Comprehensive Determination of Trans Fats in Cookies using SP-2560 and SLB-IL111 GC Columns after Silver-Ion SPE Fractionation

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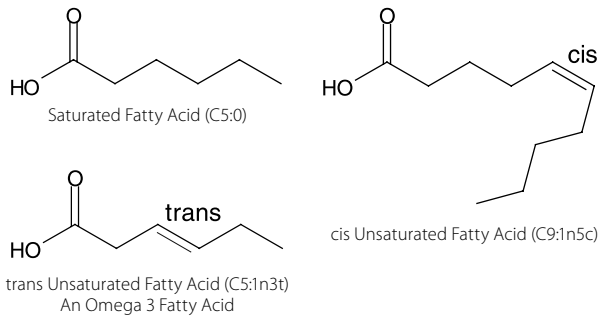
Trans Fat Labeling

Trans fats (trans unsaturated fatty acids) are fatty acids that contain at least one double bond in the trans orientation. This orientation causes carbon atoms to align in a straight configuration, similar to saturated fatty acids. Most naturally occurring unsaturated fatty acids have double bonds in the cis orientation, which is a bent configuration. Trans fats do not occur naturally in plant-based oils and fats. However, they can be produced commercially through a hydrogenation process, which is used to prevent foods from spoiling, and to produce specific structure effects (1). Examples of the structures of saturated, trans unsaturated, and cis unsaturated fatty acids are shown in **Figure 1**.

Figure 1. Fatty Acid Structures



Concerns have been raised for several decades that the consumption of trans fats may have contributed to an epidemic of coronary heart disease (1). In response, many government agencies instituted requirements that trans fat content must be listed on the nutritional labels of foods and some dietary supplements. Some regulations state if a food contains less than 0.5 g of trans fat per serving, then "0 g" is allowed to be listed on the label (2). If partially hydrogenated fats/oils are used in making the product, small amounts of trans fat will still be present (below 0.5 g even though 0 g is stated on the label).

Extraction and Analysis Methodologies

There are many references available for the preparation of samples for trans fat analysis. In 2007, the American Oil Chemists Society (AOCS®) published method Ce 1k-09 for the preparation of a variety of matrices for the analysis of lipids, including trans fats. This method describes the use of an acid digestion and alkali hydrolysis to release the fats and oils from the sample matrix, followed by methylation using boron trifluoride-methanol (BF_3-methanol) (3). The fats are then analyzed by GC as their corresponding fatty acid methyl esters (FAMEs). The GC analysis method is chosen based on the sample matrix. In the case of vegetable oils and fats, AOCS Method Ce 1h-05 can be used. This method outlines the use of a 100 m highly polar cyanopropyl silicone capillary column for the single analysis of trans isomers, saturated, cis/trans monounsaturated, and cis/trans polyunsaturated fatty acids (4). The length and high polarity of the column are necessary to facilitate resolution of the cis and trans monounsaturates in the oleic (C18:1) region.

Silver-Ion SPE (Ag-Ion SPE) Fractionation

Silver-ion (argentation) chromatography was originally pioneered for lipid analysis by Morris, who demonstrated the first practical applications by separating lipids based on degrees of unsaturation using TLC (5). In Ag-Ion SPE, silver ions are anchored onto strong cation exchange (SCX) phase functional groups through electrostatic interaction. As the FAME sample passes through, the SCX-silver counter-ions form specific polar complexes with double bonds of unsaturated FAMEs. The strength of the interaction increases with the number of double bonds, so saturated FAMEs are poorly retained. For unsaturated FAMEs, cis are retained more strongly than trans due to their greater steric accessibility to the SCX-silver ion phase. These differences in interaction strength are then used to separate FAMEs by degree of unsaturation, and also by cis/trans configuration.

In this application, a sample of commercially purchased cookies was extracted and methylated using the method described in AOCS Ce 1k-09. The extract was then fractionated using Discovery® Ag-Ion SPE, and the fractions were analyzed on two 100 m capillary columns, the highly polar SP™-2560 and the extremely polar SLB™-IL111.

Extraction of Cookie Sample

A sample of commercially purchased cookies was ground, and a 1 g sample was subjected to acid digestion and alkali hydrolysis followed by methylation, as described in AOCS Official Method Ce 1k-09 (3). A summary of the extraction and derivatization process is described in **Table 1** (page 8).

