Journal of Chromatography, 536 (1991) 75–84
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2017

Applications of preparative high-performance liquid chromatography in oleochemical research

ANDREAS BRUNS
Henkel Research Corporation, 2330 Circadian Way, Santa Rosa, CA 95407 (U.S.A.)

ABSTRACT

The application of preparative high-performance liquid chromatography to oleochemical and related products is discussed with examples that cover the field of glycerol and fatty acid derivatives, fatty alcohol ethoxylates, tocopherols, guanidines and glucose telomers. Columns of 21.5 to 100 mm I.D. and 250 to 400 mm length are used for reversed-phase and normal-phase chromatography. Semi-preparative separations with 100-mg column loads are presented as well as technical-scale applications with 15-g raw material injections per separation cycle.

INTRODUCTION

Products that are based on natural raw materials often show quite complex compositions. This is especially true for oleochemical products. Nevertheless, "pure" substances are required for many research or analytical applications and, therefore, powerful separation and purification techniques, like preparative high-performance liquid chromatography (Prep-HPLC), are of high interest in this field. The preparative application of HPLC is relatively young and that is why its use as a routine technique is still rapidly growing [1,2]. The amount of purified material that is required for a certain purpose specifies the scale of Prep-HPLC that has to be used [1].

Instead of discussing the more theoretical aspects of Prep-HPLC, such as the calculation of column loadabilities [3–5], this paper outlines the broad applicability of Prep-HPLC to oleochemical products by presenting a variety of different separation and purification examples.

EXPERIMENTAL

Four different chromatography systems were used for these Prep-LC applications: (1) Labomatic MD 80/100 medium pressure pump, Labochrom MP 20 pulse dampner, Labochrom pressure controller, Labochrom injection valve, Labochrom PGC columns, Jobin Yvon "Iota" refractive index (RI) detector with preparative cell and Isco "Foxy" fraction collector. (2) Waters 600 pump, Wisp 710 autosampler, Jobin Yvon "Iota" RI detector with preparative cell and Isco "Foxy" fraction collector. (3) Waters LC 3000 pump, sample injection via the pump through one of the four

0021-9673/91/$03.50

solvent lines, Waters 481 UV detector with semi-preparative cell, Gilson 201 fraction collector. (4) Merck Prepbar 100, fully explosion proof preparative/technical scale chromatography system.

All solvents were HPLC grade from Prochrom (Wesel, F.R.G.) or Merck (Darmstadt, F.R.G.). The organic solvents were evaporated with Büchi rotary evaporaters, a Christ freeze drying system was used for the evaporation of water.

All the preparative chromatographic conditions were directly scaled up from in house developed analytical conditons, for detailed Prep-HPLC conditions refer to figure captions.

RESULTS AND DISCUSSION

Glycerol derivatives

Glycerol derivatives are important oleochemical products with a broad application field. Glycerol fatty acid esters are for instance commonly used emulsifiers, whereas oligoglycerols can be used as polyols for polymer chemical applications.

The chromatograms in Figs. 1 and 2 show the purification of glycerol diacetate and glycerol diacetate monopropionate. These compounds were needed as standards in gas chromatography (GC). Both raw material contained less then 50% of the products of interest. Normal-phase chromatography on unmodified silica as the stationary phase resulted in purities $\geqslant 95\%$, as determined by GC. The two examples

