Analysis of Fatty Acids

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AOAC Official Methods

969.33	Fatty acids in oils and fats	Preparation methyl esters
996.06	Fat (total, saturated and unsaturated) in foods	GC-FID
991.39	Encapsulated fish oils	GC-FID
994.15	*cis*- and *trans*-Octadecenoic isomers and general fatty acid composition	GC-IR
985.21 etc	*trans*-Fatty acid in margarines	GC-FID

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Typical analysis

- Fat and fatty acids are extracted from food by hydrolytic method.

- Fat is extracted into ether, then methylated to fatty acid methyl esters (FAMEs).

- FAMEs are quantitatively measured by gas chromatography.

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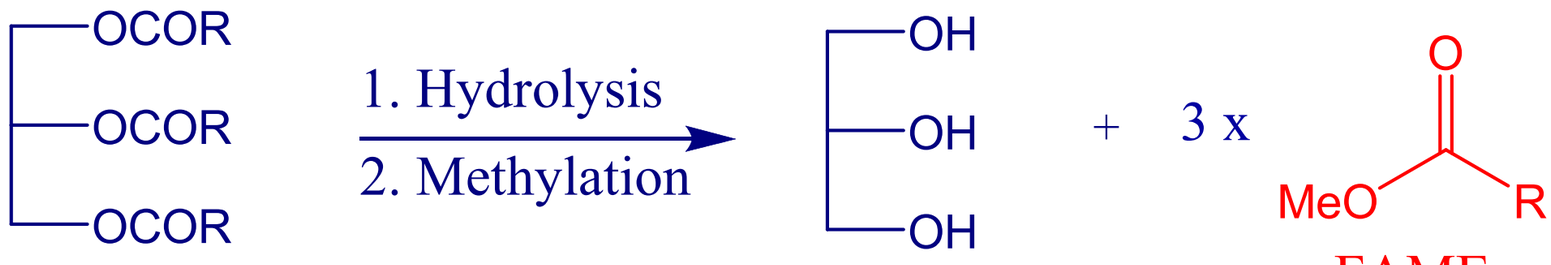
Typical analysis



R = hydrocarbon chain

GC analysis

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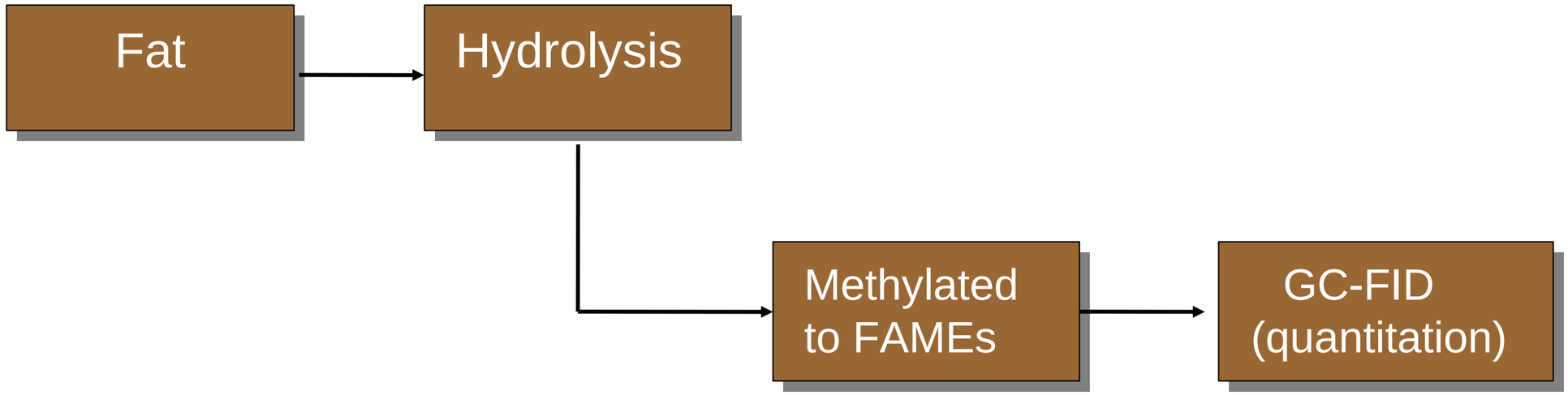
Typical analysis



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Typical analysis

Hydrolysis

Weigh sample and pyrogallic acid into the Mojonnier flask

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Typical analysis

Hydrolysis

Add internal standard, ethanol and 8.3 M HCl.

