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Technical

⁂ Analysis of Sorbitan Fatty Acid Esters by HPLC

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ABSTRACT

Sorbitan esters of several fatty acids have been analyzed by high pressure liquid chromatography (HPLC) using an RP-18 column. No derivatization was necessary. Mono-, di- and trisorbitan esters of palmitic, stearic, oleic, isostearic and sesquioleic acid have been separated using isopropanol/water as the elution mixture.

INTRODUCTION

The need for analysis of food emulsifiers arises at several stages, e.g., for production control, for comparison of emulsifiers purchased from different suppliers, and for the detection of the type of emulsifiers used in a commercial good product. Authorities may also require analyses to meet health regulations. Since most food emulsifiers are complex mixtures of several isomers and derivatives, it is quite tedious to analyze their composition (1).

Several attempts have been reported on the separation, identification and quantitative estimation of the mono-, di- and trifatty acid esters of sorbitol and of its anhydrides (2–5). Most techniques are based on pretreatment (hydrolysis) of the emulsifiers and use of gas liquid chromatography (GLC) or high pressure liquid chromatography (HPLC) as the essential quantitative method for analysis (6).

HPLC is one of the most promising techniques for the analytical characterization of these mixtures. Aitzetmüller (7) was the first to elute sorbitan esters by high pressure liquid chromatography in order to demonstrate how broad a range of applications may be covered by this approach. Fingerprint chromatograms of technical sorbitan mono- and tristearate were obtained using a silica gel column and a complex mixture of solvents. No identification of the peaks was done. Brüschweiler (8) used a μ-porasil column, an ultraviolet (UV) 220 nm detector and a mixture of isooctane/isopropanol (40:60) in order to elute the sorbitan esters. The separation was far from sufficient and no attempt to identify the peaks was made.

The following report is a continuation of previous work carried out on other food emulsifiers (9, 10), demonstrating different approaches for separating and analyzing the sorbitan esters using an RP-18 column, with isopropanol/water as an eluent and a UV detector.

EXPERIMENTAL

The emulsifiers were all commercial products purchased from several sources: Atlas Europol A.p.S. (Spans); Croda Chemicals, England (Crills); Polibasicos S.A., Mexico (Sorbac); Adumin Chemicals, Israel (Adochan); and Hamorad, Israel. The emulsifiers were sorbitan esters of palmitic, stearic, oleic, isostearic and sesquioleic acids. The eluents were isopropanol (A.R.) obtained from E. Merck, Darmstadt, West Germany, and triple distilled water.

The analyses were performed on a Spectra Physics model SP-8000 HPLC chromatograph equipped with an SP770 variable wavelength UV detector, operated at 220 nm. The separations were achieved on 25 cm × 4.6 mm id stainless steel columns prepacked with 10 μm Lichrosorb RP-18 purchased from Alltech Associates, Inc.

Sorbitan monopalmitate, monostearate, tristearate and monoisostearate were eluted with a mobile phase composed of 88% isopropanol and 12% water. Sorbitan monooleate, trioleate and sesquioleate were eluted with 85% isopropanol and 15% water. The samples were dissolved in isopropanol (up to 5% w/w) and 10 μL of solution were injected by automatic loop injector.

The percentage of the mono-, di- and triesters were calculated by weighing the area under the peaks. The response factor calculated according to injections of mono-, di- and triglycerides of stearic acid (99% from Sigma Chemicals) under the same conditions as for sorbitan esters were found to be 0.75 for mono-, 0.91 for di-, and 1.00 for triglyceride. We assume that the response factors for sorbitan esters are not far from those of glycerol esters. Therefore most calculations were done by using these response factors.

In a test experiment, pure stearic acid (99% by GLC from Sigma Chemicals) was esterified with 70 wt % aqueous sorbitol, heat stable (from Roquette Freres, Neosorb 70-02 S) and the crude product eluted through a silica column. Fractions were collected and injected to the HPLC column (1).

