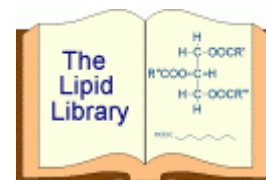


Analysis Of *Trans* Fatty Acids



Abstract: *Trans* fatty acid determination requires a skilled analyst with interpretive skills. There is no simple universal method that can be given to a trainee technician, who might be expected to analyse many samples each day.

For some years the nutritional value of fatty acids containing double bonds of the *trans*-configuration has been a matter for debate. My impression is that a consensus has emerged that dietary *trans* fatty acids are indeed harmful with estimates of potential fatalities ranging up to 20,000 per annum in the USA. This has led to pressure for the *trans*-fatty acid content of foods to be listed on labels, and it has thereby presented lipid analysts with a considerable challenge. I suspect that *trans* polyunsaturated fatty acids, which have been largely neglected (probably because of perceived problems in analysis), may be even more important in this context than *trans* monoenes.

Trans-fatty acids occur naturally in many foods, especially those from ruminant animals as in dairy products. In this instance, 11-*trans*-18:1 is the main fatty acid, although other positional isomers are also present as is a small amount of a conjugated diene - 9-*cis*,11-*trans*-18:2. These are formed as a normal part of the digestive processes by bio-hydrogenation of dietary fatty acids by microbial enzymes in the rumen. Higher proportions and more positional isomers of *trans*-fatty acids are found in commercially hydrogenated fats, centred upon the *trans*-9-18:1 isomer, though the amounts have been falling because of improved selectivity of catalysts and of a greater awareness by manufacturers. With hydrogenated vegetable oils, C₁₈ monoenes are the main components but there are usually some dienes with isolated or conjugated *trans*-double bonds. Hydrogenated fish oils contain *trans*-monoenes with a wider range of chain-lengths and probably also significant amounts of partially hydrogenated polyenoic fatty acids with *trans*-double bonds, the latter existing in the form of innumerable positional isomers.

There is no shortage of published methods, but how good are they? I have a distinct impression that a search is on for shortcuts in the methods, when none is possible. Everyone wants a method that can be given to a trainee technician, who may be expected to plough through hundreds of samples each day. In fact, *trans* fatty acid determination is a problem that still requires a skilled analyst with interpretative ability. The topic has been the subject of substantial reviews [1,2], and is discussed from a practical standpoint in my book [3].

Fourier-Transform Infrared Spectroscopy (FTIR)

Infrared (IR) spectroscopy has long been a popular method for the determination of the *trans*-content or more accurately of the content of isolated *trans*-double bonds of fats. An isolated *trans*-double bond absorbs in the IR region at a wave number of 967 cm⁻¹, equivalent to a wavelength of 10.34 μm, as a result of a deformation of the adjacent C-H bonds. When a *trans*-double bond is part of a conjugated system, the wavelength is displaced to higher wave numbers, e.g. to 983 cm⁻¹ in a *cis,trans*-diene and to 994 cm⁻¹ in a *trans,trans,trans*-triene. The approved methods for determination of *trans*-double bonds in a fat have involved preparation of the methyl ester derivatives and scanning the IR absorbance in the region 900 to 1050 cm⁻¹; the area of the *trans*-peak is compared with that of a calibration curve prepared from standard solutions of methyl elaidate. Whether low levels of conjugated isomers would be determined accurately is unlikely.

The newer technique of Fourier *transform* infrared (FTIR) spectroscopy is capable of the determination of isolated *trans*-double bonds in commercial fat and oils samples with greater ease and accuracy, since it is no longer necessary to derivatize or to dissolve in solvents prior to the analysis; automation of sample handling and data collection is also possible. Traditional IR methods draw a linear base line from which the peak area is measured, although a sample with a zero *trans* content will have a curved base line between the selected wavelengths. This inevitably leads to substantial errors that are magnified at low *trans* fatty acid concentrations.

FTIR spectroscopy is inherently more accurate than the older IR methods, and it also has the advantage of being controlled by a computer. This means that it is no longer necessary to draw linear base lines manually under the *trans* peak. Rather it is possible to use the computer to subtract a true base line derived from a similar zero-*trans* sample to calculate the area of the peak [4,5]. In addition the data can be subjected to partial least squares analysis to improve the precision. This means that accurate results can now be obtained at relatively low levels of *trans* monoenes (< 2%). The accuracy of the procedure with samples such as margarines is still limited by the content of *trans* polyunsaturated fatty acids, but the potential error is now much smaller.

