propanediol	244	0,938	260	1,000
1,2-Butanediol	245	0,942	261	1,004
1,3-Butanediol	250	0,962	264	1,015
1,3-Propanediol	251	0,965	265	1,019
1,4-Butanediol	256	0,985	271	1,042
Glycerine. . . .	302	1,161	317	1,219

* For conditions of GLC, see text.

TABLE 3. Correction Factors for the Quantitative Determination of Diesters of Diols and Triglycerides

Substances	Correction factors*	Substances	Correction factors*
Ethylene glycol dipalmitate . . .	1.00	1,2-Propanediol distearate	1.05
1,2-Propanediol dipalmitate . . .	0.99	1,3-Propanediol distearate	0.97
1,3-Propanediol dipalmitate . . .	0.95	1,2-Butanediol distearate	1.05
1,2-Butanediol dipalmitate. . . .	1.04	1,3-Butanediol distearate	0.99
1,3-Butanediol dipalmitate. . . .	1.02	1,4-Butanediol distearate	1.10
1,4-Butanediol dipalmitate. . . .	1.01	Tripalmitin.	1.15; 1.33†
Ethylene glycol distearate.	0.97	Tristearin.	1,20; 1.48†

* Calculated according to ethylene glycol dipalmitate; an average value of three determinations was taken.

† Calculated according to chromatograms taken as a sensitivity of the amplifier ×500. The remaining chromatograms were taken at an amplifier sensitivity of ×2000.

The correction factors were determined according to weighed samples of pure synthetic standards. The weight correction factors for diol diesters cited in Table 3 were determined with an accuracy within 5% rel. For tripalmitin and tristearin, the dispersion in the determination of the coefficients was 10% rel. The results obtained show that the correction factors practically do not differ for the investigated diol diesters. The coefficients for tripalmitin and tristearin (calculated according to ethylene glycol dipalmitate) depend on the degree of stabilization of the columns. When freshly prepared columns, heated for five days at 320° (see above), are used, large losses of triglycerides are observed (the correction factors are equal to 3-3.5), but as they are stabilized, the numerical values of the coefficients decrease rapidly, and after several days they become equal to 1.1-1.2 (see Table 3). Beginning with this moment we consider a given pair of columns to be suitable for quantitative analysis.

In the determination of the coefficients cited in Table 3, samples of the calibration solutions contained from 10 to 15 μg of each component. It is interesting to note that when the sample size is reduced fourfold the error in the determination of triglycerides again increases (see Table 3). This is apparently due to the fact that a constant fraction of the high-boiling components of the sample (triglycerides) is consumed in each analysis for the temporary deactivation of the active sites of the packing. This fraction is negligible when the amount of the sample is sufficient, but it becomes appreciable when the sample size is sharply reduced.

The results that we obtained show that even under the most favorable conditions, the areas of the peaks of triglycerides are lowered in comparison with the areas of the peaks of diol diesters (correction factors greater than one), although the relative content of the unoxidized carbon atoms is the same in both types of compounds. This phenomenon, associated with the nonuniformity of the evaporation of the sample and possessing a number of literature analogies [9-11], does not prevent the quantitative determination of diol lipids in the triglyceride fraction. It is necessary only to redetermine the empirical correction factors for each given pair of columns and verify them before each series of determinations.

We are using the results obtained for a quantitative determination of the content of neutral diol lipids in the triglyceride fractions, and in combination with mass spectrometry for the identification of natural diol lipids.

CONCLUSIONS

1. The conditions of gas chromatographic separation of dipalmitates and distearates of C_2-C_4 diols, tripalmitin, and tristearin were developed for the determination of neutral diol lipids in natural triglyceride fractions.

2. Empirical correction factors were found for evaluating the quantitative content of neutral diol lipids in the triglyceride fractions.

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