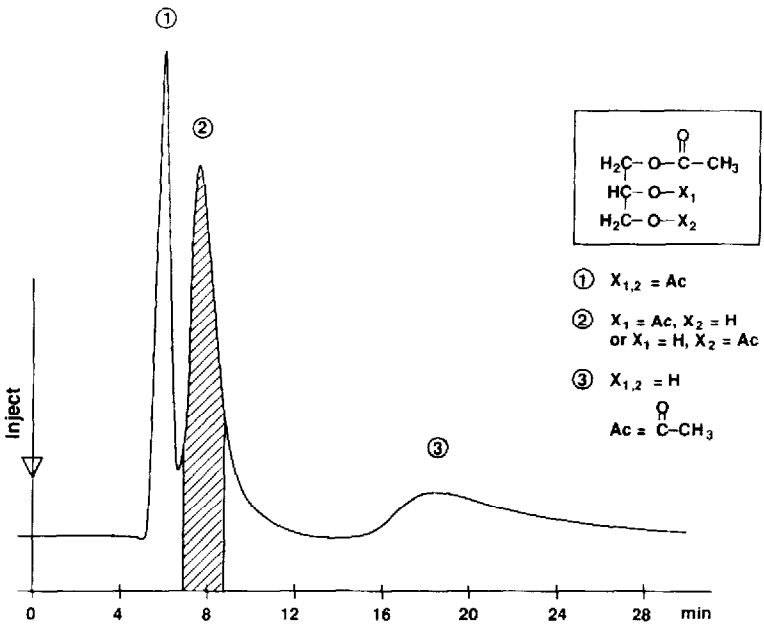

Fig. 1. Preparative chromatogram and structure for glycerol diacetate, shaded peak represents main product fraction. System: Labomatic MPLC: column: LiChroprep Si 100, 40 63 μm, 480 × 37 mm I.D., self packed; eluent: *n*-hexane–ethylacetate (20:80, v/v); flow: 35 ml/min; injection concentration and volume: 1.5 ml sample in 10 ml eluent; detection: RI

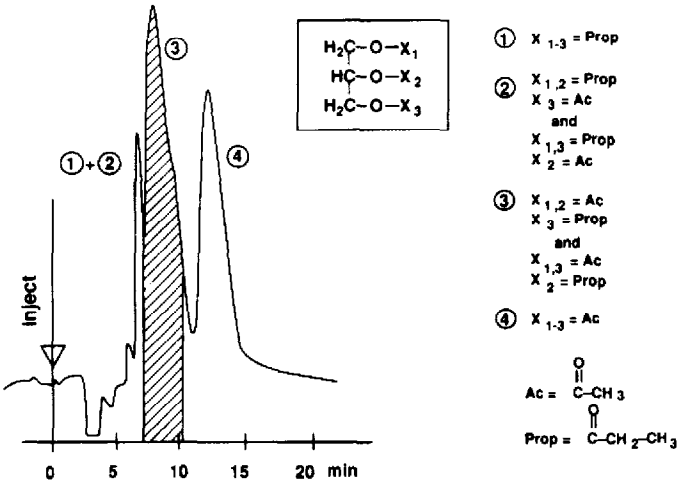

Fig. 2. Preparative chromatogram and structure for glycerol diacetate monopropionate, shaded peak represents main product fraction. System: Labomatic MPLC; column: LiChroprep Si 100, 40–63 μm, 480 × 37 mm I.D., self packed; eluent: *n*-hexane *tert.*-butyl methyl ether (80:20, v/v); flow: 62 ml/min; injection concentration and volume: 1 ml sample in 10 ml eluent; detection: RI.

demonstrate that for simple separation problems even medium-pressure equipment with large-particle-size column packing material can result in sufficient purity.

Glycerol monohydroxystearate was purified on a 50-mm I.D. cyano column in the normal-phase mode (Fig. 3). A technical-scale high-performance system was used and 97% purity (HPLC) were achieved from a 57% pure raw material. The purified product was used as GC-standard.

