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Short communication

Determination of poly(ethylene glycol) 300 in long chain free fatty acid mixtures by reversed-phase high-performance liquid chromatography

Alister D. Muir*, Martin J. Reaney, Andrew J. Aubin

Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, Sask. S7N 0X2, Canada

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Abstract

A rapid sensitive method has been developed for the detection and quantitation of poly(ethylene glycol) 300 (PEG 300) in long-chain free fatty acid mixtures that requires minimal sample preparation. The PEG 300 was separated from the free fatty acids by RP-HPLC using a water–tetrahydrofuran gradient. PEG and the free fatty acids were detected using evaporative light scattering detection. The minimum detectable level of PEG in a free fatty acid mixture was 0.0125%.

Keywords: Poly(ethylene glycol); Fatty acids

1. Introduction

Food-grade poly(ethylene glycol) (PEG) is used in a large number of food and cosmetic preparations, including baby products, lipsticks, protective creams and pharmaceuticals. PEG is also used in processed beet sugar and yeasts as a defoaming agent and in the manufacture of PEG esters of free fatty acids and related materials. Although it is permitted in food, pharmaceutical and cosmetic products, it is desirable to be able to determine its concentration in these products.

There are a number of HPLC-based analytical techniques available for the analysis of PEGs including normal-, reversed-phase- and gel permeation HPLC [1], however, most of these techniques were developed for the compositional analysis of PEGs and only a few have been adapted for the analysis of PEGs in oils and consumer products.

Although several methods are available for the analysis of low-molecular-mass PEGs (PEG 300) in aqueous solutions [2–4], the options for quantitation of PEG in vegetable oils and non-polar materials are limited. Coupková et al. [5] reported a method for RP-HPLC based on a Spheron 40-S polymeric sorbent for the analysis of PEG in surfactants and detergents. In 1981, Kudoh et al. [6] reported a semi-preparative HPLC method for the determination of PEG in non-ionic surfactants using a 50×2 cm reversed-phase RP-18 column and refractive index detection. Since these methods were developed, there have been new developments in the detection of non-UV-absorbing compounds, including evaporative light scattering detection (ELSD). ELSD has now become a useful technique for the detection of fatty acids and similar compounds [7], since it eliminates

*Corresponding author.

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the necessity to form derivatives. This detection method has also recently been applied to the compositional analysis of low-molecular-mass PEGs and their monomethyl ethers [8].

Initial experiments in our laboratory to detect and quantitate PEG 300 in vegetable oils, and long-chain free fatty acids, using gel permeation HPLC chromatography confirmed the expected observation that the molecular mass ranges for PEG 300, monoglycerides and free fatty acids overlapped and that positive determination of PEG was not possible. Therefore, other options were explored for the determination of PEG by HPLC in non-polar samples. Since the requirement for analysis of PEG in free fatty acids arose as a result of developments in the industrial processing of free fatty acids, it was desirable that the new analytical method be simple and not require extensive sample preparation or extraction of the PEG from the sample matrix prior to analysis. In this paper, we report a simple method for the detection and quantitation of PEG in a free fatty acid mixture from hydrolyzed sunflower oil, which is rich in linoleic acid.

2. Experimental

2.1. Chemicals

HPLC-grade water was obtained by passing RO-treated water through a Super Q water system (Millipore Canada, Mississauga, Canada) prior to use. HPLC-grade tetrahydrofuran (THF) was obtained from VWR Canada. PEG 300 (FG, NF grade, Sentry Brand) was obtained from Union Carbide. A free fatty acid mixture that was rich in linoleic acid was prepared in the laboratory from sunflower oil by hydrolysis with potassium hydroxide in water. The hydrolyzed soaps were acidified to pH 3.0 using 70% phosphoric acid. The aqueous layer was removed and the fatty acids were washed twice with brine. Traces of water were removed by heating to 100°C under vacuum. The fatty acid-rich mixture was analyzed by titration and found to have 87% free fatty acids. The residual non-free fatty acid material is believed to be primarily monoglycerides.

2.2. HPLC–ELSD analysis

An Alliance 2690 separations module equipped with vacuum solvent degassing (Waters Canada, Mississauga, Canada), and a PL-EMD-960 ELSD system (Polymer Laboratories, Amherst, MA, USA), controlled by Millennium software (Waters Canada), was used for all analyses. The following ELSD settings were used: Attenuation=1, time constant=1 s, temperature=90°C; gain=6 and nebulization gas=air at 4.5 l/min.

2.3. Sample preparation

Samples were filtered and diluted 2:1 (v/v) with THF, to ensure that all components would remain sufficiently soluble during reversed-phase chromatography. Serial dilutions of PEG 300 in a linoleic acid-rich free fatty acid mixture were generated to determine the limits of detection.

2.4. Chromatography

Chromatographic separation was achieved on a reversed-phase Symmetry C_{18} column (150×3.0 mm) from Waters Canada. The mobile phase consisted of two solvents: Solvent A (water) and solvent B (tetrahydrofuran). A 10-μl injection volume was used for all samples and standards. The column was eluted with the following gradient: 0 min, 5% B; 3.5 min, 5% B; 10 min, 90% B; 15 min, 90% B; 20 min, 5% B; at a flow-rate of 0.4 ml/min.

