Fractionation of cis and trans Fatty Acid Isomers for Food Samples Analysis with Hexane/Acetone on Silver SPE Material

Craig Aurand, Olga Shimelis, An Trinh, Len Sidisky, and Michael Ye
Supelco, Div. of Sigma-Aldrich, Bellefonte, PA 16823

Toshiro Kaneko
Sigma-Aldrich Japan K.K, Tennoz Central Tower 4F,
2-2-24 Higashi Shinagawa, Shinagawa-ku Tokyo 140-0002, JAPAN

Noriko Shionoya, Tomoji Igarashi, Yoshiaki Hirata, and Hirofumi Goto
Japan Food Research Laboratories, 4-5-13,
Osu, Naka-ku, Nagoya, 460-0011, JAPAN

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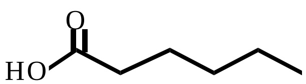
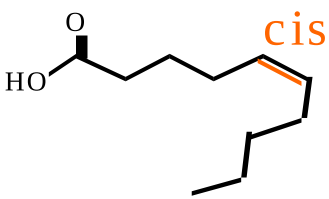
Introduction

It is well-known when vegetable oils are hardened into shortenings and margarine, some of the naturally occurring cis-fats will be converted into trans-fats. Shortenings and margarine are used in commercially baked products and fast foods for flavor preservation and spoilage prevention. As study showed, trans-fats will adversely affect blood lipids levels – increase LDL ("bad") cholesterol, thus increasing risk of coronary heart disease. It was estimated that replacing trans-fats with natural vegetable oils could prevent up to 100,000 premature deaths annually (http://www.hsph.harvard.edu/reviews/transfats.html)

Types of Fatty Acids

Structure	Common Sources	Health Effects
Saturated Fatty Acids (no double bonds)		
	**Palm kernel, Palm oil, Coconut (tropical oils), Butter, Hydrogenated Oils and Shortenings**	**Raise LDL cholesterol, and increase risk of cardiovascular disease**
Mono and Polyunsaturated Fatty Acids (≥ 1 *cis* double bond)		
	**Fluid/Liquid oils such as Soybean, Canola, Olive, Sunflower and Corn oils.**	**Lower LDL cholesterol, associated with reduced risk of cardiovascular disease.**
Trans Fatty Acids (≥ 1 *trans* double bond)		
	**Partially Hydrogenated Oils, Shortenings, Margarines, and Chips**	**Raise LDL cholesterol like saturated fat, may also lower HDL. Associated with increased risk of cardiovascular disease and possibly type II diabetes.**

Introduction (contd.)

US FDA issued a regulation that requires food manufacturers to list the TRANS FAT on the Nutrition Facts panel of foods, effect on January first of 2006.

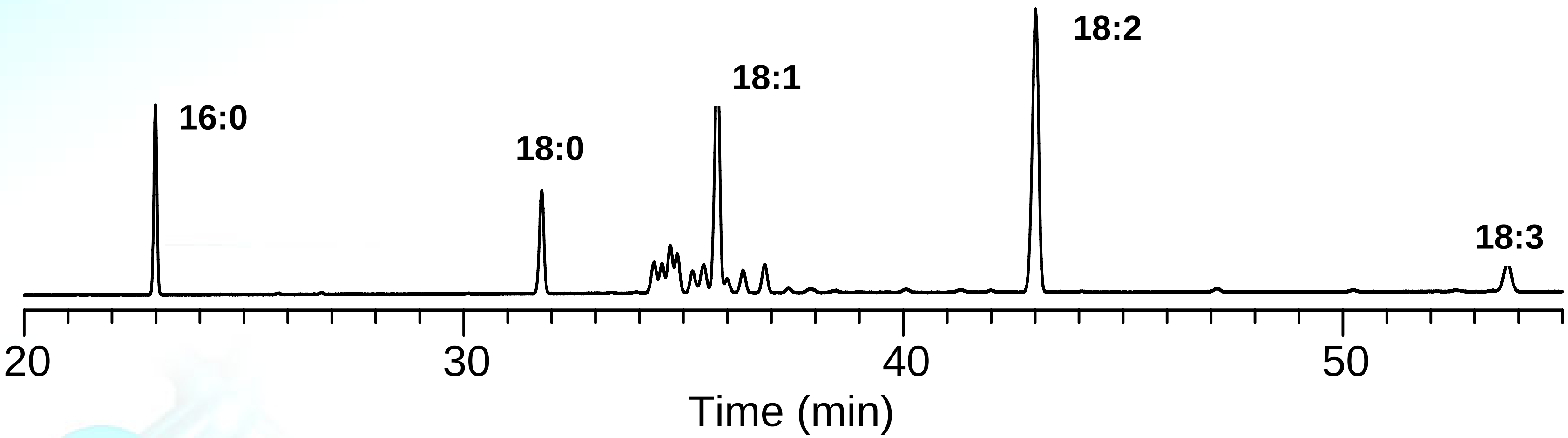
AOAC has an analytical method (AOAC 996.06) for "Fat (Total Saturated and Unsaturated) in Foods". The method specifies a sample is prepared by saponification, liquid-liquid extraction and trans-esterification and analyzed on a 100 m GC column.