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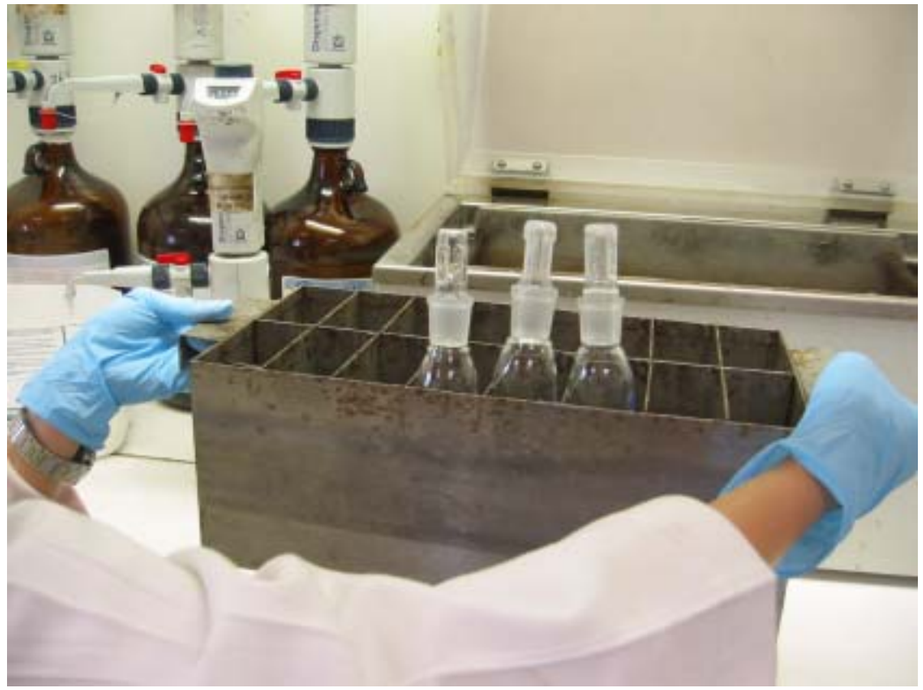
Typical analysis

Hydrolysis

Hydrolyse the sample at 70 – 80 ºC



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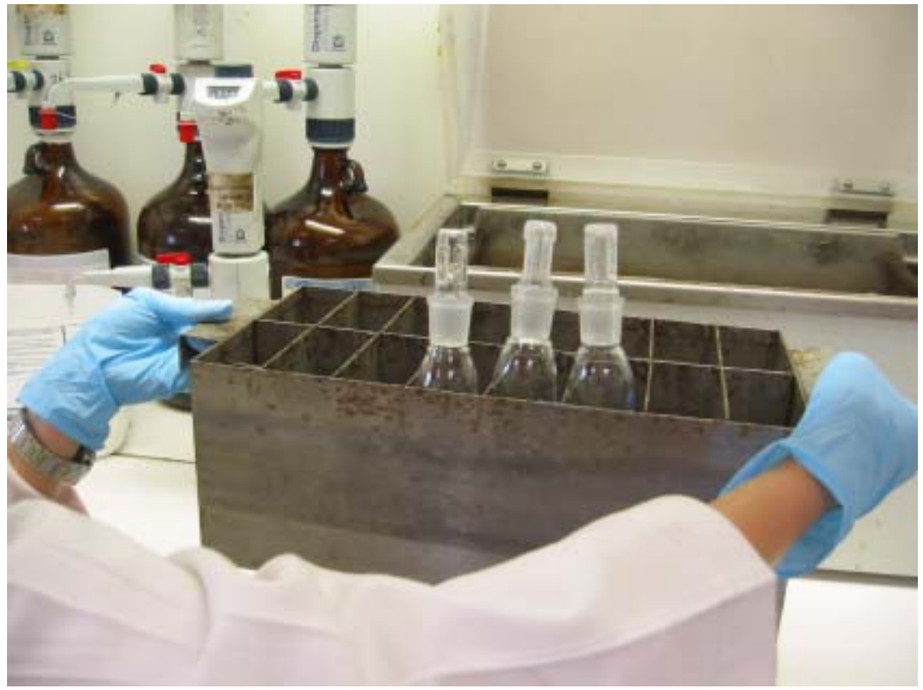
Typical analysis

