Application Note
Quantitation of Trans-Fat in Food

Trans fatty acids are partially hydrogenated oils found in a variety of processed foods. Twenty years ago, food scientists considered these fats to be relatively harmless, but research starting in the late 1980's showed that trans fat raises LDL cholesterol, may lower HDL ("good cholesterol") levels and is a likely promoter of heart disease. In 2002, the National Academy of Sciences recommended that people consume as little trans fat as possible and the food industry in combination with the FDA have started a labeling program to assist in the effort.

The most efficient method of quantitating trans fat is to extract the oils and analyze the mixture with gas chromatography. After isolation, the oil fraction is saponified and esterified according to AOAC 969.33 and AOCS Ce1c-89 procedures. Using chromatographic conditions standard for this analysis results in an assembly of completely- and partially-resolved peaks in the chromatogram (Chrompak 7488, 50 meter length, 250 µ diameter).

Quantitation of a single, well-resolved peak is straightforward with the chromatographic software. Even if retention time variability is present, setting a wider integration window for particular analytes can accommodate the variability. In some instances, however, a range of peaks needs to be summed in order to cover a sequence of incompletely resolved compounds. This case is illustrated in Figure 1.

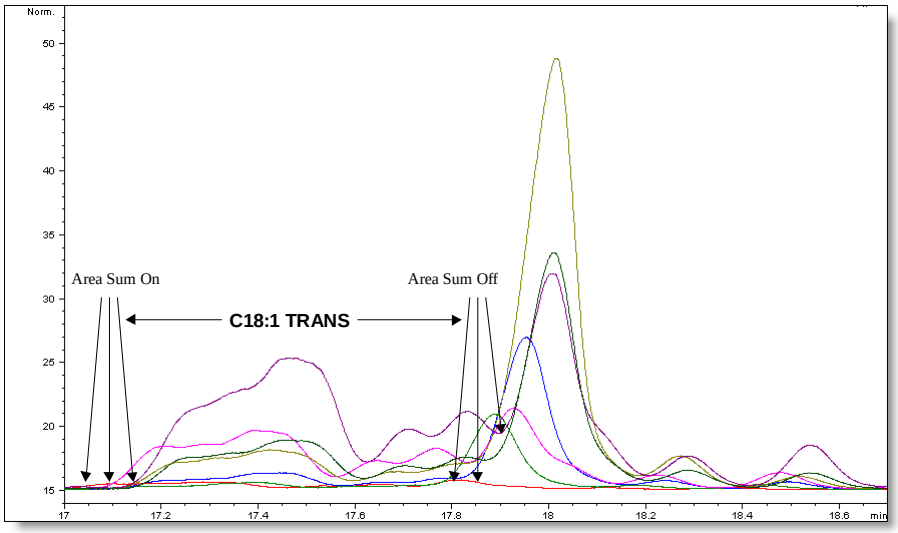

Figure 1: Retention times in several chromatograms have changed such that peak start and end marks cannot be set with confidence

When area summation is used for quantitation, retention time inconsistency demands that integration must be redone manually. For automated evaluation of samples, this manual review of the chromatogram is not practical. It is also not practical to completely eliminate the retention variability of the column and conditions even with pressure modulation hardware. We can, however, use the mathematical alignment technology in LineUp™ to automatically correct the retention shift immediately after data collection.

The software can be called as part of a method and will process raw chromatograms: alignment is done against one or more target chromatograms and a correlation that specifies the fit quality is computed. The highest correlation alignment is accepted and the resulting aligned chromatogram is written to a new file in native format, into the directory containing the original file. A different file name is used to avoid overwriting the original data. One version of LineUp works with Agilent GCs spanning the 5890, 6850 and 6890 models and contains ChemStation macros to facilitate the processing. A second version is optimized for AIA files from any single-channel detection chromatograph (*e.g.*, PerkinElmer, Waters, Varian, Thermo); utilities aid automatic processing of data from EZChrom *Elite*™ or derivative products (*e.g.*, Shimadzu, Beckman).

With LineUp, automated alignment can be incorporated into any company site without requiring specialized training of the technician. Chromatograms from different sites can be aligned without requiring that instruments be set up in any particular way ahead of time.

The results of alignment are shown in Figure 2. Elimination of the retention time shift allows a single integration method to process the data without user intervention, including peak summation.

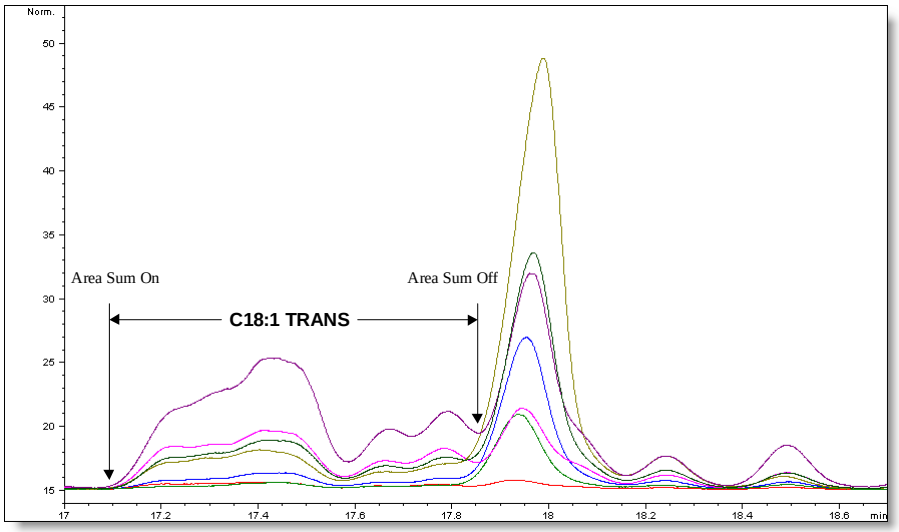

Figure 2: After alignment, the chromatogram can be processed using exact times for starting and stopping the area summation.

Post-run chromatographic alignment is a practical method of insuring compliance with the intentions of the chromatographic method. The minimization of retention time variation is fully automatic.

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