Comments on the Methodology for the Separation and Quantitation of *cis*-6 (petroselinic) and *cis*-9 (oleic) 18:1 Acids

Sir:

The separation and quantitation of *cis*-6 (petroselinic) and *cis*-9 (oleic) 18:1 acids have attracted much attention from lipid researchers for at least two reasons. The first reason is the potential usefulness of the former acid for industrial applications: after ozonolysis, the C_{12} fragment may be used for the production of soaps or detergents, whereas the C_6 diacid (adipic acid) can be a raw material for the manufacture of nylon. The second reason is that the separation of the two octadecenoic isomers has been very difficult because of their similar chromatographic behavior and has been a challenge to lipid analysts for decades.

The methods that have been employed to identify and quantitate the two *cis* 18:1 isomers can globally be classified into four analytical categories. The first, and certainly the oldest, was to isolate the *cis*-octadecenoic acids by preparative gas–liquid chromatography (GLC), by argentation thin-layer chromatography (Ag–TLC), or by both methods, followed by oxidative cleavage of the ethylenic bonds and further analysis of the resulting fragments by GLC. This was the basis of the comprehensive studies of Umbelliferae seed lipids by Kleiman and Spencer (1) and by Seher and Gundlach (2). The second method is based on slight but sufficient differences in the migration rates of the two isomers (as methyl esters) during Ag–TLC. This is exemplified in the studies by Nikolova *et al.* (3), who used silica gel plates, and by Breuer *et al.* (4), who used alumina-coated plates. Improved separations of the methyl esters were obtained at temperatures below −18°C, whereas phenacyl derivatives were easily resolved at ambient temperature on silica gel plates with low levels of impregnation with silver ions, with either overnight or multiple developments (5). Quantitation could be made by densitometry after charring the spots. In lieu of Ag–TLC, high-performance liquid chromatography in the silver-ion mode of the phenacyl derivatives also leads to excellent separations of the two isomers and also of the *cis*-11 (*cis*-vaccenic) acid (6). In a third set of methods, ^{13}C nuclear magnetic resonance spectroscopy, either in combination with GLC (7,8) or alone (9), allows quantitation of both the *cis*-6 and *cis*-9 18:1 isomers.

However, many more studies have been devoted to the separation and quantitation of these isomers by direct GLC to provide rapid and simple methods that any laboratory can apply routinely with no particular or expensive equipment. In earlier attempts, several derivatizations at the level of the ethylenic bonds have been proposed to improve the resolution of the two isomers. Without trying to be exhaustive, we can cite the trimethylsilyloxy adducts (10–12), the epoxide (13), and the dimethyldisulfide (14) derivatives of the methyl esters. Unfortunately, the resolution has generally remained rather poor, and a mass spectrometer has frequently been needed to distinguish between fragments specific to the two isomers.

Based on retention data published for the whole series of *cis*-18:1 isomers by Scholfield (15) and Christie (16) for several stationary phases, it was realized in 1992 that the differences between the equivalent chainlengths of the two isomers were sufficient to obtain a more or less good separation of the methyl esters on cyanoalkyl polysiloxane-coated capillary columns, and two independent papers describing this separation, by Wolff and Vandamme (17) and by Griffiths *et al.* (18), were published that year. A considerable improvement in the resolution was obtained when isopropanol was substituted for methanol in the derivatization procedure, leading to a near base-line resolution of petroselinic and oleic acid isopropyl esters, and also of *cis*-vaccenic acid (17). Later, it was shown that butyl (19,20) and phenylethyl (21) esters of these isomers could also be base-line resolved. The derivatization procedures with isopropanol, butanol, or phenylethanol are as simple as those with methanol; they are completed in *ca.* 1 h with acidic catalysts and in a considerably shorter time with alkaline catalysts. The isomers can be analyzed by GLC in less than 10 min (isothermal) or 25 min (temperature programming). Use of 100-m cyanoalkyl polysiloxane capillary columns operated under optimal conditions (rather low temperatures) may also lead to the separation of petroselinic and oleic acids in the simple form of methyl esters, but the time of analysis will probably be considerably longer (22).

Consequently, very simple methods exist to separate and simultaneously quantitate *cis*-6, *cis*-9, and *cis*-11 18:1 acids with no more than a gas chromatograph equipped with a flame-ionization detector and reagents that are habitual in all lipid laboratories.

However, a paper appeared recently in this journal, entitled "A Simple and Rapid Method for Concurrent Determination of Petroselinic and Oleic Acids in Oils" (23). It is necessary to quote the procedure from this article. "The method is based on transesterification of the fatty acids to methyl esters, epoxidation of the double bonds, and opening the oxirane ring to obtain the corresponding chlorohydrin derivatives. After silanization of the hydroxy groups, the mixture is analyzed

using high-resolution gas chromatography (HRGC)–mass spectrometry; obviously, simultaneous HRGC determination of fatty acid composition must be carried out." What is obvious is that the method is neither simple nor rapid. Four steps are necessary for the derivatization, two GLC analyses are needed, and the fragments can be quantitated only with a mass spectrometer. Moreover, the derivatization procedure does not allow separation of petroselinic and oleic acids by GLC. The authors (and the reviewer) were apparently not aware of the recent developments in this field, which is confirmed by the list of references in their paper that stops precisely in 1992.

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