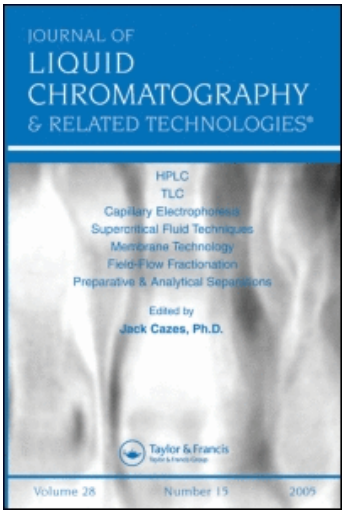

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Determination of the Mono and Diethanolamides of Palmitic Acid and of Soybean Oil Fatty Acids by High Performance Liquid Chromatography

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DETERMINATION OF THE MONO AND DIETHANOLAMIDES OF PALMITIC ACID AND OF SOYBEAN OIL FATTY ACIDS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

HPLC separations and quantitative analyses are described for palmitic and soya monoethanolamides and diethanolamides synthesized from free fatty acids, methyl esters and triglycerides. Both reverse phase and adsorption techniques were employed, using a differential refractometer for detection. Starting materials and crude reaction products are analyzed without treatment or preliminary separations. The methods described are simple and rapid and can be used to monitor the course of condensation reactions as well as final products compositions. In some instances separation of homologous fatty acid alkanolamides was achieved, enabling comparison of the yields obtained from individual fatty acid precursors.

INTRODUCTION

Direct determination of the content of surface active compounds in industrial products is very important. This simplicity saves tedious manipulations and time operation needed for isolation and purification of the components in the crude product. HPLC technique is an advance in the efforts to improve conventional methods.

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Fatty alkanolamides are a widely used class of nonionic surface active agents. Particularly certain fatty diethanolamides are popular as foam builders for alkylaryl sulfonates and condensation products of higher fatty acids with diethanolamine have become important in surface active systems (1). These products are found in formulations ranging from laundry detergents, industrial cleaners to high-quality shampoos (2). The diethanolamides obtained from coconut fatty acids and from lauric acid have been incorporated into commercial products which find greatest use in the textile and cosmetic fields (3).

The fatty alkanolamides are products of condensation between an alkanolamine and a fatty acid or its derivative (methyl ester, triglyceride). The synthesis path of a monoethanolamide can be described as follows:

a) $R'COOH + NH_2CH_2CH_2OH \longrightarrow R'CONHCH_2CH_2OH + H_2O$

b) $R'COOR'' + NH_2CH_2CH_2OH \longrightarrow R'CONHCH_2CH_2OH + R''OH$

R' - long hydrocarbon chain

R" - methyl or ethyl group

Condensation of a carboxylic acid with diethanolamine is not a simple process because of the presence of three functional groups on the diethanolamine (4). Examination of the products has indicated that, in addition to the expected alkanolamide, they contain amine-esters (5,6). Sometimes at high temperatures formation of tertiary amines is known to occur (4,7,8). A second process which leads to formation of N-ethanolamides is amidation of fatty esters (practically methyl ester) in the presence of sodium methoxide (9,10). In 1958 G.C. Tesoro reported a method of using the ester or glyceride of the fatty acid (11). This synthesis is based on the preparation of N-substituted aliphatic amides by heating the ethyl ester of the corresponding acid with the desired amine (12).

In this study we report a satisfactory separation and determination of ethanolamides by using the HPLC technique. Measurements conditions such as eluent composition and flow rate, column packing material were empirically determined for each type of reaction product.