3. Results

3.1. Optimization of sample preparation

For effective chromatographic separation of PEG from the free fatty acids, we determined that the sample must have some polar solvent solubility, which was achieved by diluting the sample with THF. At PEG concentrations above 0.5%, diluting the sample 1:4 (v/v) with THF gave satisfactory chromatography but limited the detectable levels of PEG. For samples with less than 0.5% PEG, a dilution of two parts free fatty acid mixture to one part THF gave the lowest SD (±0.18%) for three

replicate injections, compared to higher or lower dilutions.

3.2. Chromatographic separation of PEG 300 from free fatty acid mixtures

PEG 300 could be separated from and detected in the presence of free fatty acids (Fig. 1) using the gradient described. Analysis of free fatty acid samples prepared by a method that did not involve the use of PEG indicated that there were no compounds present in this material that could cause interference with PEG determination.

3.3. Minimum detectable concentration

The minimum detectable concentration of PEG 300 in the free fatty acid mixture was determined to be 0.0125% at a signal-to-noise ratio of eight.

3.4. Quantitation of PEG 300 in free fatty acid mixtures

The linoleic acid-rich free fatty acid mixture was spiked with PEG to give concentrations ranging from 0.0125 to 10% PEG, for the generation of a calibration curve. Each standard solution was analyzed in triplicate. The ELSD response was found to be non-linear at concentrations below 0.2% PEG (0.0125–0.2% PEG 300) ($y=-0.58+319.3x+49860x^2-98678x^3$, $R^2=1.00$). At higher concentrations (1–10% PEG 300), the detector response was linear ($y=3.722x-3.65$, $R^2=0.99$).

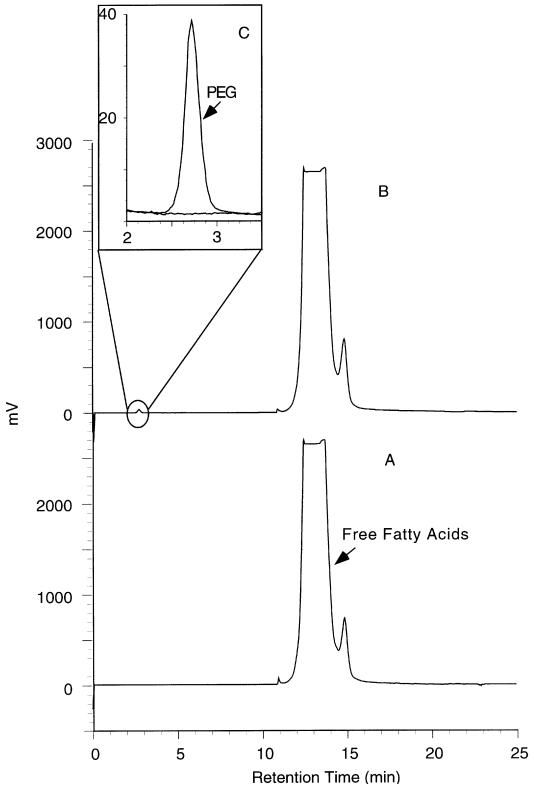

Fig. 1. Chromatogram of a linoleic acid-rich free fatty acid mixture before (A) and after (B) the addition of 0.1% PEG 300. Insert (C) illustrates the superposition of chromatogram B over chromatogram A. Chromatographic conditions are as described in the text.

4. Discussion and conclusion

PEG 300 can readily be detected in crude free fatty acid mixtures by reversed-phase HPLC with minimal sample preparation. This was achieved by first eluting the column with a high aqueous context solvent to elute the PEG 300, followed by a fast gradient to a high organic concentration to elute the less water-soluble free fatty acids. An ELSD temperature of 90°C was required to fully evaporate the high aqueous solvent in the PL-EMD-960 detector.

Trathnigg and Kollroser [8] suggested that the lowest nebulization temperature at which the mobile phase was sufficiently evaporated was desirable when using the SEDEX 45 detector. However, a direct comparison is not possible since, in the PL-EMD-960, the entire sample passes through the light path of the detector, while in the SEDEX 45 detector, only a proportion of the sample actually passes the light detector. The loss of sensitivity previously observed [8] may be due in part to the effects of sample splitting. In our system, the higher temperature was required due to the high water content, which, in turn, was required to achieve effective separation of the PEG from the fee fatty acids.

Concentrations of PEG 300 as low as 0.0125% can reliably be detected.

References

[1] K. Rissler, J. Chromatogr. A 742 (1996) 1.
[2] A. Oliva, H. Aramas, J.B. Farina, Clin. Chem. 40 (1994) 1571.
[3] Z. Moldovan, J. Lebrato-Martinez, M.V. Delgado-Luque, E. Otal-Salaverri, J. Liq. Chromatogr. 18 (1995) 1633.
[4] M.J. Sebastio, J.M.S. Cabral, M.R. Aires-Barros, Biotechnol. Techniques 9 (1995) 503.
[5] M. Coupková, K. Janes, J. Sanitrak, J. Coupek, J. Chromatogr. 60 (1978) 73.
[6] M. Kudoh, S. Fudano, S. Yamaguchi, J. Chromatogr. 205 (1981) 473.
[7] T.A. Foglia, K.C. Jones, J. Liq. Chromatogr. Rel. Technol. 20 (1997) 1829.
[8] B. Trathnigg, M. Kollroser, J. Chromatogr. A 768 (1997) 223.