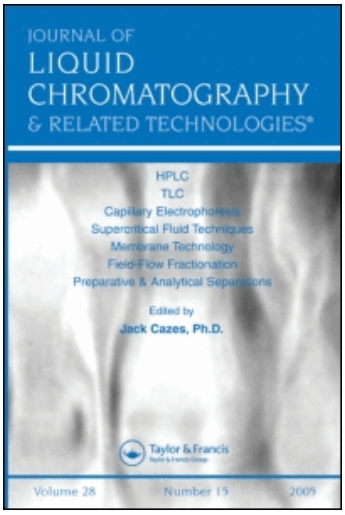

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Albert A. Ben-bassat[a]; Tamar Wasserman[a]
[a] Israel Fiber Institute, Jerusalem, Israel

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SEPARATION AND DETERMINATION OF MONOALKANOLAMIDES OF SOYBEAN OIL FATTY ACIDS BY RP-HPLC OF THE CRUDE REACTION PRODUCT

Albert A. Ben-Bassat* and Tamar Wasserman
Israel Fiber Institute
P. O. Box 8001
Jerusalem 91 080, Israel

ABSTRACT

A reverse-phase HPLC technique, previously developed by us, was used to achieve satisfactory separation and determination of monoalkanolamides of the soybean oil fatty acids. Four reaction products, each containing a mixture of similar monoalkanolamides of the soybean fatty acids, were analyzed without pretreatment. Structurally, the four monoalkanolamides series differ in their alkanol moiety, having two or three carbon atoms in the alkanol chain which is substituted or unsubstituted. A series of N-methyl monoethanolamides was also prepared and HPLC analyzed. In each case, the separated components were isocratically eluted with a ternary solvent system of tetrahydrofuran-acetonitrile-water at pH 2.6 (37.5:37.5:25 v/v/v), followed by detection with a differential refractometer. In all cases, the elution order was in accordance with the length as well as with the degree of unsaturation of the hydrophobic chain of the monoalkanolamides. The sequence of the eluted components was elucidated by chromatographing, under the same conditions, of the corresponding commercially purchased pure fatty acid methyl esters. As control compounds, the corresponding stearoyl monoalkanolamides, prepared in our laboratory, were individually chromatographed. The HPLC results showed that, in addition to the expected monoalkanolamides, unreacted methyl esters and sometimes amine-esters were separated and detected. For each alkanolamine used, the amidation reaction was discussed.

INTRODUCTION

A great number of fatty alkanolamides exhibit the properties of nonionic surfactants.Some are applied as foam boosters and stabilizers in liquid detergents. Alkanolamides from higher fatty acids are found in the formulation of various commercial products: household detergents, high quality shampoos, industrial cleaners, wetting and thickening agents for textile processes.

The fatty alkanolamides are products of condensation between an alkanolamine and a fatty acid or its derivative (methyl ester, triglyceride). Generally, this amidation reaction is complicated by the competitive reactivity of the hydroxyl group of the alkanolamine. The reaction with a monoalkanolamine is simpler compared to that with a dialkanolamine. The composition of the reaction product depends also on the molar ratio of the reactants and on the reaction conditions employed. This explains the absence of a standard method for determination of any type of alkanolamide. In most cases, the raw materials used in the industry of nonionic surfactants are composite, and then the reaction product is a mixture of the corresponding main derivatives, unreacted starting compounds and sometimes by-products. Many investigators have attempted to find suitable methods for isolation and determination of nonionic surfactants (1-3). The analytical efforts included: thin layer chromatography (4-7) and gas-liquid chromatography (8-13). The elution of alkanolamides by gas chromatography requires their conversion into volatile derivatives before analysis. Direct determination of the composition of complex reaction product is of great importance, since it saves tedious manipulations and time performance needed in cases of systematic analytical methods (14,15,16). The advantages of HPLC techniques are their simplicity and the mild conditions which assure direct determination of the product composition without structural changes. Homologous series of several fatty mono-and-diethanolamides

(17), as well as of fatty monoisopropanolamides (18) have been separated by HPLC. Recently, Nakamura (19) reported on a HPLC technique for separation of certain nonionic, cationic and anionic surfactants.

The present work is an extension and further application of previously reported HPLC technique which enabled us to achieve a satisfactory separation and determination of the ethanolamides of soybean oil fatty acids (20) and quantitative monitoring of stearoyl monoalkanolamides synthesis (21).