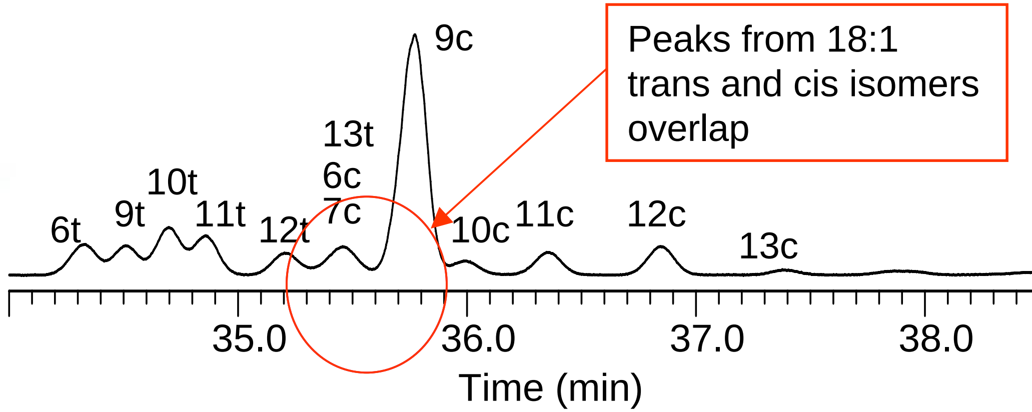
However, there are several cis and trans monoenes that overlap under the GC conditions, reducing the accuracy of the method (see following chromatograms).

The Limit of Current Analytical Method

Overlap of trans/cis monounsaturated octadecenoic fatty acids on the GC chromatogram

Extracted Fat from Margarine





Introduction (contd.)

Silver chromatography has traditionally been used to separate saturated and unsaturated compounds. W. Christie first introduced Ag-Ion SPE to separate fatty acid methyl esters by the degree of unsaturation in 1989. Japanese Food Research Laboratory developed a method on an in-house prepared Ag SPE to separate fatty acid isomers by cis/trans configuration up to trienes in 2005. Thus it opens a door for a pre-separation of cis/trans isomers prior to GC analysis.