An aliquot of the resulting extract was then fractionated using Discovery Ag-ion SPE. The volume of the extract fractionated was calculated to deliver 1 mg of fat to the SPE cartridge. This calculation was made based on nutritional content reported on the product label, and the sample size and solvent volumes used for extraction. After fractionation, samples were dried and reconstituted in 1 mL of hexane for GC-FID analysis. **Table 2** (page 8) contains a summary of the fractionation procedure.

(continued on page 7)

Figure 2. SP-2560 Chromatograms

sample/matrix:	1 g of commercially purchased cookies was ground and subjected to acid digestion and alkali hydrolysis, followed by methylation as described in AOCS Official Method Ce 1k-09
SPE tube:	Discovery Ag-Ion SPE tubes, 750 mg/6 mL (54225-U)
conditioning:	4 mL of acetone; allow solvent to gravity drip completely through tube; discard eluant; 4 mL of hexane; allow solvent to gravity drip completely through tube; discard eluant
sample addition:	1 mL of extract; discard any eluant that drips through tube
elution:	(Fraction 1) 6 mL of hexane:acetone (96:4); collect eluant in a fresh container with slight vacuum; (Fraction 2) 4 mL of hexane: acetone (90:10); collect eluant in a fresh container with slight vacuum; (Fraction 3) 4 mL of 100% acetone; collect eluant in a fresh container with slight vacuum
eluate post-treatment:	evaporate each fraction at room temperature using nitrogen; reconstitute each fraction to 1 mL of hexane
column:	SP-2560, 100 m x 0.25 mm I.D., 0.20 µm (24056)
oven:	180 °C
inj. temp.:	250 °C
detector:	FID, 250 °C
carrier gas:	hydrogen, 1 mL/min
injection:	1 µL, 10:1 split
liner:	4 mm I.D., split type, single taper wool packed FocusLiner™ design