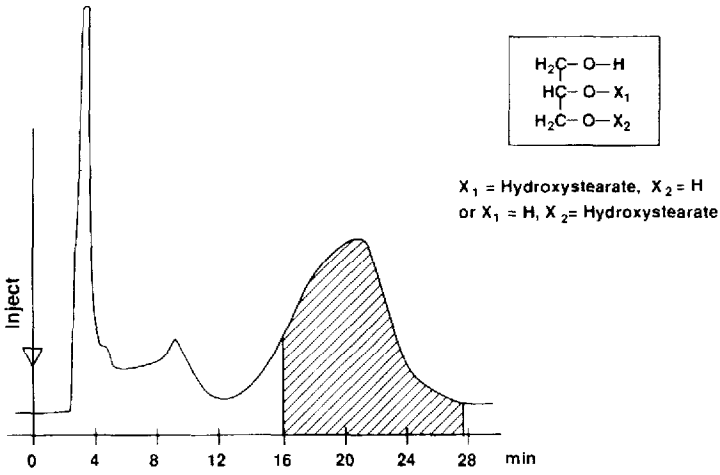

Fig. 3. Preparative chromatogram and structure for glycerol monohydroxystearate, shaded peak represents main product fraction. System: Merck Prepbar 100; column: LiChroprep CN, 25–40 μm, 250 × 50 mm I.D., LiChroprep CN guard column; eluent: *n*-hexane–2-propanol (95:5, v/v); flow: 120 ml/min, injection concentration and volume: 3.4% in 15 ml heptane–2-propanol (67:33, v/v); detection: UV 230 nm.

In the analytical HPLC chromatogram of an oligoglycerol sample the first four peaks could be assigned to the oligomers with $n = 1$–4 by retention time comparison with commercially available standards (Deutsche Solvay-Werke, Solingen, F.R.G.). The identities of two later-eluted components were questionable. Fig. 4 shows the preparative chromatogram of this oligoglycerol sample, where the unidentified peaks are marked with $n = ?$. Fractionation on a 22.5 mm I.D. diol-modified silica column and identification by GC–mass spectrometry (MS) (after silylation) showed that they corresponded to the higher oligomers with $n = 5$ and $n = 6$.

Fatty acid derivatives

Dicarboxylic acids are for instance used for polymer production. Purification to $\geq 95\%$ for the use as a GC-standard was the goal for a C_{16}-dicarboxylic acid. In addition the byproducts should be isolated and identified spectroscopically. The methyl ester derivative of this sample was used for Prep-HPLC (Fig. 5). The main compound, a C16:1 dimethylester with the double bond in position 3, was isolated in >98% purity (NMR). The structures were assigned with the aid of GC–MS (after OsO_4-silylation [6]) and showed that all components were C_{16}-diacid methyl esters with zero, one or two double bonds in different positions (see structure assignments in Fig. 5).

High purity oleic acid is a valuable raw material for further synthetic applications. Liquid chromatography was tested as an alternative or additional technique to purification of this material by distillation. Whereas in the above example 100-mg amounts of the sample were injected into a 22.5-mm I.D. silica column, we used column loads of 10 g raw material on a 100-mm I.D. reversed-phase column for the purification of oleic acid (Fig. 6). For a raw material that contained 85% oleic acid,