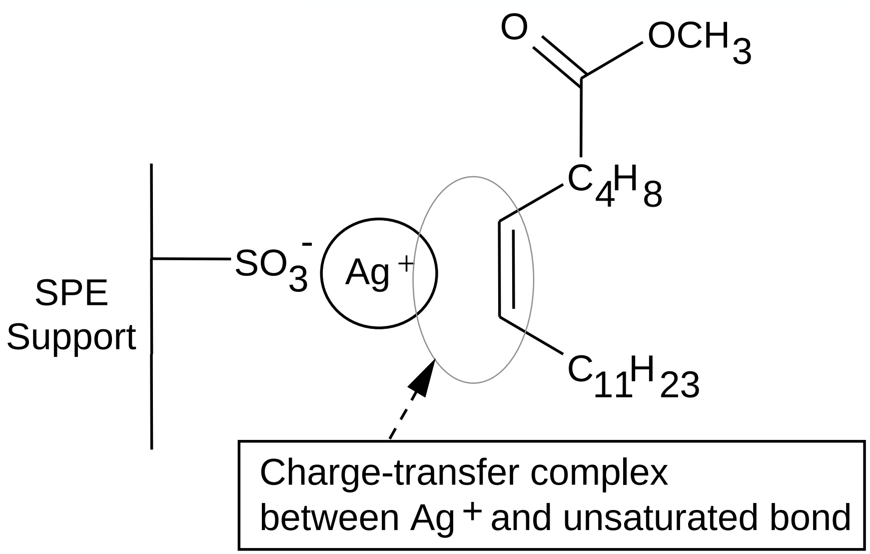
Results and Discussion

Properties of Ag-Ion SPE from Supelco

- Stable silver-loading
  - No silver bleed when common organic solvents are used
- Stable color
  - No effect of light-exposure on the Ag-Ion packing material
  - Long shelf-life
- Capacity of one 750 mg SPE tube – up to 1 mg of total FAMEs
- Reproducible resolution of cis/trans monoene FAMEs

Interaction Mechanism

- Charge – transfer
- Unsaturated compound – electron donor, Silver - electron acceptor
- One silver = 2 double bonds OR
- One silver = one double bond and one carboxyl group
- *Cis*-fatty acid isomers form stronger complexes than *trans*
- Conjugated polyenes form less stable complexes
- Strength of interactions increases with the number of double bonds



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Ag-Ion SPE Method for cis/trans Separation

1.	Condition	4 mL acetone
2.	Equilibrate	4 mL hexane
3.	Sample Load	1 mL of 1 mg/mL FAMEs in hexane at 5 mL/min.
4.	Elution **Fraction 1**	6 mL hexane:acetone (96:4 v/v)
5.	Elution **Fraction 2**	4 mL hexane:acetone (90:10 v/v)
6.	Elution **Fraction 3**	4 mL acetone
7.	Elution **Fraction 4**	4 mL acetone:acetonitrile (97:3)

8. Evaporate fractions, reconstitute in hexane for GC injection

- Fraction 1 targets saturated FAMEs and trans monoenes
- Fraction 2 targets cis monoenes and T/T dienes
- Fraction 3 targets C/C, C/T, T/C dienes, most triens

