

140). Thus, the IOM definition includes both conjugated and non-conjugated double bonds in the *trans* configuration, whereas FDA's definition only includes *trans* fatty acids with nonconjugated double bonds. In addition, the IOM report considers conjugated linoleic acid as a collective term for geometric and positional fatty acids in which the double bonds (*trans* and/or *cis*) are conjugated. In the IOM report, the categories, *trans* fatty acids and conjugated linoleic acid, overlap. Under FDA's definition, conjugated linoleic acid would be excluded from the definition of *trans* fat. Thus, using FDA's regulatory chemical definition, the categories "*trans* fatty acids" and "conjugated fatty acids" are mutually exclusive. The definition of *trans* fatty acids, excluding fatty acids with conjugated double bonds, is consistent with the way that *cis* isomers of polyunsaturated fatty acids are defined in redesignated § 101.9(c)(2)(iii).

D. Methodology

(Comment 20) One comment asked whether the Association of Official Analytical Chemists (AOAC) Official Method 996.01 can be used for measuring *trans* fat in foods. The comment noted that, at present, AOAC Official Method 996.01 is the ideal method for the measurement of total fat, saturated fat, and mono- and polyunsaturated fat in foods. The comment noted further that AOAC Official Method 996.01 was originally intended for cereal products containing 0.5–13 percent total fat and that recently, a study by Ali et al. (Ref. 30) demonstrated its applicability to all types of food matrices with fat contents ranging from 0.7 to 97.5 g/100 g food. The comment noted that the method of Ali et al. (Ref. 30) used an SP-2560 fused silica capillary column (100 meters (m) x 0.25 millimeter (mm)) and can be used for the accurate determination of *trans* fatty acids. The comment noted that if appropriate gas chromatography (GC) operating conditions are selected, the SP-2560 column as well as columns of similar polarity give a very good separation of *cis* and *trans* isomers.

FDA notes that, as currently written, AOAC Official Method 996.01 is not suitable for quantifying *trans* fatty acids for food labeling purposes because the capillary column specified (i.e., 30 m x 0.25 mm id., 0.2 µm film, non-bonded 90 percent cyanopropyl, 10 percent phenyl siloxane) is not sufficiently long to obtain adequate separation of the *cis* and *trans* fatty acids. Ali et al., (Ref. 30) modified the method and used a 100 m flexible fused silica column (SP-2560,

100 m x 0.25 mm id., 0.20 µm film thickness) to obtain better separation of isomers in food samples. Specifically, better resolution in the complex 18:1 and 18:2 regions was obtained with the longer column. FDA has found that when appropriate operating conditions are selected, the SP-2560 column and other columns of similar polarity give a very good separation of *cis* and *trans* isomers. We point out, however, that the modification described by Ali et al., (Ref. 30) has not been subjected to a collaborative study and is not an official method.

It is important to note that FDA regulations do not specify the methodology that firms are to use in obtaining values for nutrition labeling purposes. Rather, under § 101.9(g)(2), FDA determines compliance with nutrition labeling rules by using appropriate analytical methods "as given in the 'Official Methods of Analysis of the AOAC International' 15th Ed. (1990) or, if no AOAC method is available or appropriate, by other reliable and appropriate analytical procedures." Firms may choose to use a method other than that which the agency uses to determine compliance, but the firm would be subject to, for compliance purposes, a method the agency considers appropriate under § 101.9(g). With respect to analysis of fats (including *trans* fat), FDA laboratories utilize the most recent editions (including revisions of methods from the Association of Official Analytical Chemists International (AOAC; *Official Methods of Analysis of AOAC International*, 17th edition, Revision 1, 2002; AOAC International, Gaithersburg, MD) (Ref. 143) and the American Oil Chemists Society (AOCS; *Official Methods and Recommended Practices of the AOCS*, 2002–2003 Methods-Additions and Revisions, AOCS Press, Champaign, IL) (Ref. 144)).

(Comment 21) Several comments asked that FDA recognize AOAC Method 996.06 as modified in the *Journal of the Association of Official Analytical Chemists* in January 2000, as a suitable method for the analysis of *trans* fatty acids for food labeling purposes.

FDA points out that recommendations for the modification of AOAC Official Method 996.06 (Ref. 105) were published in the *Journal of the Association of Official Analytical Chemists* (Ref. 106). The recommendations are based on the work of DeVries et al. 1999 (Ref. 107). DeVries and coworkers report that while quantitation of fat in foods has been performed successfully with AOAC Official Method 996.06, a number of

situations have been encountered that render the following method note inaccurate: "For any unknown or uncalibrated peaks, use the nearest calibrated fatty acid response factors and conversion factors" (Ref. 107). Specifically, the identification of extraneous compounds and availability of additional standard fatty acid methyl esters combined with mass spectral data led to the recommendation of modifications in AOAC Official Method 996.06.

Specific recommendations for modifications include recommendations that the column requirements for the method be changed to a performance-based specification such that a capillary column capable of separating adjacent peaks of C18:3 and 20:1 and the fatty acid methyl ester trio of adjacent peaks of C22:1, C20:3 and C20:4 with a resolution of 1 or greater be used. Column SP-2560, 100 m x 0.25 mm with a 0.20 µm film was identified as a suitable column.

The recommendations referenced in the paragraph above have now been incorporated into AOAC Method 996.06 (*Official Methods of Analysis of AOAC International*, 17th edition, Revision 1, 2002; chapter 41.1.28A) (Ref. 105). This method is suitable for use in a wide range of food matrices for measuring *trans* fat for labeling purposes.

AOAC Method 996.06 cited above for *trans* fat analysis is the most current AOAC gas chromatography method available and FDA will consider it an appropriate method under § 101.9(g)(2) for determining compliance with nutrition labeling provisions for *trans* fat. AOAC Method 996.06 is not included in the 15th edition (1990) of *Official Methods of Analysis of AOAC International* (which is incorporated by reference in § 101.9(g)(2)) because the process of development and validation of this method was not completed until 1996. Therefore, AOAC Method 996.06 as it is reported in Revision 1, 2002 of the 17th edition of *Official Methods of Analysis of AOAC International* (Ref. 105) may be used as an "other reliable and appropriate analytical procedure" as provided for in § 101.9(g)(2). FDA intends to propose amendments in the future on the edition of the AOAC method listed in § 101.9(g)(2) and other needed revisions of § 101.9.

(Comment 22) One comment noted that detection methodology is not sophisticated enough to accurately measure *trans* fat in all food products. The comment stated that significant work is needed to validate the AOCS methods for food matrices other than fat and oils.