Temperature-Enhanced Alumina HPLC Method for the Analysis of Wax Esters, Sterol Esters, and Methyl Esters

Robert A. Moreua*, Karen Kohouta, and Vijay Singhb

*ERRC, ARS, USDA, Wyndmoor, Pennsylvania, and bDepartment of Agricultural Engineering, University of Illinois, Urbana, Illinois 61801

ABSTRACT: Previous attempts at separating nonpolar lipid esters (including wax esters, sterol esters, and methyl esters) have achieved only limited success. Among the several normal-phase methods tested, a single recent report of a method employing an alumina column at 30°C with a binary gradient system was the most promising. In the current study, modification of the alumina method by increasing the column temperature to 75°C improved the separation of standards of wax esters and sterol esters. Elevated column temperature also enhanced the separation of FAME with differing degrees of unsaturation. Evidence was also presented to indicate that the method similarly separated phytosterol esters, based on their levels of unsaturation. With the increased interest in phytosterol- and phytostanol-ester enriched functional foods, this method should provide a technique to characterize and compare these products.

Numerous HPLC methods have been reported for the separation of polar and nonpolar lipids (1,2). Approaches to the separation of nonpolar lipid classes have included the use of Diol (3,4), cyanopropyl (CN) (5,6), and alumina (7) columns. Among the nonpolar lipid classes, wax esters (a wax ester is defined as a FA esterified to a fatty alcohol) and sterol esters (including phytosterol fatty acyl esters) have proven to be the most difficult to separate, and the evidence reported in the latter alumina method (with a column temperature of 30°C) indicates that it is the most promising one (7). While employing the alumina method to separate grain seed extracts that were high in both wax esters and phytosterol esters, we noted that when the temperature of the alumina column was increased (up to 75°C) the separation between wax esters and sterol esters was enhanced. In addition, the phytosterol esters were subfractionated into several distinct peaks that appeared to be related to the degree of unsaturation. With the increased interest in phytosterol- and phytostanol-ester enriched functional foods, this method should provide a way to characterize and compare the types of sterol esters in these products.

MATERIALS AND METHODS

Lipid standards and jojoba oil were purchased from Sigma Chemical Co. (St. Louis, MO). Benecol and Take Control margarines were purchased from local grocers and were extracted with hexane (150 mg sample per 30 mL hexane), rinsed in a separatory funnel with an equal volume of water, dried under N2, and redissolved in hexane (with 0.01% BHT) for HPLC injection. CookSmart, a phytosterol ester-enriched cooking oil, was purchased on-line from Procter & Gamble (Cincinnati, OH) during a limited test-market period. Corn fiber oil (unrefined) was extracted as previously described (3).

RESULTS AND DISCUSSION

Standards of hydrocarbon (squalene), wax ester (stearyl stearate), and sterol ester (cholesterol stearate) were separated at a column temperature of 25°C (Fig. 1) using an Aluspher column and a gradient method very similar to that reported by Nordbäck and Lundburg (7). A fourth standard, methyl stearate, was also included in this mixture, and it appeared as a shoulder that preceded the peak of sterol ester. Increasing the column temperature to 50°C enhanced the separation of all four components, and further increasing it to 75°C had little effect on the first three peaks but increased the retention time of cholesterol stearate from about 6 to about 9 min.

Samples of four phytosterol ester-rich food products were injected into the HPLC system using this alumina method at
75°C (Fig. 2), and the major components in each were TAG, eluting as a broad peak at 17 to 20 min. The chromatograms obtained for all four samples also contained multiple peaks in the retention time region of 7 to 16 min, and these peaks appeared to be subfractions of phytosterol esters. Benecol, a spread containing phytostanol (completely saturated phytosterols obtained by hydrogenating phytosterols) esters, had a major peak at 8 min, and TakeControl, another spread containing phytosterol esters, had a major peak at 12 min. CookSmart (a phytosterol ester containing cooking oil that was test-marketed for a short time by Procter & Gamble) had a major peak at about 10 min. Corn fiber oil (3), which contains both phytosterol and phytostanol esters, had major peaks at 10 and 12 min. The Benecol and TakeControl chromatograms indicate that multiple peaks of phytosterol esters may be separated based on the degree of unsaturation, partially eluting in order of increasing number of carbon-carbon double bonds in the esters.

Jojoba oil is an unusual seed oil that contains no TAG, and the storage form of lipid in the oil is entirely wax esters (20- and 22-carbon FA esterified to 20- and 22-carbon fatty alcohols) (8). The chromatogram of jojoba oil contained a major broad peak at 5 min (Fig. 2), similar to that of the wax ester standard in Figure 1.

Various FAME were then injected in the 75°C alumina method (Fig. 3). A mixture of methyl esters of four saturated FA (myristate, palmitate, stearate, and arachidate) eluted as a single peak at about 5 min. A second mixture of 18-carbon methyl esters of stearate, oleate, linoleate, and linolenate