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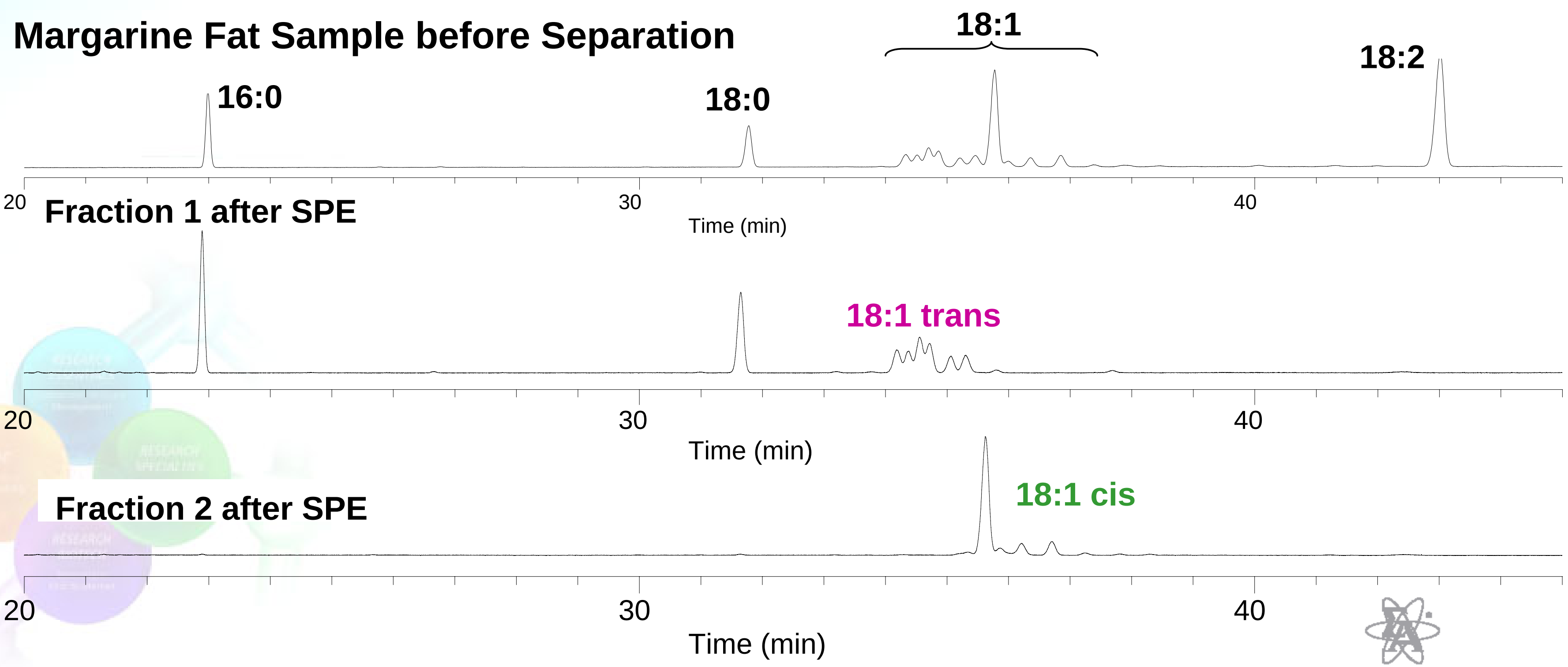
Moisture Exposure Testing of Ag-Ion SPE

- Cartridge was washed with 1 mL water prior to conditioning step to simulate residual moisture effect on separation.
- Loading and elution were done under normal phase conditions
- Fractionation of cis/trans isomers was not affected by residual moisture