We are reporting the HPLC analysis of four structurally different series of monoalkanolamides of soybean oil fatty acids. In each case, the monoalkanolamides were directly determined in the crude reaction product. The chromatographed products were prepared by aminolysis of methanolyzed soybean oil (a mixture of fatty methyl esters, Henkel) with different monoalkanolamines under mild conditions. Recently, we have introduced certain improvements (21) to previously reported preparation methods (22-25). The HPLC conditions such as column packing material, eluent composition and flow rate were empirically determined. The HPLC data showed that, in certain cases, the monoalkanolamides with the same alkanol moiety were obtained in proportion almost to that of the corresponding methyl esters precursors.

EXPERIMENTAL

Materials

The chemicals were used as purchased. Tetrahydrofuran and acetonitrile (HPLC grade) were obtained from Bio-Lab., Ltd., Jerusalem, Israel. For preparation of the eluent, deionized water was freshly distillated twice, first in the presence of potassium permanganate. Perchloric acid (Merck, Darmstadt, W. Germany) was used to adjust the pH of the water to 2.6. Methanolyzed soybean oil was obtained from Henkel, KGaA, Dusseldorf, W. Germany. Sodium

methylate (purified) was obtained from Fisher Scientific Company, New Jersey, U.S.A. Fatty acid methyl esters, used as control standards, were purchased as follows: methyl palmitate, methyl stearate (approx. 98% purity) from Henkel, KGaA, Dusseldorf, W. Germany; methyl myristate, methyl oleate, methyl linoleate and methyl linolenate (99%) from Sigma Chemicals Co., St. Louis, Mo., U.S.A. The alkanolamines used, 1-Amino-2-propanol, 3-Amino-1-propanol, 2-(Methylamino) ethanol and 2-Amino-2-methyl-1-propanol were over 99% pure and obtained from Aldrich Chemical Company, Milwaukee, Wisc., U.S.A. The four stearoyl monoalkanolamides, chromatographed individually for comparison with the chromatographic properties of the corresponding stearoyl component in the analyzed products, were prepared and purified in our laboratory (21).

Instrumentation

Ir spectra were recorded with a Perkin-Elmer 257 Grating Infrared Spectrophotometer using a thin layer of the tested material or film from its chloroform solution. The HPLC analyses were run on a Varian 5030 system. A reverse-phase type RP8 Merck stainless steel column (115 mm x 4 mm I.D.) packed with Ultrasphere Octyl F (10 microns) was used. The column was operated at ambient temperature. Differential Refractometer-R401 (Waters Associates) was used to detect the separated and eluted compounds. Retention times, peak areas and percentage amounts of the components were recorded with a Hewlett-Packard 3390A integrator.

HPLC Procedure

The crude reaction products were analyzed without pretreatment. The samples were dissolved in the eluent (3-4% w/v) and 20µl of the solution were injected via a loop injector. The separated compounds were eluted isocratically at a pressure of about 160 psis, using an eluent composition of tetrahydrofuran-acetonitrile-water at pH 2.6 (37.5:37.5:25v/v/v). The

eluent flow rate was 1.0 ml/min. In order to assure a complete elution of the separated compounds, the HPLC measurements were carried out up to 30 minutes.

Preparation of the Soybean Oil Fatty Acid Monoalkanolamides.

Four reaction products, each containing a mixture of saturated and unsaturated fatty acid monoalkanolamides, were prepared. Moderate conditions were chosen. The assembly used for performance of the aminolysis process was as reported (21).

0.5% (w/total weight of the reactants) of pure and dry sodium methylate were introduced in the reaction vessel, followed by 0.33 mole of the corresponding alkanolamine. Gradual heating and constant stirring were applied to obtain a clear solution (approx. 70°C). Then 0.3 mole (average value) of soybean oil fatty acid methyl esters were added. Passing a constant flow of nitrogen through the reaction mixture, the temperature was elevated to 90°C and the reaction continued isothermally up to 2 hours. The nitrogen served to prevent oxidative changes in the unsaturated fatty acid chains, as well as to expel the methanol co-product. Finally, the temperature was decreased to 80°C and, with vigorous stirring, 1.5 ml of distilled water was added. The viscous product was transferred to a bottle for storage.

RESULTS AND DISCUSSION

The four amidation products were directly analyzed by reverse-phase HPLC. Each product contained monoalkanolamides of the soybean oil fatty acids, the corresponding unreacted starting methyl esters and sometimes a variable amount of amine-esters as by-products. The series of the monoalkanolamides were as follows:

SMA - $R'CONHCH_2CH(OH)CH_3$

SMB - $R'CONHCH_2CH_2CH_2OH$

SMC - $R'CON(CH_3)CH_2CH_2OH$

SMD - $R'CONHC(CH_3,CH_2OH)CH_3$

R' = long hydrocarbon chains: Myristyl, linolenyl, linoleyl, oleyl, palmityl, stearyl.

