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CHROMATOGRAPHIC ANALYSIS OF FATTY ACID DIMERS

COMPARISON OF GAS–LIQUID CHROMATOGRAPHY, HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND THIN-LAYER CHROMATOGRAPHY WITH FLAME IONIZATION DETECTION

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SUMMARY

For monitoring fatty acid polymerization and isolation processes, a sufficiently rapid and reproducible analytical procedure is still lacking. This paper compares the results of the analysis of mixtures of monomeric and oligomeric fatty acids by means of gas–liquid chromatography, high-performance liquid chromatography, and thin-layer chromatography with flame ionization detection. For serial analyses, the last mentioned method appeared to be the most suitable.

INTRODUCTION

The composition and chemical structure of dimeric fatty acids prepared by thermal polymerization of unsaturated fatty acids has been investigated by various methods. The presence of components such as unreacted monoenoic and dienoic acids and their stereoisomers, linear dimeric, monocyclic and dicyclic dimeric and higher oligomeric fatty acids is well known[1]. In the dimerization process and for the quality control of end-products, analytical determination of the main component classes, *i.e.* monomers, dimers and trimers, is sufficient.

At first, molecular-still separations[2,3] and column liquid chromatography[4–6] were used and, in some cases, thin-layer chromatography (TLC) was used[6,7]. During the early and prolific days of instrumental chromatographic development, attempts were made to use gel permeation chromatography (GPC)[8–14], from which all three main component classes could be determined. Nevertheless, the extended time-scale for GPC separations on different gels is a disadvantage[7,11,12,14]. Efforts to apply gas–liquid chromatography (GLC) were hindered at first by the high retention of the

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trimeric component, so that its content was evaluated from the mass differences[11,13,15,16]. In spite of the possibility of obtaining complete separation by GLC with the aid of temperature programming[17,18], the main disadvantages of GLC are (i) the need to sample derivatize in order to produce the corresponding methyl esters, and (ii) the necessity to operate at temperatures of 350–400°C.

Surprisingly, papers reporting the use of high-performance liquid chromatography (HPLC) and thin-layer chromatography with flame ionization detection (TLC–FID)[19,20] for oligomeric fatty acid separations could not be found in the literature until recently. In this paper, we discuss the suitability of the application of these chromatographic methods in the control of the production of fatty acid dimers. For comparison, GLC separations were also used to test both of the above-mentioned techniques on selected samples.

EXPERIMENTAL

Reagents and chemicals

Samples of fatty acid dimers were prepared in our Institute during the study of unsaturated fatty acid dimerization[21] according to methods previously published[22]. Fatty acids from rape oil with low erucic acid content were used, having a high enough content of C_{18} unsaturated fatty acids. Dimerization was performed at 260°C under nitrogen for 2 h, applying bentonite as catalyst. From the reaction mixtures, fractions of unreacted monomers and enriched dimers were isolated by thin-film molecular distillation for further technological experiments, application testing and analysis. The remaining distillation residue was purified, bleached and also tested and analysed. Some of the isolated fractions of monomers and dimers, showing purities of 93–96%, were used as standards for identification in analysis or were used for the preparation of model mixtures for analytical method testing for reliability and accuracy.

The samples of commercial fatty acid dimers were obtained from Koospol (Foreign Trade Agency, Prague, Czechoslovakia); a list is included in Table I, along with a comparison of the compositions calculated by the manufacturers with those found by us using GLC.

TABLE I
CALCULATED COMPOSITIONS OF COMMERCIAL FATTY ACID DIMERS AND THEIR MONOMER (M), DIMER (D) AND TRIMER (T) CONTENT (IN MASS %) FOUND BY GLC

Product	*Calculated composition*			*Composition found by GLC*		
	M	*D*	*T*	*M*	*D*	*T*
Empol 1014	1	95	4	0.7	95.3	4.0
Empol 1018	1	81–83	17–19	2.6	81.8	15.6
Empol 1022	3	75–80	20–22	2.6	76.2	21.2
Hystrene 3695	1	95	4	3.6	87.6	8.8
Dimak S	5–7	67–69	24–28	5.1	68.0	26.9
Dimak DS	7–10	72–76	15–18	8.7	71.7	19.6
Emery 3020	–	–	–	5.2	72.2	22.6
Pripol 1022	–	80	20	3.5	73.8	22.7

All chemical used were of reagent grade; all solvents were dried and distilled in glass before use.