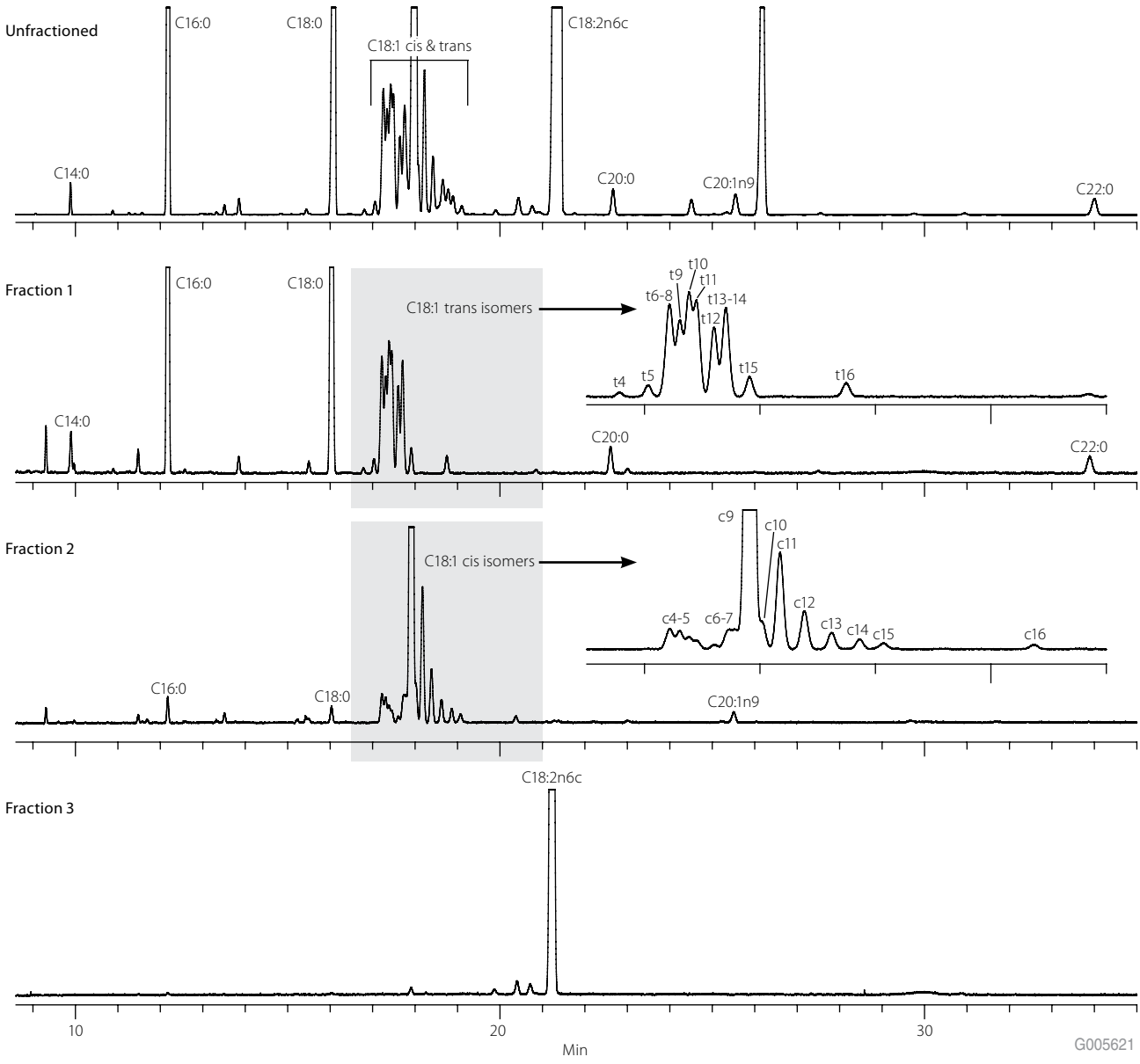
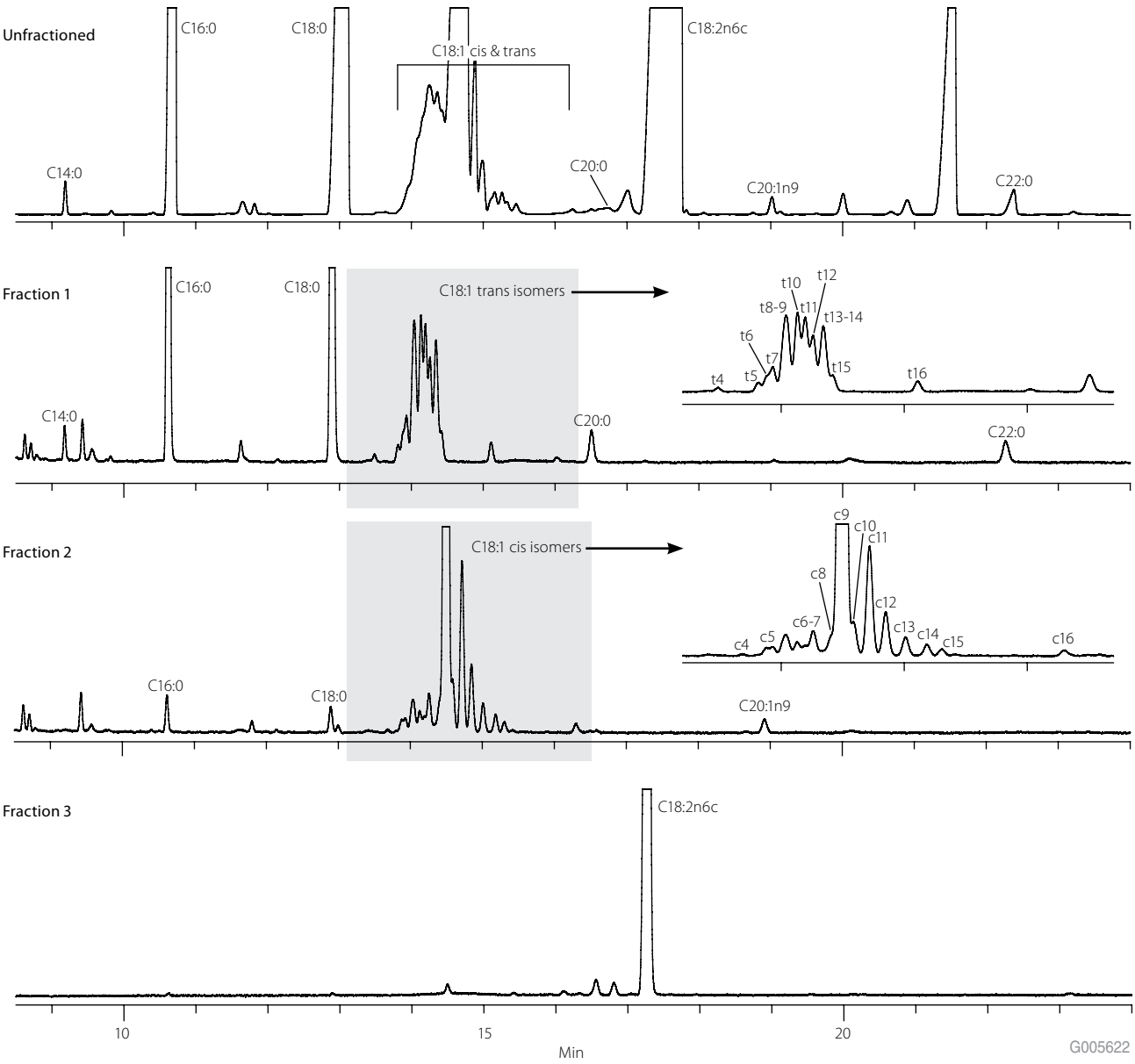