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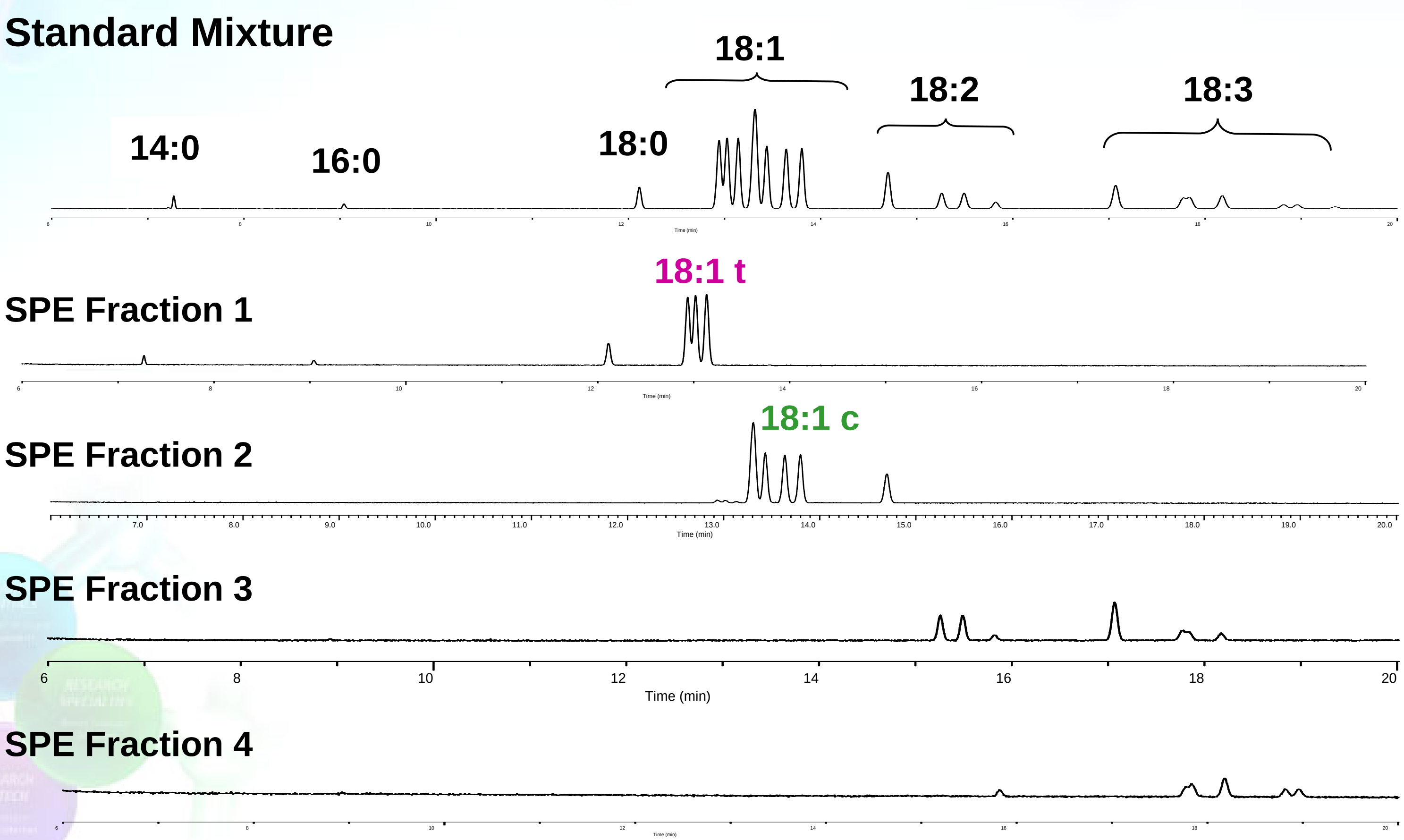
Fractionation of the Standard FAME Mixture

Standard sample, total FAMEs at 1 mg/mL



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GC Conditions using SP-2560, 75 m x 0.18 mm I.D.

Use of shorter GC column (SP-2560, 75 m) with hydrogen carrier gas significantly decreased the time required for the analysis.

oven:	180 ˚C, isothermal
inj.:	220 ˚C
det.:	FID, 220 ˚C
carrier gas:	hydrogen, 40 cm/sec. at 180 ˚C
injection:	0.5 µL, 100:1 split
liner:	4 mm I.D., split, cup design

Results of Fractionation of Standard Mix of FAMEs (% recovery)

	Elution	18:0	18:1t	18:1c	18:2tt	18:2 c/t	18:2cc	18:3ttt	18:3
1	6 mL Hexane:acetone (96:4)	100	98.1	0.4					
2	4 mL Hexane:acetone (90:10)		1.90	99.60	100				
3	4 mL Acetone					100	50	100	40
4	4 mL Acetone:acetonitrile (97:3)						50		55
	TOTAL	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**95**

Note: more polar elution solvent is needed to completely elute 18:3ccc isomer.

Fat Extraction Procedure for Potato Chips

- Ground and extract with 4 x 4 mL petroleum ether
- Evaporated and reconstituted into toluene
- Trans-esterified using 7% BF_3/MeOH
- Re-extracted into hexane after completion of reaction, dried over anhydrous Na_2SO_4
- Loaded into Ag-Ion SPE 750 mg/6 mL