EXPERIMENTAL

Materials

The chemicals were used without further purification. Tetrahydrofuran and acetonitrile (HPLC grade) were obtained from Bio-Lab Ltd. Laboratories, Jerusalem, Israel. N-hexane, 2-propanol and acetic acid (analytical grade) were purchased from Frutarom, Laboratory Chemicals Ltd., Haifa, Israel. Deionized water was twice distillated. The first distillation was carried out in the presence of potassium permanganate and followed by a simple distillation. Perchloric acid (analytical grade, Frutarom) was used to adjust the pH of the water to 2.6. Monoethanolamine, palmitic acid (E. Merck, Darmstadt, W. Germany) and sodium methoxide (Aldrich Chemical Co., Milwaukee, Wis., U.S.A.) were synthesis grade. Diethanolamine (B.D.H., Chemical Ltd., Poole, England) was technical grade. Methylated soybean oil and glyceryl tripalmitate were obtained from Shemen Co., Israel. Soybean oil purified was purchased commercially.

The reference compounds were purchased as follows: methyl palmitate (92-94%) and methyl stearate (92-94%) from Henkel KGaA, Düsseldorf, W. Germany; methyl oleate, methyl linoleate and methyl linolenate (all approx. 99%) from Sigma Chemicals Co., St. Louis Mo., U.S.A.

The examined products were prepared according to methods reported in the literature (2,3,4,10,11). When the starting material was glyceryl tripalmitate, methyl esters or triglycerides of soybean fatty acids - 0.3% W/W of sodium methoxide was used as catalyst.

HPLC Procedure

The qualitative and quantitative analyses were run on a Varian 5030 HPLC system. Differential Refractometer - R401, Waters Associates was used to detect the separated and eluted compounds. In the present research different columns were used in order to achieve good separation of the components in various products:

A) Reverse-phase type Varian Micro Pak MCH-10 steel column (30cm x 4mm) packed with a monomeric C_{18} (octadecyl) bonded onto 10μ silica gel. This column was used when the starting material was palmitic acid or glyceryl tripalmitate.

B) Reverse-phase type RP-18 steel column (30cm x 4mm) prepacked with 10 microns LiChrosorb, Cat. No. 9333 Merck, Darmstadt, W. Germany. This column was used for separation of ethanolamides prepared from methylated soybean oil.

C) Varian Micro Pak Si-5 (packed with 5μ silica) column (30cm x 4mm) was used in the case of soybean oil (mixture of triglycerides) as starting material.

The columns we operated at ambient temperature. Components were eluted isocratically at a pressure of about 115 psi. Optimum eluent compositions were arrived at experimentally, in consideration of the column type and nature of the substances determined. The examined samples were dissolved in eluent or THF (2-5% W/V) and 20 μl of the solution were injected via loop injector. In certain cases solution was achieved by gentle warming.

The detector was connected to a Hewlett-Packard 3390 A integrator to record peak areas, retention times and percentage compositions. The detector response was not calibrated for the different materials measured due to the lack of pure reference materials. The refractive indices of the eluents were close to 1.37, while those of fatty acids and their derivatives range from 1.44-1.47 (13). Replicate measurements showed that the

values for retention time and for percentage amount varied in a range of ± 2%.

Small peaks, representing less than 2% of the products, appeared in some of the chromatograms but were ignored for the purpose of this report. Free ethanolamine and diethanolamine eluted with the solvent front and were not determined.

In order to assure that the elution of the components is complete, for each series of HPLC measurements one or two samples were analyzed up to 30 minutes.

RESULTS AND DISCUSSION

HPLC Results

1. Palmityl Monoethanolamide

The content of this alkanolamide was determined in products obtained through two different ways:

1a. Reaction of palmitic acid with monoethanolamine (1:1.1 molar ratio) under the following conditions: 3 hours at 160°C.

1b. Reaction of glyceryl tripalmitate with monoethanolamine (1:3.3 molar ratio) under the following conditions: 2 hours at 100°C.

HPLC measurements conditions for 1a and 1b: for each case the reaction product was dissolved in tetrahydrofuran (5% W/V) and analyzed on column A. The components were eluted with tetrahydrofuran, acetonitrile and water (47.5:36.5:16 V/V). The corresponding results are presented in Tables I and II.