For GLC separations, all samples were converted to methyl esters using the current esterification procedure of reacting 0.2 g of fatty acid dimer with 50 ml of methanol plus three drops of concentrated sulphuric acid and heating for 3 h under reflux on a water-bath; the resulting methyl esters were isolated after addition of 100 ml of water by extraction into 3 × 50 ml of diethyl ether. For samples with a substantial concentration of higher oligomers present, the sample to be esterified can be promoted by the addition of 1–2 ml of chloroform.

For analyses by HPLC and TLC–FID, original free fatty acid samples were applied without any modification.

Instruments and operating conditions for GLC, HPLC and TLC–FID

Corresponding data are summarized in Table II.

RESULTS AND DISCUSSION

GLC separation

Using GLC, various commercial samples and fractions from fatty acid dimer preparation were analysed. Because of the lack of standards, the separated peaks were assigned to monomers, dimers and trimers according to their retention order given by the relative volatility. This identification is in agreement with the literature[17,18]. The evaluation of GLC results was made from peak-area measurements by applying empirical corretion factors taken from the calculated and found compositions of commercial products. By choosing the dimer as a relative standard (K_D = 1.0), the factors for monomer and trimer peak-area corrections were K_M = 0.9 and K_T = 2.0, respectively. From the very high K_T value caused by the very low FID response, it may be assumed that some of the less volatile trimer or higher oligomers are retained on the column.

An example of GLC separation is shown in Fig. 1. The retention temperatures under our operating conditions were 140–160°C for unreacted fatty acids (*i.e.* isomeric monomers in one main peak and some trace peaks of different shape, their asymmetry being determined by the isomer distribution), *ca.* 260°C for dimers (where a slight asymmetric peak includes all linear and cyclic isomers), and *ca.* 330°C for trimers. Some results of GLC separations are summarized in Table I. The compositions of commercial products, as determined by our procedures, were comparable with those stated in product specifications confirmed by GLC.

HPLC separation

At first, attempts were made to separate fatty acid dimer samples by reversed-phase liquid chromatography (RPLC) on C_2, C_8 and C_{18} type packings using a methanol–water mobile phase. In comparison with GLC separations, more peaks appeared, especially on the C_{18} bonded phase. However, assignment of the individual oligomers was not as simple as in GLC. In addition, the retention time of the trimer fractions was very long, indicating the possible presence of higher oligomers, which could be completely retained on the column. Therefore, application of the back-flushing technique was unavoidable.

TABLE II

INSTRUMENTS AND OPERATING CONDITIONS

	GLC	*HPLC*	*TLC–FID*
Instruments	Fractovap GV-200	LC chromatograph with 52.00 pump and Rheodyne 7120 sampling valve	Iatroscan TH-10 (two instruments: Mark II and Mark III)
	C. Erba, Milan, Italy	H. Knauer, Bad Homburg, F.R.G.	Iatron Labs., Japan, distributed by Newman-Howells, Winchester, U.K.
Integrator	–	–	For Mark II: Spectra-Physics System I Computing Integrator; for Mark III: analogue built-in integrator
Detector	FID, operated at 350°C	Differential refractometer 61.00 (Knauer)	FID, scanning speed of 0.42 cm/s, H_2 flow of 180 ml/min, air flow of 2100 ml/min
Column	Dual, stainless steel, 60 cm × 4 mm I.D.	stainless steel, 25 cm × 4.6 mm I.D.	–
Separation media	Column packing of 3% JXR on Chromaton N HMDS 100–120 mesh	Column packing of LiChrosorb NH_2 (Merck), 10 μm	Silica gel of 5-μm grain size on Chromarod S II
Operating temperature	Programmed from 120 to 350° at a rate of 8°C/min	Ambient under detector thermostating	Ambient
Mobile phase	N_2 carrier gas at rate of 60 ml/min	Methanol + 0.05% acetic acid at a flow-rate of 1.0 ml/min corresponding to 5 MPa inlet pressure approx.	(A) *n*-Pentane–diethyl ether–acetic acid (85:15:1) (B) *n*-hexane–diethyl ether–formic acid (94:3:3) (C) Methylene chloride–methanol–acetic acid (98:0.5:1.5)
Sample application	Injected volume: 0.2–1 μl of sample diluted by *n*-hexane	Injected volume: 20 μl of 5% solution in the mobile phase	Spotted volume: 1 μl of the sample solution (25–300 mg in 1 ml of chloroform)
Remark	Methyl esters were prepared using diazomethane or methanol/sulphuric acid reagent	Back-flushing system was used applying six-way two-position Rheodyne 7010 valve coupled with column inlet and outlet	One-step elution was performed in saturated developing chamber, migration distance of the front from the start was 10 cm