RESULTS AND DISCUSSION

Several commercially available sorbitan esters of fatty acids have been injected into an RP-18 column using various combinations of eluents based on isopropanol and water. Figure 1 demonstrates some of the typical separations obtained for commercial so-called "sorbitan monooleate" (Span 80). It can be seen that by decreasing the isopropanol proportions in the eluent mixture, better separation can be obtained up to an eluent mixture of 85:15 isopropanol/water in which three groups of peaks can be identified. The

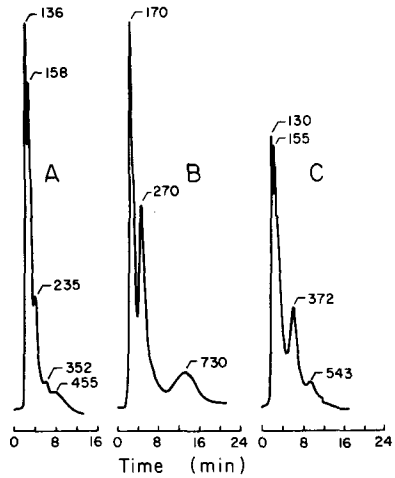

FIG. 1. Fingerprint chromatograms of commercial sorbitan monooleate (Span 80) eluted on RP-18 column using UV detector (220 nm) and isopropanol/water mixtures as eluent. (A) Isopropanol/water (92:8): sorbitan monooleate at 136 sec, sorbitan dioleate at 158 sec, sorbitan trioleate at 235 sec. (B) Isopropanol/water (85:15): sorbitan monooleate at 170 sec, sorbitan dioleate at 270 sec, sorbitan trioleate at 730 sec. (C) Isopropanol/water (80:20): sorbitan monooleate isomers at 130 and 155 sec, sorbitan dioleate at 372 and 543 sec.

monooleate esters are the first to be eluted, followed by the dioleate esters. The tri- or polyoleate esters are eluted last as broad unresolved peaks. When the eluent mixture is of 92:8 isopropanol/water, there is only slight separation between the mono-, di- and triester peaks. Two more unidentified peaks appear at 352 and 455 sec, most probably due to the higher esters of the sorbitan. When the percentage of water is higher than 15%, the mono- and diesters appear in several peaks due to the different isomers. The trioleate is not eluted under these conditions.

In order to obtain enriched fractions of each of the isomers and mainly, as pure as possible, sorbitan monostearate (rather than the di- or trihomologs of the sorbitan esters), a controlled reaction between pure (99%) stearic acid (Sigma Chemicals) and sorbitol 70 wt % was carried out. The esterification was carefully controlled. The crude product was eluted through a silica gel liquid cholumn (1) and fractions were collected and reinjected to the HPLC column. Figure 2 demonstrates the differences

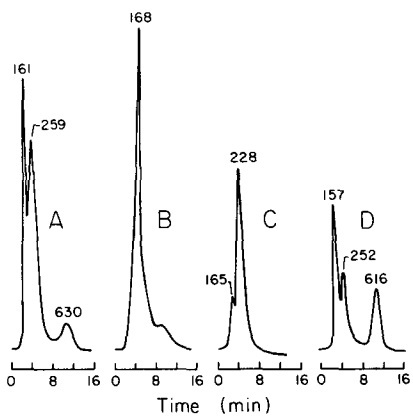

FIG. 2. Sorbitan monostearate prepared from pure stearic acid: (A) crude product, (B) pure monoisomer of "sorbitan monostearate" from column fractionation, (C) pure diisomer, (D) enriched mixture of the triisomers.

between the crude product and the collected fractions. Using the above method allowed the collection of almost pure monoisomer (Fig. 2B) and pure diisomer (Fig. 2C). The triisomers were collected in an enriched mixture with the mono- and the diisomers. Verification and identification of the collected isomers was accomplished using GLC techniques (1).