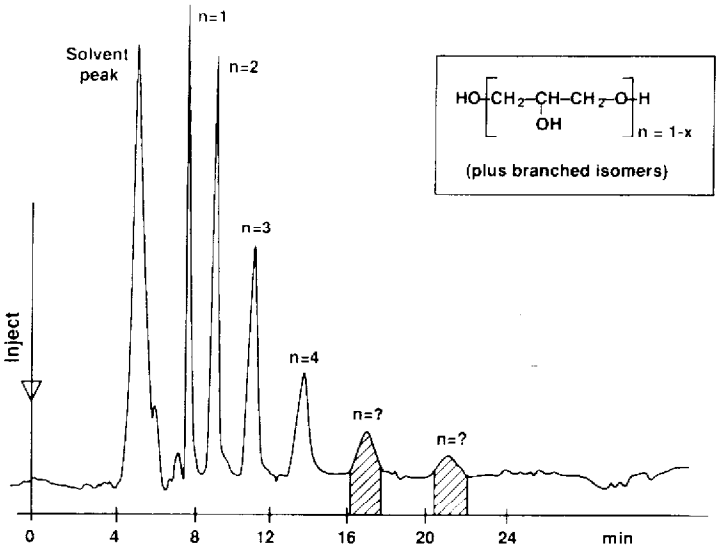

Fig. 4. Preparative chromatogram and structure for oligoglycerols, shaded peaks represent fraction cuts. System: Waters 600; column: LiChrosorb Diol, 7 μm, 250 × 22.5 mm I.D.; eluent: acetonitrile–water (85:15, v/v); flow: 20 ml/min; injection concentration and volume: 7% in 0.9 ml eluent; detection: RI.

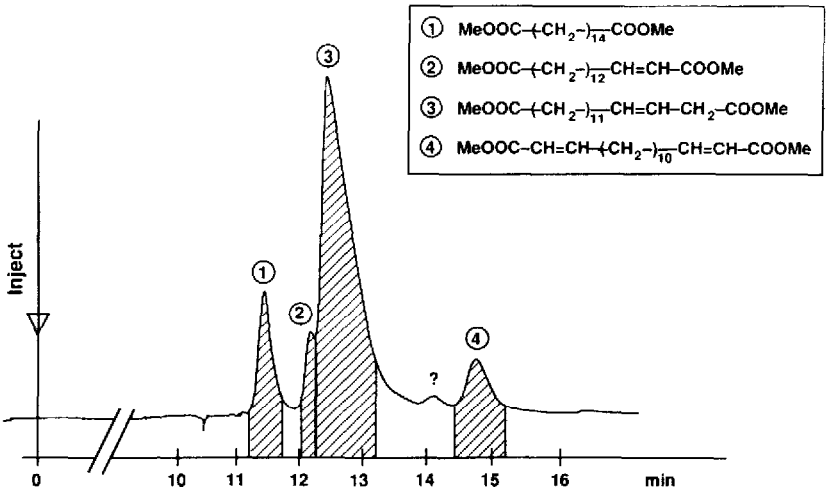

Fig. 5. Preparative chromatogram and structures for C_{16}-dicarboxylic acid methylesters, shaded peaks represent fraction cuts. System: Waters 600; column: LiChrosorb Si 60, 7 μm, 250 × 22.5 mm I.D.; eluent: *n*-hexane–ethylacetate (90:10, v/v); flow: 20 ml/min; injection concentration and volume 20% in 0.5 ml eluent; detection: RI. Me = Methyl.