The results presented in Tables I and II show clearly that in the case of glyceryl tripalmitate a greater amount of amine-ester was obtained.

2. Palmityl Diethanolamide

The content of this alkanolamide was determined in products obtained by two different reactions:

TABLE I

HPLC Analysis Data for the Product of the Reaction Between Palmitic Acid and Monoethanolamine (a)

Component	Retention Time (min)	Amount %
Palmityl monoethanolamide	2.6	94.2
Palmitic acid	3.0	2.4
Amine-ester (possibly) (b)	6.9	3.4

(a) The flow rate of the eluent was 1.3 ml/min.

(b) Broad peak.

TABLE II

HPLC Analysis Data for the Product of the Reaction Between Glyceryl Tripalmitate and Monoethanolamine (a)

Component	Retention Time (min)	Amount %
Palmityl monoethanolamide	2.6	81.4
Palmitic acid	3.0	2.5
Amine-ester	6.4	16.0
Glyceryl mono(di)palmitate	7.0	traces

(a) The flow rate of the eluent was programmed: 1.3 ml/min for two minutes then increased at a rate of 0.1 ml/min for 28 minutes.

2a. Reaction of palmitic acid with diethanolamine (1:1.1 molar ratio) under the conditions: 3 hours at 160°C.

2b. Reaction of glyceryl tripalmitate with diethanolamine (1:3.3 molar ratio) under the conditions: 3 hours at 145°C.

The products were examined under the following conditions: the crude product was dissolved in tetrahydrofuran (5% W/V) and separated on column A. The components were eluted with tetrahydrofuran, acetonitrile and water (47.5:36.5:16 V/V). The results are presented in Tables III and IV (see also Figure 1).

3. In the present work HPLC measurements were also carried out for separation and determination of monoethanolamides or diethanolamides prepared from methylated soybean oil (a mixture of the methyl esters of soybean fatty acids). The principal soybean fatty acids are: linoleic, oleic, palmitic, linolenic and stearic. The chromatograms obtained enabled to calculate the ethanolamides content in the crude reaction product. For information and comparison of the results the starting material (methylated soybean oil) was analyzed by HPLC (Table V). The products of the following reactions were examined:

3a. Reaction of methylated soybean oil with monoethanolamine under the conditions: one hour at 95°C.

3b. Reaction of methylated soybean oil with diethanolamine under the conditions: three hours at 130°C.

The HPLC conditions were: the examined product was without further treatment dissolved in the eluent and separated on column B. The components were eluted with tetrahydrofuran, acetonitrile and water (43:43:14 V/V). The results are presented in Tables V, VI (see Figure 2) and VII.

The results obtained show that in addition to the corresponding ethanolamides, the examined products contain also amounts of the starting material and of amine-esters. It may be concluded that the monoethanolamides were obtained in a higher yield than the corresponding diethanolamides.

TABLE III

HPLC Analysis Data for the Product of the Reaction Between Palmitic Acid and Diethanolamine (a)

Component	Retention Time (min)	Amount %
Palmityl diethanolamide	5.2	60.4
Palmitic acid	6.4	1.6
Amine-ester	11.6	32.0
Undefined (b)	13.6	2.0

(a) The flow rate of the eluent was 0.6 ml/min increased to 1.0 ml/min during 15 minutes.

(b) Broad peak.

TABLE IV

HPLC Analysis Data for the Product of the Reaction Between Glyceryl Tripalmitate and Diethanolamine (a)

Component	Retention Time (min)	Amount %
Palmityl diethanolamide	2.4	63.1
Palmitic acid	2.9	2.3
Amine-esters	5.7	25.0
Glyceryl mono(di)palmitate	6.8	2.4

(a) The flow rate of the eluent was programmed: 1.4 ml/min for two minutes increased to 3.0 ml/min during additional 18 minutes and constant flow of 3.0 ml/min for further 10 minutes.