Much of the published work being done with FTIR spectroscopy linked to gas chromatography has been impressive, especially those papers from Mossoba's laboratory (*cf.* reference 5). However, the cost of the equipment seems to have deterred others from following this path. Certainly, it has many uses as a research tool, especially for *trans* polyunsaturated fatty acids, so I expect to continue to see new published applications.

Gas Chromatographic Methods

The alternative method preferred by most analysts is probably gas chromatography of the methyl ester derivatives on fused-silica capillary columns coated with highly polar stationary phases such as SP-2340TM, SP-2560TM or CP-Sil 88TM, ideally in columns that are 100 m in length. With such columns, two overlapping series of peaks corresponding to the *trans*- and then the *cis*-monoenes are seen for each chain-length. The analyst must decide where the point of division between each group lies, as it is well established that there is some overlap of isomers. Therefore, drawing this line inevitably carries a degree of arbitrariness. New stationary phases are reported from time to time that may ease but do not eliminate the problem. While conjugated and some other partially hydrogenated isomers can sometimes be identified, it is doubtful whether every fatty acid containing a *trans*-double bond can be recognised. In addition, small changes in chromatographic conditions can influence relative retention times and increase the difficulties. Ratnayake *et al.* [6] have shown how separate analyses by IR spectroscopy and GC can be combined with a 'fiddle factor' (my term not theirs) to improve accuracy, but difficulties remain.

If these limitations are recognised, consistent results with at worst a small constant error are achievable, although this is still a technique for the skilled analyst rather than the beginner.

Reversed-Phase Chromatography

HPLC in the reversed-phase mode is a somewhat neglected technique for the separation of *cis*- and *trans*-isomers, with elution in the order stated, that has given excellent resolution in skilled hands [7]. The position as well as the configuration of the double bonds is relevant to the elution order, and there is some danger of *trans*-isomers in which the double bond is roughly central overlapping with *cis*-isomers in which the double bond is at either end of the molecule, but no

more so than with other techniques. When used as a preliminary fractionation method prior to analysis by capillary GC, some useful separations have been achieved.

Silver Ion Chromatography

Silver ion chromatography (see our web pages on this topic), either in the form of TLC or HPLC, is probably capable of better resolution of corresponding *trans*- and *cis*-forms of a given positional isomer of a mono-unsaturated fatty acid than any other technique. HPLC techniques in which the silver ions are bound to an ion-exchange support afford better resolution, as an interaction between the silver ions and the double bonds only is observed and any adsorption effects are minimal.

Many years ago, a colleague and I [8] described a method for *trans* analysis involving separation of a saturated plus *trans* band (methyl ester derivatives) by silver ion TLC combined with GC analysis of the sample before and following the separation; the saturated fatty acid components of the sample served as an internal standard. This was subsequently updated to silver ion HPLC [4]. By using a short silver ion column, we could get complete separation of saturated, and *trans*- and *cis*-monoenoic derivatives in under 10 minutes. The analysis was completed by collecting fractions for quantification by GC. Supelco Inc. now market disposable solid-phase extraction columns for the same purpose.

It is my contention that silver ion chromatography is the only technique to have the potential for complete and accurate analysis of *trans* fatty acids, when used in combination with capillary gas chromatography. This has been confirmed by a recent inter-laboratory study [9]. It may be essential for *trans* dienes [10]. I have heard that AOCS and IUPAC have avoided such methodology because it was felt that silver ion TLC in particular is not suited to routine analysis of large numbers of samples. This is arguable since the technique has been used in hundreds of laboratories around the world for over forty years. Silver ion HPLC and solid-phase extraction techniques are clean, simple and rapid, and they surely deserve more consideration.

Food processors and retailers are increasingly making claims that *trans* fatty acids have been eliminated from their products. If these claims are to be checked, the authorities that set standards are going to have to look at silver ion methodology. It may not be suitable for novices, but it will keep skilled analysts in gainful employment.

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