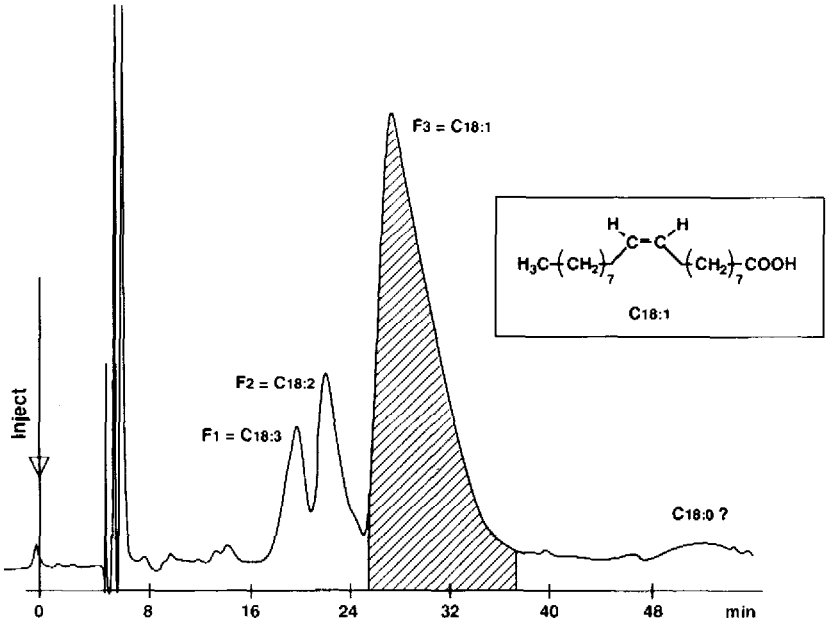

Fig. 6. Preparative chromatogram and structure for oleic acid purification, shaded peak represents main product fraction. System: Merck Prepbar 100; column: LiChroprep RP 18, 5–20 μm, 400 × 100 mm I.D.; eluent: methanol–water–acetic acid (90:9.9:0.1, v/v/v); flow: 400 ml/min; injection concentration and volume: 10 g raw material in 50 ml methanol; detection: RI.

this approach resulted in oleic acid with 95% purity (GC) and 4% palmitic acid as a byproduct, which could be separated by distillation. Until now, the purity of technical-grade oleic acid was limited by the inability of distillation processes to separate fatty acids that differ only in the number of double bonds. As this application demonstrates, the combination of distillation and chromatographic purification allows purification of oleic acid to $\geqslant 98\%$.

Fatty alcohol ethoxylates

Fatty alcohols are direct derivatives of fatty acids and are used as raw material for ethoxylation processes, resulting in non-ionic surfactants. These products show a broad distribution of ethoxylate oligomers. Pure C_{12}-fatty alcohol-based ethoxylate oligomers with $n > 8$ were needed as reference materials for physical tests (the oligomers with $n \leqslant 8$ are commercially available from Nikko Chemicals, Tokyo, Japan).

Fig. 7 demonstrates the preparative chromatogram. The separation conditions were scaled up from thin-layer chromatography by using unmodified silica as the stationary phase and butanone with a significant percentage of water as the eluent. These unusual conditions served the purpose for the preparative separation and enabled the production of 200 to 500-mg amounts of the $n = 9$–15 ethoxylate oligomers in 90–99% purity (HPLC).

Tocopherols

Natural vitamine E is derived from vegetable oils and is comprised of a mixture of D-α-, -β-, -γ- and -δ-tocopherols (Fig. 8). Considerable quantities of the D-γ-to-

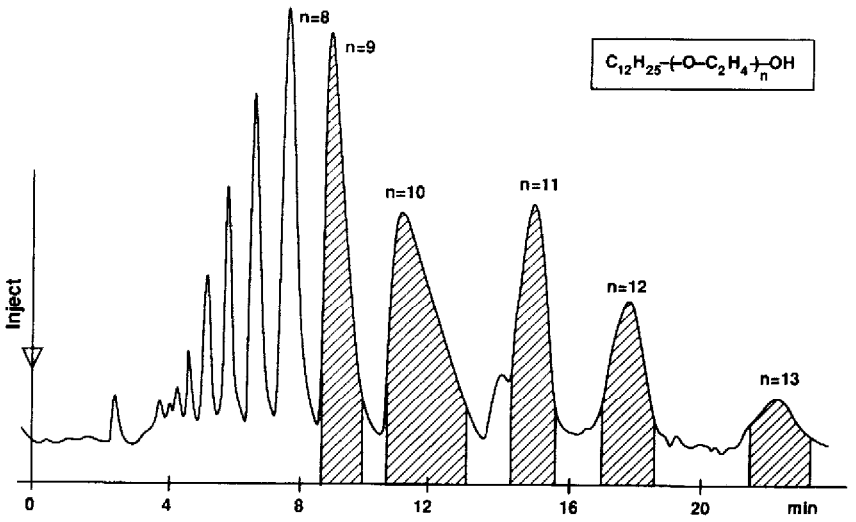

Fig. 7. Preparative chromatogram and structure for C_{12}-fatty alcohol ethoxylates, shaded peaks represent fraction cuts. System: Waters 600; column: LiChrosorb Si 60, 7 μm, 250 × 22.5 mm I.D.; eluent: butanone–water (92:8, v/v); flow: 18 ml/min; injection concentration and volume: 40% in 0.5 ml eluent; detection: RI.