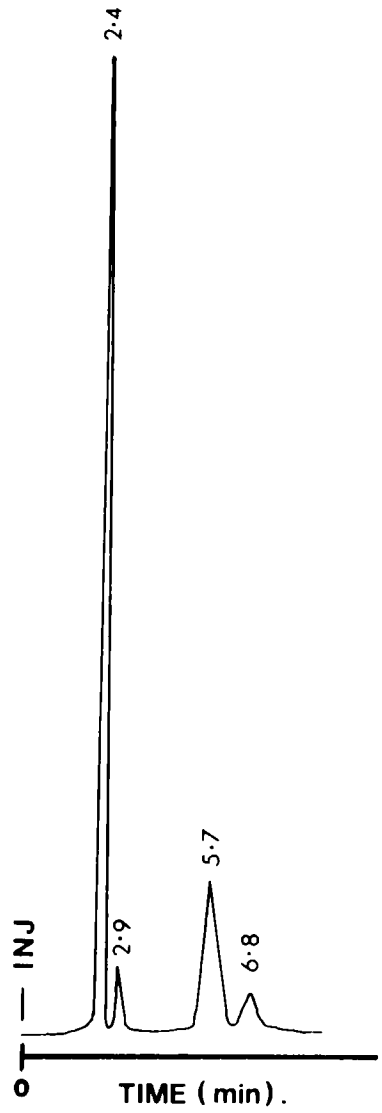

FIGURE 1: Reversed-phase HPLC chromatogram of the product of the reaction between glyceryl tripalmitate and diethanolamine (see Table IV).

TABLE V

HPLC Analysis of Methylated Soybean Oil (a)

Component	Retention Time (min)	Amount %
Methyl linolenate	6.5	8.5
Methyl linoleate	7.4	56.5
Methyl palmitate Methyl oleate	9.0	31.0
Methyl stearate	11.7	3.0

(a) The flow rate of the eluent was: 0.6 ml/min increased to 1.0 ml/min in the first 15 minutes and 1 ml/min for further 5 minutes.

TABLE VI

HPLC Analysis Data for the Product of the Reaction Between Methylated Soybean Oil and Monoethanolamine (a)

Component	Retention Time (min)	Amount %
Linolenyl monoethanolamide	4.2	8.0
Linoleyl monoethanolamide	4.5	55.7
Palmityl monoethanolamide Oleyl monoethanolamide	5.1	28.4
Stearyl monoethanolamide	5.9	6.4
Methyl esters of soybean fatty acids	7.2-8.9	1.4

(a) The flow rate of the eluent was 0.7 ml/min.

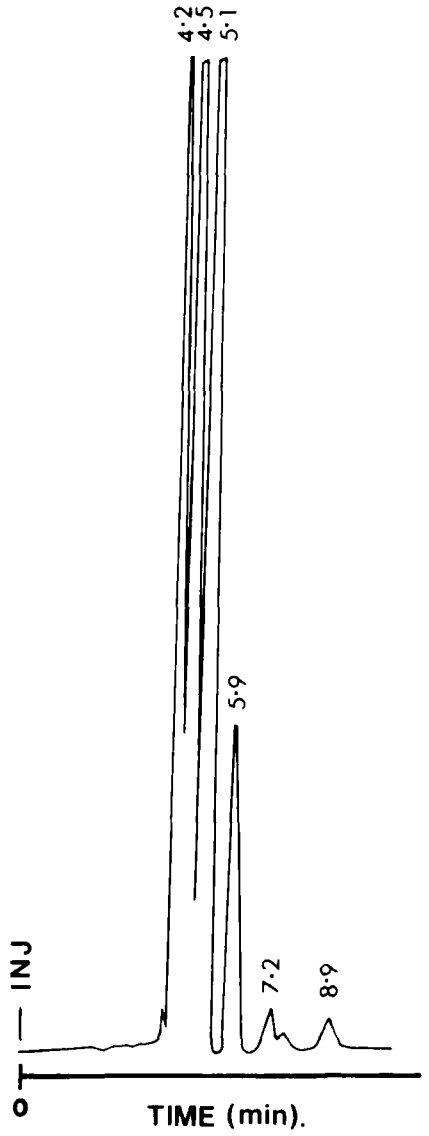

FIGURE 2: Reversed-phase HPLC chromatogram of the product of the reaction between methylated soybean oil and monoethanolamine (see Table VI).