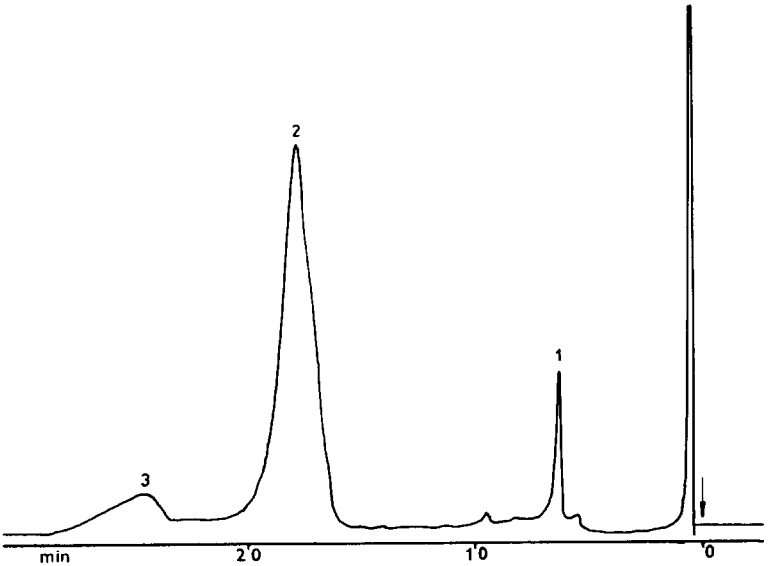

Fig. 1. GLC separation of a commercial fatty acid dimer mixture after the conversion to methyl esters. Peaks: 1, monomers; 2, dimers; 3, trimers.

Much more reliable results have been achieved on NH_2-bonded phases, where separation proceeds according to carboxyl group number, evidently due to the expected ion-exchange mechanism of the separation in methanol to which acetic acid has been added (0.01–0.1%, v/v). The retention is strongly influenced by the acetic acid concentration: while at concentrations above 0.1% all components were poorly separated and eluted in a short time, at 0.05% a sharp separation appeared in approximately 20 min.

Examples of HPLC separations are shown in Fig. 2. In the chromatograms, there are five characteristic peaks. Peak 1 is a trace component (<1%) of apolar nature, presumably representing products of decarboxylation. Peak 2 is a system peak generated by acetic acid freed from the column-packing by the separated carboxylates. Monocarboxylic fatty acid components are included in peak 3; its shape is mostly asymmetrical due to the presence of various isomers. Peak 4 contains all of the dicarboxylic components. After elution, tricarboxylic components together with other higher oligomers are displaced from the column by reversed elution, applying the back-flushing valve: thus, peak 5 contains all the remaining components.

For the quantitative evaluation of peak areas, correction by using relative detector responses should have been considered, but the non-availability of pure standards and the absence of appropriate n_D data in the literature[1] made such corrections impossible. Nevertheless, even without correction, the results coincided with the GLC analyses of commercial products: from this, one can deduce that the difference in n_D data between oligomers is small. On the other hand, the peak areas from independent and parallel UV detection, applied to several samples, showed a strongly increased response to dimer and trimer fractions, proving the presence of cyclic structures[1].