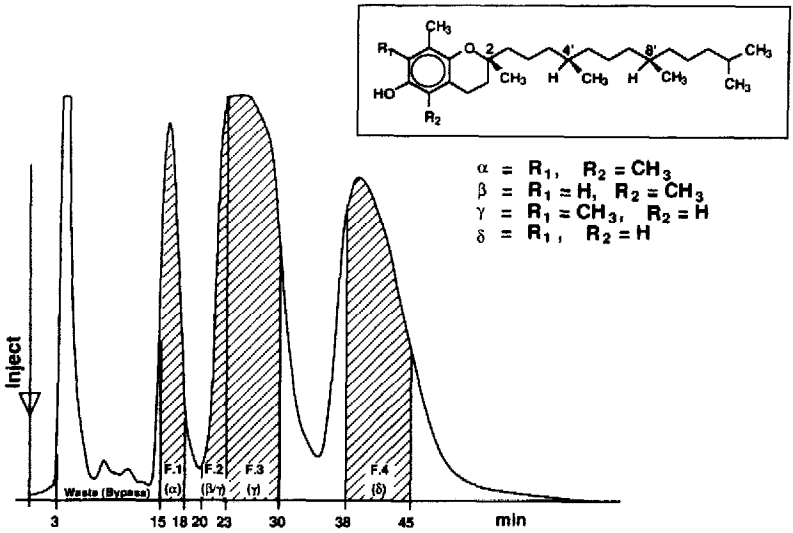

Fig. 8. Preparative chromatogram and structure for tocopherols, shaded peaks represent fraction cuts. System: Merck Prepbar 100; column: LiChroprep Si 60, 25–40 μm, 400 × 100 mm I.D.; eluent: *n*-hexane–*tert.*-butyl methyl ether (97:3, v/v); flow: 450 ml/min; injection concentration and volume: 15 g raw material in 50 ml eluent; detection: UV 205 nm.

copherol had to be isolated in a purity > 90% for application tests. The raw material contained only 70% tocopherols, 60% of which was the D-γ-analogue.

For the purification a technical-scale separation with a 100-mm I.D. column and unmodified silica as the stationary phase [7] was used (Fig. 8). The analytical HPLC data showed the D-γ-fraction to have a purity of 95%.

Guanidines

Products that have interesting metal extraction properties by an ion-pair extraction mechanism [8,9] can be synthesized from oleochemical feedstocks. Bis(2-ethyl-hexyl)guanidine (for structure see Fig. 9) was used as a model compound to study the metal loading behavior of these products.

This guanidine was purified on a 21.5-mm I.D. column in the reversed-phase mode, using a polymer-based stationary phase. This was because of the low pH that had to be used in order to control the peak tailing of the basic compounds. The use of sulfuric acid for pH control originated from the analytical conditions, in which an ion-pairing system was applied to control tailing. A buffer–ion-pairing system did not appear to be very convenient for preparative-scale chromatography and that was how sulfuric acid was found to work well for the purpose. The peak area ratios of the main product (shaded peak in chromatogram, Fig. 9) and the byproducts (trialkyl guanidines and bisguanidines according to NMR and MS) do not reflect the real weight percent ratios. This is due to detector overload, which causes a non-linear response.

Bis(2-ethyl-hexyl)guanide (25 g) was isolated in >98% purity (purity assignments according to NMR mole percent). This shows that even with small-scale Prep-HPLC equipment it is possible to purify significant amounts of products conveniently, as long as the instruments are fully automated.