Figure 3. SLB-IL111 Chromatograms

column: SLB-IL111, 100 m x 0.25 mm I.D., 0.20 µm (29647-U)
oven: 168 °C
Other conditions are the same as Figure 2.



(continued from page 5)

Analysis on 100 m SP-2560 and SLB-IL111 Columns

The established AOCS method for analysis of fats in vegetable-based oils and fats requires GC analysis isothermally at 180 °C. This temperature has been determined to provide optimal resolution on a cyanopropyl silicone column (such as the SP-2560) of the C18 FAME isomers usually found in partially hydrogenated vegetable oils (6). The SLB-IL111 is made from an ionic liquid stationary phase, and has demonstrated selectivity characteristics indicating it to be more polar than the SP-2560. Studies of cis/trans FAMEs on this column determined 168 °C as the optimal operating temperature for this application (6).

Chromatograms of unfractionated cookie extract, and each of the three fractions collected using Ag-Ion SPE, are presented in **Figure 2** (SP-2560) and **Figure 3** (SLB-IL111). Peaks were identified using retention time and direct comparison to standards. As shown, Ag-ion SPE fractionation was able to separate the saturates and C18 trans monoenes into fraction 1, the cis monoenes into fraction 2, and the dienes into fraction 3.

The nutritional label for the cookies included partially hydrogenated cottonseed and soybean oils as ingredients. Based on this information, the fatty acid profile obtained for this sample was as expected; with cottonseed oil accounting for the small amount of C14:0 FAME, and the soybean oil as the source of the

small amount of C22:0 FAME detected. Both oils contributed to the higher levels of the C16:0, C18:0 and C18:1 FAMEs. Note the presence of the C18:1 trans isomers in fraction 1. These are the result of the partial hydrogenation of these oils.

Differences were observed in the elution patterns of the C18:1 cis and trans isomers between the two columns. For example, in Ag-Ion SPE fraction 1, C18:1Δ11t was better resolved from C18:1Δ12t on the SP-2560. In the unfractionated sample, C20:0 elutes after C18:2n6c on the SP-2560 and before on the SLB-IL111. This is due to the stronger dipole-induced dipole interaction exhibited by the SLB-IL111. At the same time, the weaker dispersive interaction demonstrated by the higher polarity SLB-IL111 resulted in faster overall elution of the FAMEs compared to the SP-2560.

Table 1. Summary of Extraction and Derivatization

1. Homogenize sample and weigh 1 g into a 50 mL round bottom flask. Add several boiling chips.
2. Add 5 mL of 1.3 M methanolic hydrochloride (HCl). Attach condenser and reflux for 15 min after mixture has started to boil.
3. Turn off heat, remove condenser and add 5 mL of 2.3 M methanolic sodium hydroxide (NaOH) to flask. Reattach condenser and reflux again for 15 min.
4. Turn off heat, remove condenser, and add 14 mL of 10% BF_3-methanol. Reattach condenser and reflux for 5 min.
5. Turn off heat, remove condenser, and add 5 mL of hexane. Remove flask from mantle and let it cool to room temperature.
6. Add saturated sodium chloride (salt) solution (aqueous) to the flask until organic layer (top) is in the neck.
7. Stopper the flask and shake. Remove top later, pass through sodium sulfate bed and save for fractionation using Ag-Ion SPE.