HPLC Study

The starting material, methanolyzed soybean oil, was chromatographed under the reported conditions and the elution order of the methyl esters as well as their percentage amounts were determined (Figure 1). For comparison, the corresponding pure (over 99%) methyl esters, purchased com-

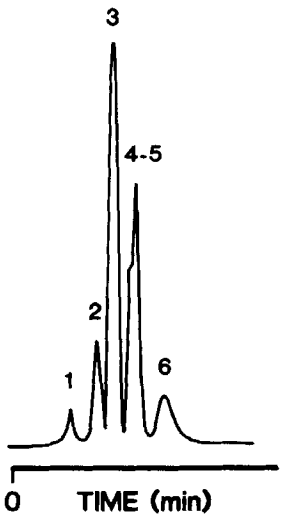

FIGURE 1: Reversed phase HPLC chromatogram of the methanolyzed soybean oil used as starting material. Peaks: 1 = methyl myristate (1.1%); 2 = methyl linolenate (10.5%); 3 = methyl linoleate (50%); 4 - 5 = methyl palmitate and methyl oleate (34.4%); 6 = methyl stearate (4%).

mercially, were individually chromatographed and the retention time determined. The HPLC analysis of each reaction product was performed in duplicate and, for all measurements, an average deviation of 2% for the values of percentage amount of the components was obtained. For each type of monoalkanolamides, the corresponding stearoyl monoalkanolamide, synthesized and purified in our laboratory (21), was individually chromatographed and its retention time was in accordance to that of the stearoyl component in the crude reaction product (Table I).

1. HPLC Analysis of Product SMA

The HPLC data showed the existence of the corresponding monoalkanolamides of the following fatty acids (Figure 2): myristic (0.6%), linolenic (5.2%), linoleic (35.9%), palmitic and oleic (26.5%) and stearic (4.0%). The retention

TABLE I

The Retention Times of the Control and of the Determined Stearoyl Monoalkanolamide for Four Reaction Products

Stearoyl Monoalkanolamide	Retention Time (min)(a) Reaction Products: (b)			
	SMA	SMB	SMC	SMD
Control (synthesized and purified)	3.73	3.94	3.98	4.12
Determined in the crude reaction product	3.80	3.95	4.00	4.15

(a) Average value
(b) General formulas of the four series of monoalkanolamides:

SMA - $R'CONHCH_2CH(OH)CH_3$

SMB - $R'CONHCH_2CH_2CH_2OH$

SMC - $R'CON(CH_3)CH_2CH_2OH$

SMD - $R'CONHC(CH_3,CH_2OH)CH_3$

R' = fatty hydrocarbon chains: myristyl, linolenyl, linoleyl, oleyl, palmityl, stearyl.

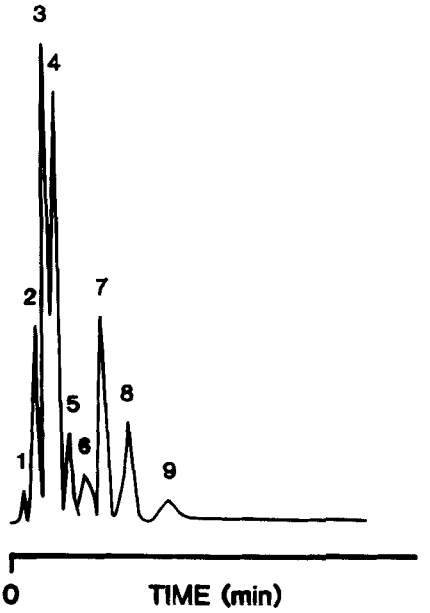

FIGURE 2: Reversed phase HPLC chromatogram of the product SMA. Peaks: The corresponding monoalkanolamides $R'CONHCH_2CH(OH)CH_3$: 1 = myristoyl; 2 = linolenoyl; 3 = linoleoyl; 4 = palmitoyl and oleoyl; 5 = stearoyl; (6 - 9) Unreacted starting fatty acid methyl esters.

TABLE II

Total Percentage Amount of the Monoalkanolamides Determined During the Preparation of Products SMA, SMB, SMC and SMD (a)

Reaction Time(min)	Total Monoalkanolamides Amount (b) %			
	SMA	SMB	SMC	SMD
30	63.0	68.5	70.7	43.6
60	68.0	70.0	72.9	46.0
90	71.0	69.5	74.5	46.9
120	72.2	68.6	77.0	48.0

(a) See footnote (b) of Table I.