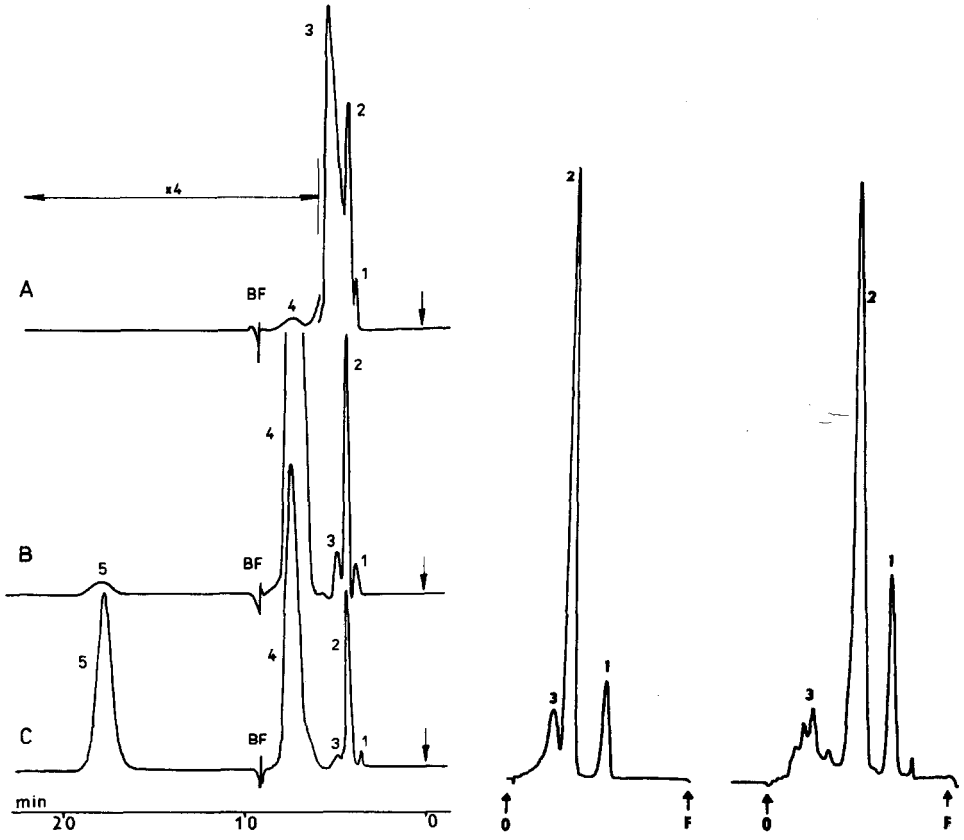

Fig. 2. HPLC separation of fractions isolated from the reaction mixture in fatty acid dimerization experiment. A, monomer fraction as first fraction from molecular distillation; B, dimer fraction as main product from molecular distillation; C, trimer fraction in distillation residue. Peaks: 1, decarboxylation products; 2, system peak; 3, monomers; 4, dimers; 5, trimers and other higher oligomers. BF = back-flush; ×4 = amplification degree of detection. HPLC analyses were performed applying original samples, *i.e.* in the form of free acids only.

Fig. 3. TLC–FID chromatogram of fatty acid dimer mixture on Chromarod S II using mobile phase B. O = Origin (starting point), F = elution front. Peaks: 1, monomers; 2, dimers; 3, trimers and higher oligomers.

Fig. 4. TLC–FID analysis of the same sample as in Fig. 3. Chromarod S II, first elution in mobile phase B (10 cm), second elution in mobile phase C (2.5 cm above the starting point); for description, see Fig. 3.

TLC separation with FID quantification

In optimizing the separating conditions, several mobile phase systems were tried. They were combined from various organic solvents (alkanes, benzene, chloroform, dichloromethane, diethyl ether, ethyl acetate and acetone) and formic or acetic acids. After numerous preliminary experiments in two independent laboratories, each laboratory decided to use its own mobile phase (A and B in Table II).

The main factor that complicated the resolution of the individual oligomer

classes is the presence of one or more free carboxylic groups affecting the shape of the particular adsorption isotherm. This is markedly evident in neutral solvent systems, where the separated zones are often broad, deformed and exhibit tailing toward the origin. In non-polar solvents, such as *n*-hexane, all components remain unresolved at the origin. Diethyl ether, ethyl acetate or acetone increase the R_F value of all oligomers, which migrate in one broad and deformed peak. Chromatography in benzene, dichloromethane or chloroform gives a group of fused peaks in the first third of the migration path along the Chromarod.