Table 2. Summary of Ag-Ion SPE Cleanup

1. Condition Ag-Ion SPE tube with 4 mL of acetone and allow solvent to drip completely through the tube; discard eluant.
2. Equilibrate Ag-Ion SPE tube by passing 4 mL of hexane completely through the tube; discard eluant.
3. Load 1 mL of sample extract onto the tube. Any eluant coming through the tube may be discarded.
4. Elute fraction 1 with 6 mL of hexane:acetone (96:4) and collect eluant. Be sure all solvent has been drawn through the tube. This fraction should contain any saturates, trans monoenes, and cis/cis and trans/trans conjugated linoleic acids (CLAs).
5. Elute fraction 2 with 4 mL of hexane: acetone (90:10) and collect eluant. Be sure all solvent has been drawn through the tube. This fraction should contain any cis monenes, trans/trans dienes, and cis/trans and trans/cis CLAs.
6. Elute fraction 3 with 4 mL of 100% acetone. This fraction should contain any cis/cis dienes, other dienes, and most trienes.
7. Dry each fraction down at room temperature using nitrogen, and reconstitute in 1 mL of hexane for GC analysis.

Note: Gravity should be sufficient for each step, with vacuum applied briefly only at the end of steps 4, 5, and 6, to help draw the elution solvent completely out of the SPE tube.

Conclusion

In this application, we have demonstrated the extraction of lipids as fatty acids, and subsequent detection of trans fats from a cookie sample made using partially hydrogenated vegetable oils. Ag-Ion SPE was used to fractionate the extracted sample into saturated FAMEs, and trans monoenes, cis monoenes, and dienes. Subsequent analysis of the monoene fractions on a highly polar SP-2560 column and an extremely polar SLB-IL111 column provided resolution of many of the C18:1 cis/trans geometric-positional isomers, with different elution patterns observed. The columns should be considered complimentary in the analysis of these isomers, with application-specific resolutions considered when choosing which one to use.

References

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8. *Discovery Ag-Ion SPE for FAME Fractionation and Cis/Trans Separation*; Supelco Technical Report (T406062 IRV).

Featured Products

Description	Qty.	Cat. No.
Discovery Ag-Ion SPE Tubes, 750 mg/6 mL	30	54225-U
SP-2560 GC column, 100 m x 0.25 mm I.D., 0.20 µm	1	24056
SLB-IL111 GC column, 100 m x 0.25 mm I.D., 0.20 µm	1	29647-U

Related Information

The 8-page Technical Report *SLB-IL111 for Fatty Acid Methyl Ester (FAME) Applications* (T411139, ODZ) contains 13 chromatograms and describes the benefits of this column for detailed separations of cis/trans FAME isomers, and for edible oil analysis. Request a no-charge copy by email (*techservice@sial.com*), or download a no-charge .pdf (*sigma-aldrich.com/il-gc*).

Related Products

Description	Cat. No.
Calibration Standards	
Supelco 37-Component FAME Mix 10 mg/mL (total wt.) in methylene chloride, 1 mL (visit ***sigma-aldrich.com/fame*** for composition details)	47885-U
Derivatization Reagents	
BF_3-Methanol, 10% (w/w), 20 x 1 mL	33356
Methanolic HCl, 3N, 20 x 1 mL	33355
Solvents for Pesticide Residue Analysis	
Acetone	34480-2.5L
n-Hexane	34484-2.5L
Methanol	34485-2.5L
Analytical Reagents	
Sodium hydroxide, purum p.a., >97.0%, 1 Kg	71692-1KG
Sodium chloride, purum p.a., >99.5%, 1 Kg	71381-1KG
Extraction Glassware	
50 mL single neck, round bottom flask, 24/40 female	Z414484
Modified Friedrichs condenser, 30 cm L x 54 mm O.D., 24/40 male	Z553654

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