(b) Average value.

time of the determined and of the control (specially prepared) stearoyl-NH(2-hydroxy-propyl) amides were in accordance: 3.80 min and 3.73 min, respectively (Table I). Table II represents the total percentage amount of the determined monoalkanolamides at various reaction times. Unreacted starting fatty acid methyl esters were also detected (27.8%) in the final crude product. No by-products, such as amine-esters, were obtained.

2. HPLC Analysis of Product SMB

Following the progress of the aminolysis reaction, it was found that the total percentage amount of the corresponding monoalkanolamides (see footnote in Table I) increased for the first 60 minutes and afterwards slightly decreased up to 120 minutes (Table II). According the the possible rearrangements of the fatty alkanolamides (26), this decrease was probably due to the formation of amine-esters (7% in the final crude product). In the present case, the hydroxyl group of the used amine is not steric hindered. The HPLC data for products SMA and SMB showed that the aminolysis with 3-amino-1-propanol was more rapid than that with 1-amino-2-propanol. Figure 3 represents the HPLC chromatogram of product SMB.

3. HPLC Analysis of Product SMC

The HPLC chromatogram of the final crude product exhibited a composition of the corresponding monoalkanolamides (77%), unreacted starting methyl esters (15.4%) and amine-esters as by-products (7.6%). The chromatograms of the samples withdrawn at definite reaction times showed a higher yield of the monoalkanolamides compared to that of the monoalkanolamides corresponding to products SMA and SMB (Table II). This can be explained by the higher reactivity of 2-(Methylamino)ethanol as amidation reagent.

4. HPLC Analysis of Product SMD

According to the HPLC data, the corresponding fatty monoalkanolamides were obtained, under the same preparation conditions, in moderate yields (Table II).

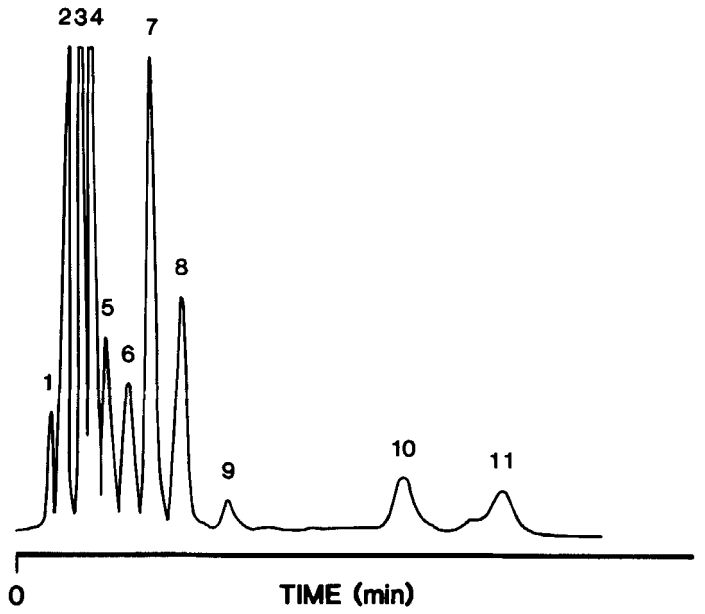

FIGURE 3: Reversed phase HPLC chromatogram of the product SMB. Peaks: The corresponding monoalkanolamides $R'CONHCH_2CH_2CH_2OH$: 1 = myristoyl; 2 = linolenoyl; 3 = linoleoyl; 4 = palmitoyl and oleoyl; 5 = stearoyl; (6-9) Unreacted starting fatty acid methyl esters; (10 - 11) Amine-esters.

The relatively low rate of the amidation reaction is probably due to steric hindrance on the reactivity of the amino group of the alkanolamine, or to an intramolecular hydrogen bond between the hydroxyl group and the amino nitrogen. This is supported by the relatively high amount of unreacted starting methyl esters (49.5%) and the very low yield of amine-esters, by-products of the competitive esterification process (2.5%).

SUMMARY

HPLC conditions are described for the separation and quantitation of soybean fatty acid monoalkanolamides in crude products prepared by aminolysis of

methanolyzed soybean oil with structurally different alkanolamines. Unreacted starting methyl esters and sometimes amine-esters, as by-products, were separated and detected. The developed method affords simple and rapid analyses for complex mixtures and enables the following of the course of synthesis of monoalkanolamides in commercial products.

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