TABLE VII

HPLC Analysis Data for the Product of the Reaction Between Methylated Soybean Oil and Diethanolamine (a)

Component	Retention Time (min)	Amount %
Linolenyl diethanolamide	4.4	5.0
Linoleyl diethanolamide	4.8	45.4
Palmityl diethanolamide Oleyl diethanolamide	5.3	21.0
Stearyl diethanolamide	6.2	5.0
Methyl esters of soybean acids	7.0-10.0	16.0
Amine-esters	13.2-16.6	4.0

(a) The flow rate of the eluent was programmed: 0.6-1.0 ml/min for 15 minutes followed by a constant rate of 1 ml/min for additional 5 minutes.

The mono- and-diethanolamides elute, as expected, in the same relative order as the corresponding methyl esters but at lower retention volumes, due to their greater polarities. Confirmation of the identities of the ethanolamide peaks can be found in their relative abundances compared with the starting methyl esters. These are equal ($\pm$ 2%) for the methyl esters, mono- and di-ethanolamides, implying incidentally similar reactivities for the various fatty methyl esters with ethanolamines.

4. Additional application of HPLC in the present work was the determination of mono or diethanolamides derived from purified soybean oil. This oil is a mixture of the triglycerides of the corresponding fatty acids (above mentioned).

TABLE VIII

HPLC Analysis Data for the Product of the Reaction Between Soybean Oil and Monoethanolamine (a)

Component (group)	Retention Time (min)	Amount %
Triglycerides	3.4	0.4
Diglycerides	3.8-4.3	2.4
Amine-esters	4.8	4.1
Monoglycerides	6.9	1.7
Monoethanolamides (b)	8.4	91.1

(a) The eluent composition was: n-hexane, isopropanol and acetic acid (80:20:1 V/V).

(b) Overlapping resulted in a broad peak.

TABLE IX

HPLC Analysis Data for the Product of the Reaction Between Soybean Oil and Diethanolamine (a)

Component (group)	Retention Time (min)	Amount %
Triglycerides	3.4	0.4
Diglycerides	3.7-3.8	2.9
Fatty acids	4.3	2.9
Amine-esters	5.2	22.2
Monoglycerides	6.1	4.3
Undetermined	7.4	1.8
Diethanolamides (b)	10.8	65.4

(a) The eluent composition was: n-hexane, isopropanol and acetic acid (75:25:1 V/V).

(b) Overlapping resulted in a broad peak.