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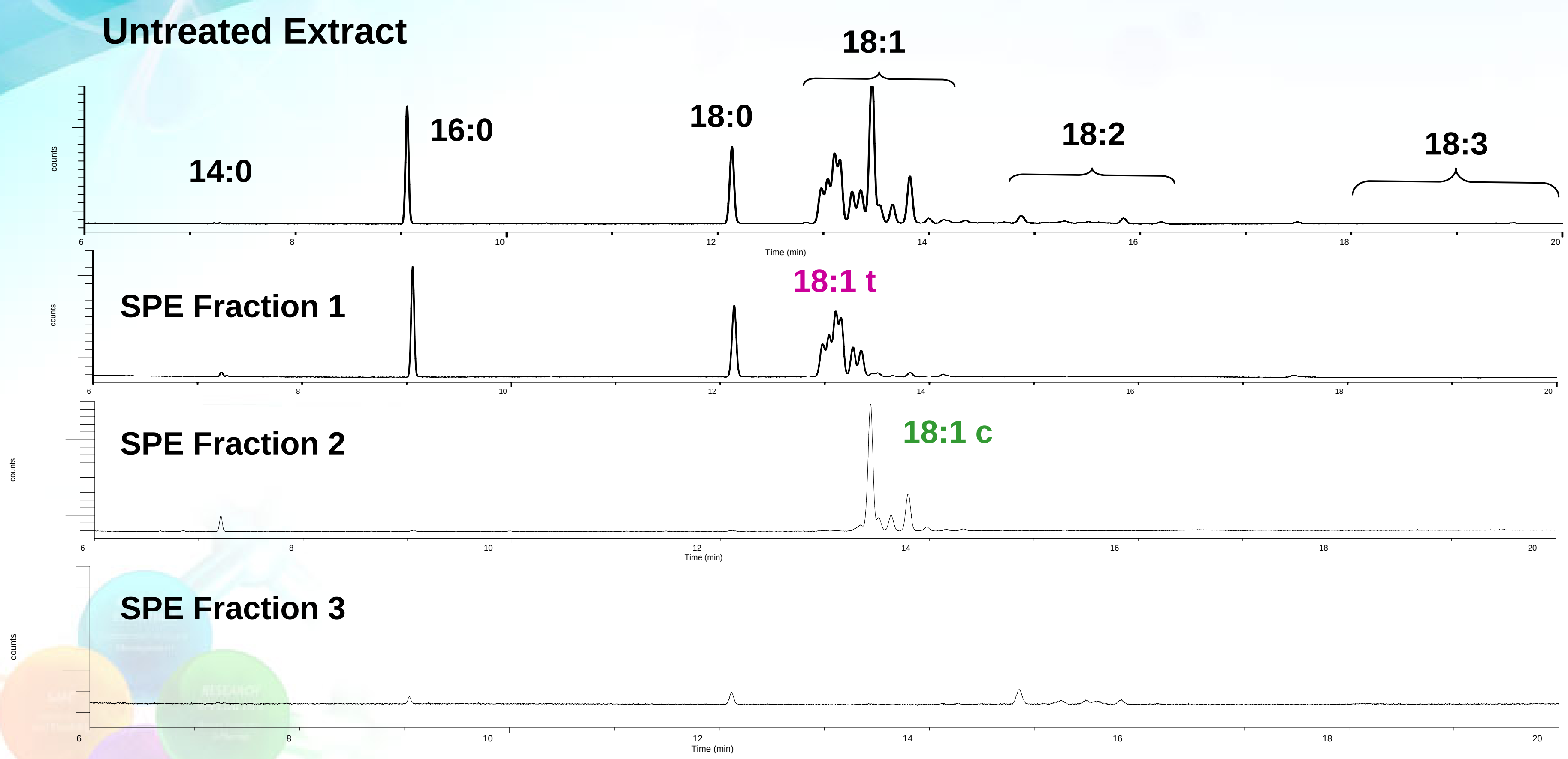
Fractionation of the Fat from Potato Chips



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Conclusion

- Ag-ION SPE completely resolves the cis/trans 18:1 fatty acids, making it possible to accurately quantify the trans fat.
- More simple technique than Ag-Ion thin-layer chromatography with no contamination from silver ions.
- Simpler fractions from complex natural samples are more easily identifiable.
- The elution protocol was proved to be robust and reproducible for variety of samples.
- The conditioning step sufficiently removed any traces of water that may affect the separation.
- Use of shorter GC column (SP-2560, 75 m) with hydrogen carrier gas significantly decreased the time required for the GC analysis.

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