Figure 3 demonstrates some of the differences found between three commercial products available in the market. Since these are food grade materials, the restrictions of their properties are quite severe and only small variations on their composition could be detected. Table I summarizes the specifications of the sorbitan esters and product distribution found by the HPLC technique. It should be noted that since the detector is based on UV and no standard pure isomers are available, no quantitative accurate composition analysis could be made. Yet, using the response factors of glycerol fatty acid esters in this case allowed us to calculate, with some degree of accuracy, the product distribution in our products. Table I is entitled therefore "Proposed product distribution values for mono-, di- and trisorbitan esters of fatty acids," however, it is clear that none of the products contain more than 56% monoester fatty acids even though the product is claimed to be monoester and known as "sorbitan monooleate". Up to 25% of tri- and higher fatty acid isomers were detected in the commercial products (sorbitan sesquioleate). None of the commercial products contained less than 9% of sorbitan triesters.

When commercial so-called "sorbitan trioleate" was injected under similar conditions (Fig. 4B), a significant increase in the high isomer was recorded. In a typical "sorbitan trioleate" (Span 85), the composition (as explained in Table I) calculated by weighing the areas under each peak, was as follows: 35% monoester, 33% diester and 32% triester. None of the sorbitan trioleates contained over 37% of triester isomers. Figure 4B shows an additional peak appearing prior to the monoester peak. This peak is probably due to some impurity having very high absorbance at 220 nm. The impurity tends to disappear when a small quantity of hydrogen peroxide solution is added to the sample, therefore, it is not part of the emulsifier isomers.

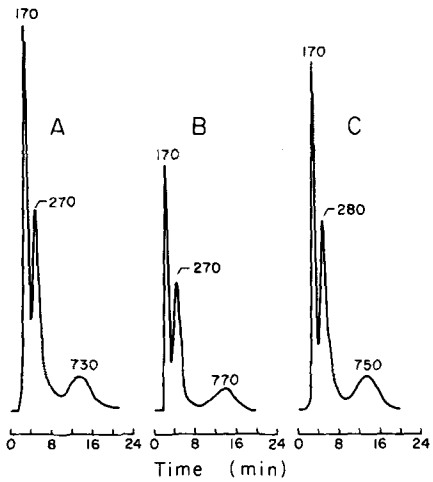

FIG. 3. Sorbitan monooleate from various commercial sources: (A) Span 80-Atlas, (B) Crill 4-Croda, (C) Sorbac 80-Polibasicos.

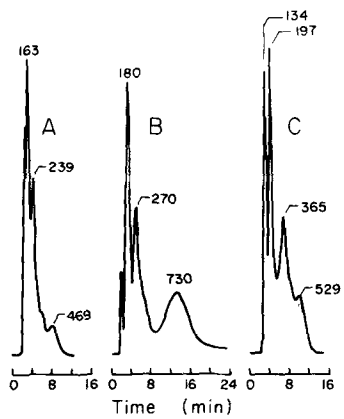

FIG. 4. Sorbitan trioleate (Span 85) eluted with isopropanol/water: (A) 92:8, (B) 85:15, (C) 80:20.

TABLE I

Proposed Product Distribution Values for Mono-, Di- and Trisorbitan Esters of Fatty Acids

	Acid value	Iodine value	Saponification value	% Monoesters	% Diesters	% Triesters
Span 40[a]	4.48	–	–	52	39	9
Span 60[b]	4.48	–	–	48	34	18
Crill 3[b]	4.75	–	154	45	38	17
Hamorad 60[b]	4.49	–	152	55	33	12
Sorbac 60[b]	–	–	156	51	40	9
Adochan 75[b]	4.56	–	156	56	33	11
Span 65[c]	–	–	–	38	31	31
Span 80[d]	5.99	64	–	52	34	14
Crill 4[d]	5.12	59	156	48	36	16
Sorbac 80[d]	5.50	57	–	44	38	18
Sorbac 85[e]	8.01	78	182	31	32	37
Span 85[e]	10.72	86	–	35	33	32
Crill 43[f]	8.02	–	159	36	38	26
Crill 6[g]	4.78	–	159	44	33	23

[a]Sorbitan monopalmitate.
[b]Sorbitan monostearate.
[c]Sorbitan tristearate.
[d]Sorbitan monooleate.
[e]Sorbitan trioleate.
[f]Sorbitan sesquioleate.
[g]Sorbitan monoisostearate.