Hydrolysis

Remove flasks from water bath. Cool to room temperature.



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Typical analysis

Extraction

Add ethanol,
Ether and shake.

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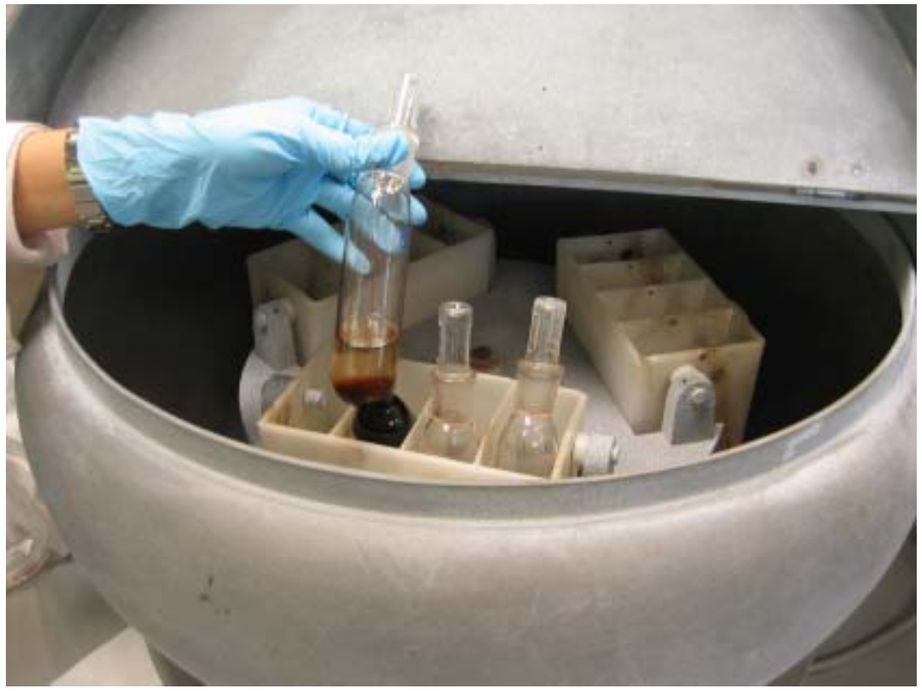
Typical analysis

Extraction

Centrifuge sample. (allow contents to sep. at least 1 h if centrifuge is not available)