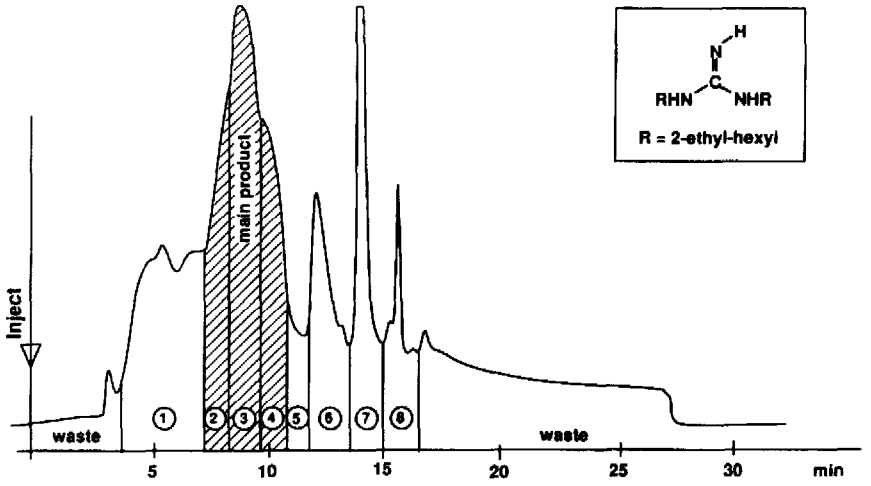

Fig. 9. Preparative chromatogram and structure for bis(2-ethyl-hexyl)guanidine, numbers represent fraction cuts, shaded peak represents main product fraction. System: Waters LC 3000; column: Hamilton PRP-1, 10 μm, 250 × 21.5 mm I.D.; eluents: A = methanol–water–sulfuric acid (75:24.95:0.05, v/v/v), B = methanol–sulfuric acid (99.95:0.05, v/v); gradient: hold 100% A 5 min, then go to 100% B in 10 min, hold 100% B for 10 min, reequilibrate 5 min; flow: 20 ml/min; injection concentration and volume: 20% in 2.5 ml solvent B; detection: UV 214 nm.

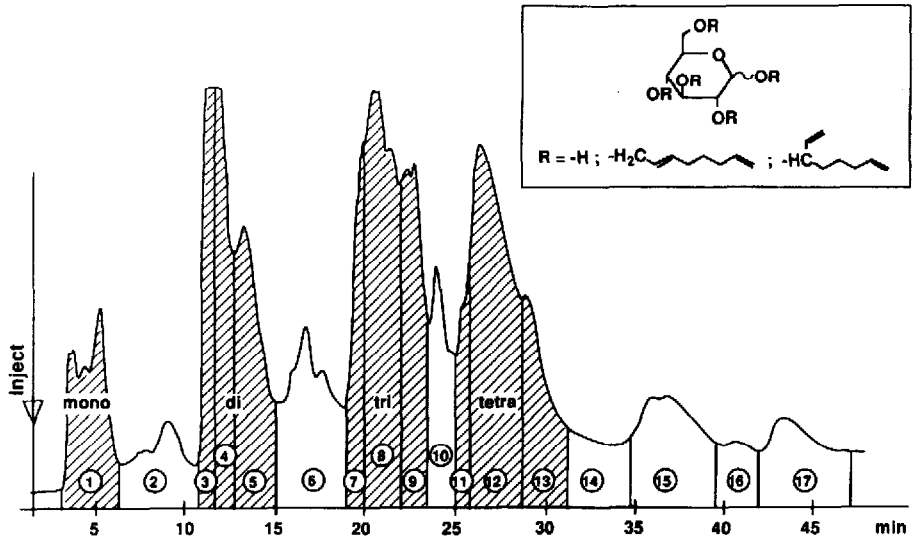

Fig. 10. Preparative chromatogram and structure for glucose telomers, numbers represent fraction cuts, shaded peaks represent main components. System: Waters LC 3000; column: LiChrosorb RP 18, 10 μm, 250 × 22.5 mm I.D.; eluents: A = acetonitrile–water (40:60, v/v), B = acetonitile; gradient: 100% A to 100% B in 20 min, hold 100% B for 30 min, reequilibrate 5 min; flow: 20 ml/min; injection concentration and volume: 20% in 3.0 ml solvent B; detection: UV 210 nm.