Sorbitan monoesters of palmitic, stearic, isostearic and sesquioleic acids were injected in slightly different conditions and similar separation was obtained. Figure 5 and Table I summarize these results.

Figure 6 presents the separation of sorbitan tristearate and sorbitan trioleate from two sources. It can be seen that the trioleate from Polibasicos shows additional diisomer. Figure 6A presents the chromatogram of sorbitan tristearate (Span 65) which dissolves in isopropanol with great difficulty.

Figure 7 demonstrates variations in product composition of several commercial sorbitan monoesters of stearic acid which is a common surfactant in both food, cosmetics and pharmaceuticals.

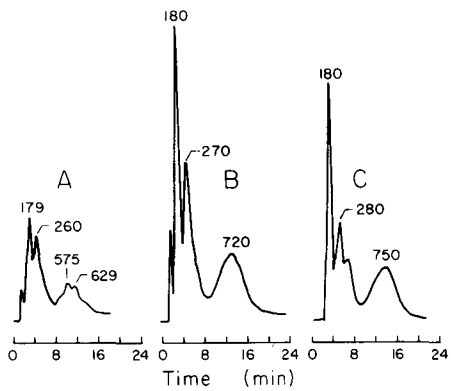

FIG. 6. Sorbitan triesters of: (A) stearic (Span 65-Atlas), (B) oleic (Span 85-Atlas), (C) oleic (Sorbac 85-Polibasicos).

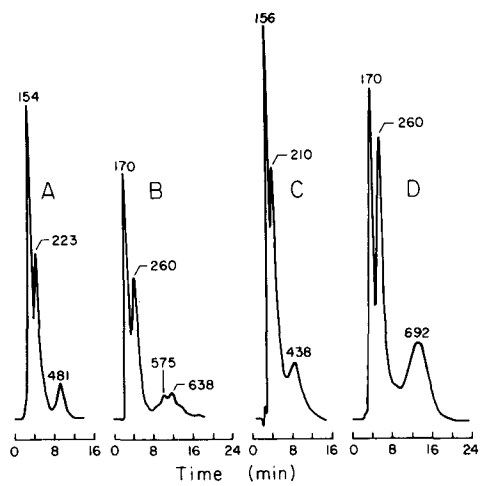

FIG. 5. Sorbitan monoesters of several fatty acids: (A) palmitic, (B) stearic, (C) isostearic, (D) sesquioleic.

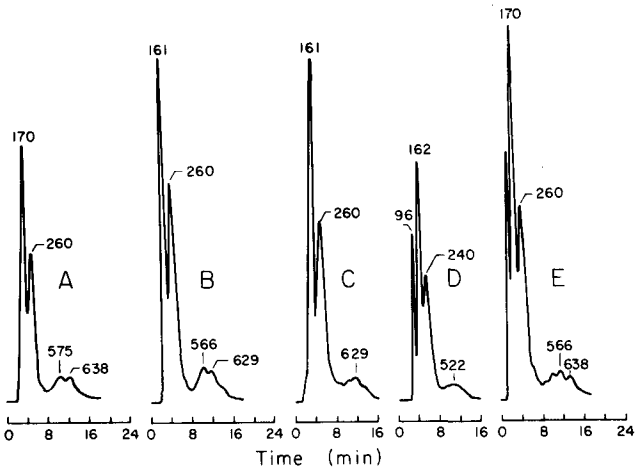

FIG. 7. Sorbitan monoesters of stearic acid from several commercial sources: (A) Atlas, (B) Croda, (C) Polibasicos, (D) Adumim Chemicals, (E) Hamorad.

It is important to note that in some of the products claimed to be monoesters, up to 26% of triester isomers were detected.

Several attempts have been made to distinguish between the triester isomers. Unfortunately, no distinctions have yet been found, and the work is still in progress.

In conclusion, this work presents a very simple and convenient method of analyzing the sorbitan esters without any derivatization. Using this technique one can characterize the product composition and can get a better idea of the internal distribution in the material.

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