After the addition of organic acids to such systems, separation improves dramatically; the peaks are narrower, better resolved and their shape is almost Gaussian, all of which provides a better basis for quantification. Satisfactory separations can be achieved in mixtures of *n*-hexane (or *n*-pentane), diethyl ether and formic (or acetic) acid, as well as in systems based on chloroalkanes and acetic acid that contain small amounts of methanol. For example, chromatography in dichloromethane–methanol–acetic acid (mobile phase C in Table II) gave a similar separation to the more widely used *n*-hexane–diethyl ether–formic acid mixtures (mobile phase B in Table II). However, the latter systems are less hazardous, have higher flow-rates and seem to be preferable due to the lower noise during scanning of Chromarods in FID. Optimum elution strength of these types of solvent systems can be achieved by adjusting the ratio of diethyl ether and organic acid. For example, mobile phase A, containing 15% (v/v) diethyl ether and 1% (v/v) acetic acid, has a very similar resolving power to mobile phase B containing only 3% (v/v) diethyl ether and the same amount of formic acid. An elution mixture with a lower diethyl ether content and a higher acid content was found to be more sensitive to changes in composition, as a result of the loss of diethyl ether due to its high volatility and its preferential sorption in the lower region of the thin layer.

The trimer fraction, remaining close to the origin (Fig. 3), can be moved by the second elution in mobile phase C over a short migration distance (*ca.* 2 cm above the starting point, see Fig. 4). As can be seen, the zone of higher oligomers is split into approximately four fused peaks.

For quantitative evaluation, no correction factors were applied, presuming the relative responses of all components in FID to be equal due to the similar structure and combustible effective carbon atom number in the molecule. This approximation, although not quite correct[19,20], proved to be admissible, as demonstrated from the analysis of samples prepared by mixing commercial products of known composition (Table III).

The reproducibility of the TLC–FID analyses is markedly dependent on the amount of sample analysed (Table IV). Poor reproducibility in the range below 1 μg of substance spotted is a well-known problem in TLC–FID quantification[23,24], therefore, it is advantageous to operate with higher loads (50 μg or more) to obtain sufficient reproducibility for minor components.

Comparison of GLC, HPLC and TLC–FID analyses

The results of the collaborative testing of three mixed model samples are shown in Table V. The individual samples were prepared by mixing pure monomer fraction A and pure dimer fraction B, the composition of which was determined by means of all three methods independently. From these data, the compositions of the tested

TABLE III

TLC–FID ANALYSES OF SAMPLES OF KNOWN COMPOSITION (MODEL MIXTURES) ON CHROMAROD S II USING MOBILE PHASE B, SPOTTED AMOUNT 100 μg

Components for model mixtures	*M (%)*	*D (%)*	*T (%)*
a Distilled monomer fraction	93.0	2.0	5.0
b Distilled dimer fraction	3.9	93.4	2.7
c Purified trimer fraction from distillation residue	0.8	19.8	79.4

Sample	*Mass proportions of components in model mixtures*			*M (%)*		*D (%)*		*T (%)*	
	a	*b*	*c*	*Calc.*	*Found*	*Calc.*	*Found*	*Calc.*	*Found*
1	1	9	0	12.8	11.1	84.3	86.6	2.9	2.4
2	5	5	0	48.5	51.6	47.7	44.4	3.9	4.0
3	9	1	0	84.1	87.9	11.1	7.9	4.8	4.2
4	1	0	9	10.0	7.0	18.1	17.8	71.9	75.2
5	5	0	5	46.9	46.9	10.9	10.2	42.2	42.9
6	9	0	1	83.8	87.7	3.7	2.8	12.5	9.5
7	0	1	9	1.1	0.9	27.2	26.1	71.7	73.0
8	0	5	5	2.4	1.9	56.6	58.0	41.0	40.0
9	0	9	1	3.6	3.0	86.0	90.7	10.4	6.3
10	3	2	4	32.2	30.6	30.2	30.7	37.6	38.7

mixtures C, D and E were calculated separately for GLC, HPLC and TLC–FID in two laboratories.

It is apparent from the results given above that the best correlation between

TABLE IV

THE DEPENDENCE OF THE REPRODUCIBILITY OF TLC–FID ANALYSIS ON THE SPOTTED AMOUNT OF SAMPLE CHROMAROD S II, TWO-STEP ELUTION IN MOBILE PHASE D (10 cm) AND MOBILE PHASE C (2.5 cm)

Spotted amount (μm)	*M*		*D*		*T*	
	Mass (%)	*C.V. (%)*	*Mass (%)*	*C.V. (%)*	*Mass (%)*	*C.V. (%)*
25	49.4	4.8	50.6	5.3	—*	—
50	50.8	3.3	47.8	3.7	1.4	35.6
100	50.2	3.5	47.1	2.6	2.7	11.6
200	47.5	2.1	50.5	2.3	2.0	8.4
300	48.7	2.2	48.9	1.2	2.4	6.5
Calculated composition	49.4		48.5		2.1	

* Very low and broad peak that cannot be integrated.