Glucose telomers

In addition to fats and oils carbohydrates are attractive natural raw materials, for instance for glucose based surfactants such as alkyl glucosides [10,11] or for the so-called glucose telomers, a new group of products synthesized from glucose and butadiene according to the telomerization mechanism [12,13].

To characterize these glucose telomers in further detail and establish analytical standards for quantitative HPLC analysis, the compounds with different degrees of substitution were separated (for structures see Fig. 10). For preparative chromatography a reversed-phase system with a 22.5-mm I.D. C_{18}-column was used. The chromatogram (Fig. 10) shows the separation of the different groups that represent the different degrees of substitution. The various isomers within each of these groups were not separated. Quantities of 4–9 g of the mono- to tetra-octadienyl-substituted glucose telomers were isolated in purities >90% (according to GC, GC–MS and HPLC). The amount of penta-substituted material (fractions 15 and 17) was very low according to the spectroscopic results.

CONCLUSION

In a broad field of applications it has been demonstrated that preparative high-performance liquid chromatography is a very versatile separation and purification tool for oleochemical products. Depending on the separation problem, there is a wide variety of chromatographic separation techniques available. In principle, any analytical HPLC procedure can be scaled up to the preparative or even technical scale. Obviously, preparative and especially technical-scale HPLC is not a low-budget technology, but it does offer very high separation efficiencies and therefore it is certainly a very helpful addition to the conventional purification techniques, like extraction, crystallization, or distillation.

ACKNOWLEDGEMENTS

Dr. W. Winkle and Dr. A. Werner-Busse are thanked for contributing analytical HPLC conditions that have been used for scale-up. The assistance of Dr. V. Puchta and Dr. D. Berg is gratefully appreciated. Special thanks are due to the members of the chromatography and spectroscopy laboratories in Düsseldorf and Santa Rosa for their skillful support.

REFERENCES

1 M. Verzele and C. Dewaele, *Preparative High Performance Liquid Chromatography*, TEC, Gent, 1986, pp. 10–26.
2 P. D. McDonald and B. A. Bidlingmeyer in B. A. Bidlingmeyer (Editor), *Preparative Liquid Chromatography (Journal of Chromatography Library*, Vol. 38), Elsevier, Amsterdam, 1987, pp. 1–95.
3 K. P. Hupe and H. H. Lauer, *J. Chromatogr.*, 203 (1981) 41.
4 G. Cretier and J. L. Rocca, *Chromatographia*, 21 (1986) 143.
5 S. Ghodbane and G. Guiochon, *J. Chromatogr.*, 450 (1988) 27.
6 H. Budzikiewicz, *Analytiker Taschenbuch*, Vol. 5, Springer, Heidelberg, 1984, pp. 135–138.
7 A. Bruns, D. Berg and A. Werner-Busse, *J. Chromatogr.*, 450 (1988) 111.
8 Y. Marcus and A. S. Kertes, *Ion Exchange and Solvent Extraction of Metal Complexes*, Wiley-Interscience, New York, 1969.

9 W. Lin, P. Mattison and M. Virnig, *U.S. Pat.*, (1985) 4 814 007.
10 W. Koenigs, E. Knorr, *Chem. Ber.*, 34 (1901) 957.
11 A. Bruns, W. Waldhoff and W. Winkle, *Chromatographia*, 27 (1989) 340.
12 A. Behr, in R. Ugo (Editor), *Aspects of Homogeneous Catalysis*, Vol. 5, Reidel, Dordrecht, Boston, Lancaster, 1984, pp. 5–48.
13 L. I. Zakharkin, V. V. Guseva, D. D. Sulaimankulova and G. NM. Korneva, *Zh. Org. Khim.*, 24 (1988) 119.