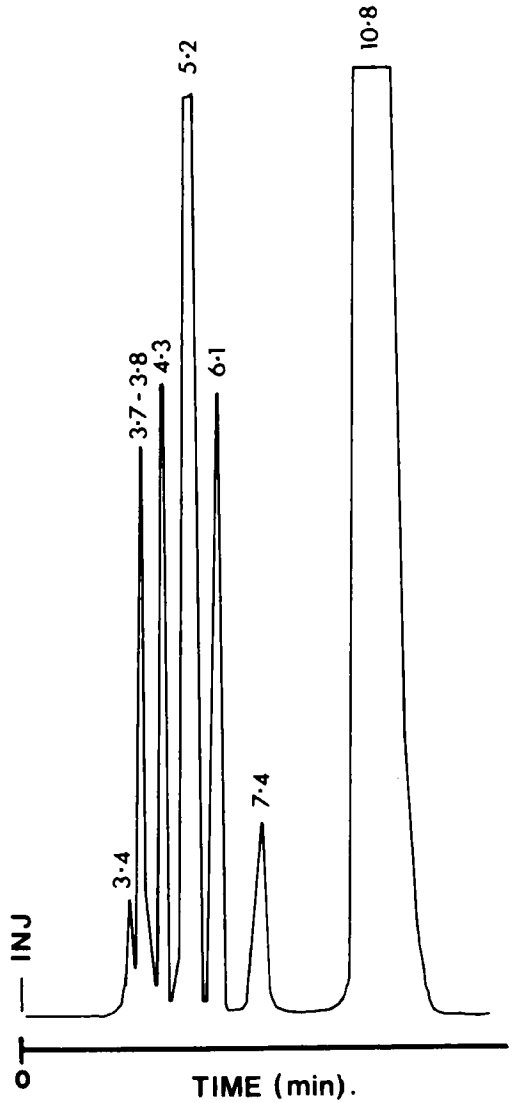

FIGURE 3: HPLC chromatogram of the product of the reaction between soybean oil and diethanolamine (see Table IX).

The products were analyzed without further treatment. The chromatograms obtained showed that in addition to the corresponding ethanolamides partially hydrolyzed glycerides and amine-esters were detected. Under the present reported HPLC conditions broad (overlapping) peaks were obtained for each group of components and integration of the total area was performed.

The products of the following reactions were analyzed:

4a. Reaction of soybean oil with monoethanolamine at 108°C for a period of two hours.

4b. Reaction of soybean oil with diethanolamine at 145°C for a period of three hours.

HPLC Determinations

In each case the crude product was dissolved in the eluent and separated on column C. The flow rate was 0.8 ml/min. The ethanolamides were eluted last without separation and their total amount was recorded. The results are presented in Tables VIII and IX (see also Figure 3).

Commercial soybean oil analyzed under these conditions contained 96.8% triglycerides (eluted as one peak) and 3.2% diglycerides.

SUMMARY

HPLC conditions are described for the analysis of fatty acid mono-and-diethanolamides and associated impurities and by-products encountered in typical syntheses. Separations and quantitation were achieved for the homologous fatty acid methyl esters as well as the corresponding ethanolamides. These methods afford simple and rapid analyses for these complex mixtures and can be used to follow the course of synthesis and to characterize commercial products.

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REFERENCES

1. Schwartz, A.M. and Perry, J.W., Surface Active Agents, Interscience Publishing Company, N.Y., 1949, p. 212.

2. Monick, J.A., JAOCS, 39, 213, 1962.

3. Kroll, H. and Nadeau, H., JAOCS, 34, 323, 1957.

4. Kritchevsky, W. (Ninol Laboratories), US Patent 2,089,212 (1937).

5. Masci, J.N. and Poirer, N.A. (to Johnson & Johnson), US Patent 2,680,753 (June 8, 1954).

6. Trowbridge, J.R., Falk, R.A. and Krems, I.J., J. Org. Chem., 20, 990, 1955.

7. Malkemus, J.D. (to Colgate-Palmolive-Peet Co.), US Patent 2,636,033 (April 21, 1953).

8. Bauer, E., US Patent 2,575,041 (November 13, 1951).

9. Meade, E.M. (Lankro Chemicals Ltd.), British Patent 631,637 (1949).

10. Meade, E.M. (Lankro Chemicals Ltd.), US Patent 2,464,094 (1949).

11. Tesoro, G.C. and Ferry, D. (to Onyx Oil & Chemical Co.), US Patent 2,844,609 (July 1958).

12. D'Alelio, G.F. and Reid, E.E., JACS, 59, 111, 1937.

13. Rosen, M.J. and Goldsmith, H.A., Systematic Analysis of Surface-Active Agents, second edition, Wiley-Interscience, N.Y., 1972, p. 531-539.