TABLE V

COMPARISON OF THE GLC, HPLC AND TLC–FID ANALYSES OF FATTY ACID MONOMERS AND DIMER FRACTIONS AND THEIR MIXTURES

TLC–FID I: one-step elution in mobile phase A on Chromarod S II, Iatroscan TH-10 Mark III; TLC–FID II: one-step elution in mobile phase B on Chromarod S II, Iatroscan TH-10 Mark II. Average values are presented: for GLC from two determinations, for HPLC from 2–3 determinations and for TLC–FID from five determinations.

Sample		*Method*	*Found*					
			M (%)		*D (%)*		*T (%)*	
A	Pure unreacted monomer fraction isolated by distillation	GLC	90.9		9.1		–*	
		HPLC	95.6		3.8		0.6	
		TLC–FID I	95.5		0.6		3.9	
		TLC–FID II	96.0		1.5		2.5	
B	Pure dimer fraction isolated by distillation	GLC	7.4		92.6		–*	
		HPLC	3.5		95.6		0.9	
		TLC–FID I	3.1		93.1		3.8	
		TLC–FID II	2.8		95.6		1.6	
			M (%)		*D (%)*		*T (%)*	
			Calc.	*Found*	*Calc.*	*Found*	*Calc.*	*Found*
C	Mixture A:B (8:2)	GLC	74.2	76.8	25.8	23.2	–	–
		HPLC	77.2	77.9	22.2	21.8	0.6	0.2
		TLC–FID I	76.5	80.2	19.3	15.8	4.2	4.0
		TLC–FID II	77.4	79.1	20.3	19.2	2.3	2.3
D	Mixture A:B (5:5)	GLC	49.2	52.3	50.8	47.7	–	–
		HPLC	49.6	49.9	49.7	49.6	0.7	0.5
		TLC–FID I	48.9	51.5	47.0	45.4	4.1	3.2
		TLC–FID II	49.4	47.5	48.5	50.5	2.1	2.0
E	Mixture A:B (2:8)	GLC	24.1	21.0	75.9	79.0	–	–
		HPLC	21.9	22.0	77.2	77.4	0.6	0.8
		TLC–FID I	21.3	21.8	74.8	74.9	3.9	3.3
		TLC–FID II	21.4	20.3	76.8	77.4	1.8	2.3

* In GLC, trace trimers concentration cannot be determined.

calculated and experimental data was found using HPLC. There is sufficient agreement of results for monomers and dimers from all methods. For trimers of trace level, the GLC response was not sufficient to be exactly evaluated; a possible explanation may be the irreversible sorption or thermal decomposition of higher oligomers during analysis at high temperatures.

Higher results of TLC–FID for trimers, compared with HPLC can be explained by the influence of different retention principles in both procedures. In the given HPLC system, strongly specific interactions with carboxyl groups is decisive, whereas in the given TLC–FID system, adsorption is predominant without specific interactions of functional groups. Because of the existence of various structures in dimers and trimers[1,22] and the possible presence of trimers having two carboxyl groups only, the response for trimer components cannot be identical in both HPLC

and TLC–FID. Moreover, the trend for more efficient separation of dimer and trimer components in TLC–FID compared with simple HPLC separation according to carboxyl group number is evident also from Figs. 2 and 4.

It is interesting to note that the TLC–FID results are obtained in two independent laboratories. In spite of different elution systems, the differences in experimental results are relatively small. The higher content of trimers in elution system A suggests that the resolution between trimers and dimers is not identical in both systems.

Conclusions

Fatty acid dimers, containing a complicated mixture of isomeric oligomers, can be analysed by GLC, HPLC and TLC–FID. As a result, the contents of monomers, dimers and trimers can be evaluated. The best correlation between calculated and found composition was achieved by means of HPLC; however, for the monitoring of dimerization process control, TLC–FID appears to be most convenient method.

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