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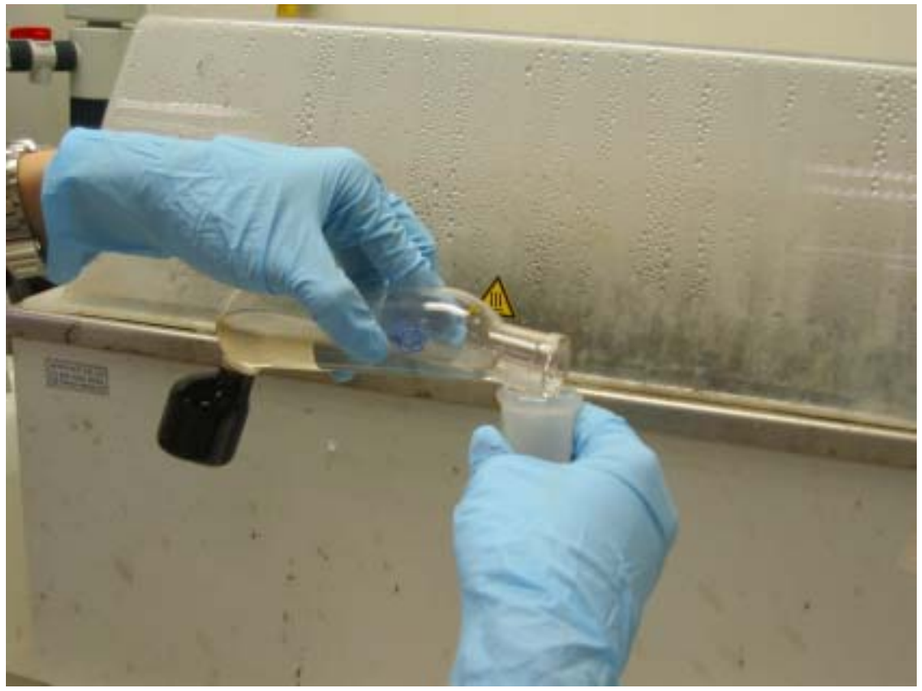
Typical analysis

Extraction

Decant the ether layer into a tube.



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Typical analysis

Extraction

Evaporate ether on water bath using nitrogen stream to aid in evaporation.

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Typical analysis

Extraction

Dissolve the residue in chloroform.

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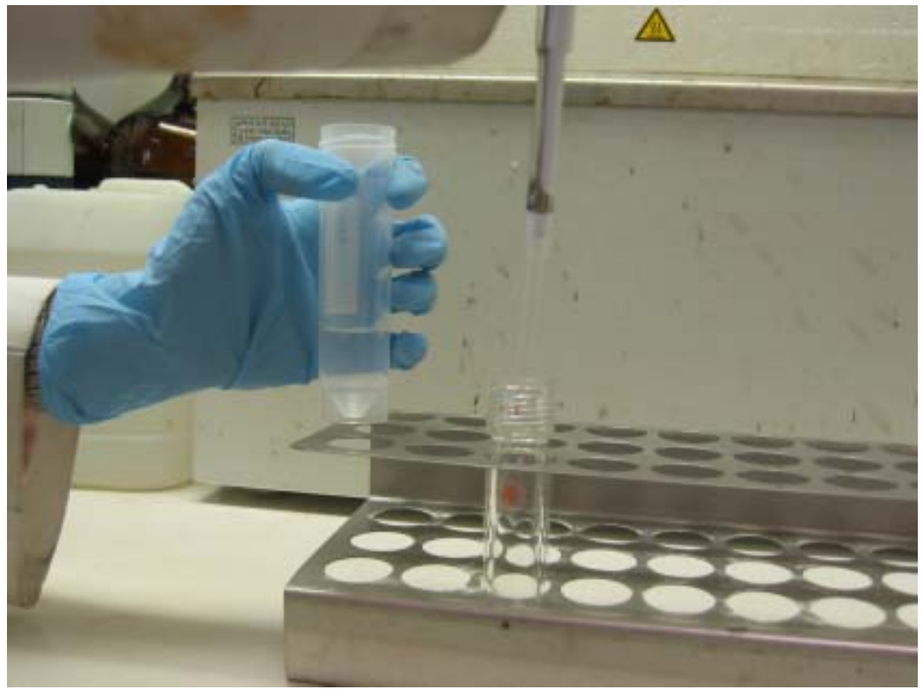
Typical analysis

Methylation

Transfer mixture to a glass vial. Evaporate to dryness.



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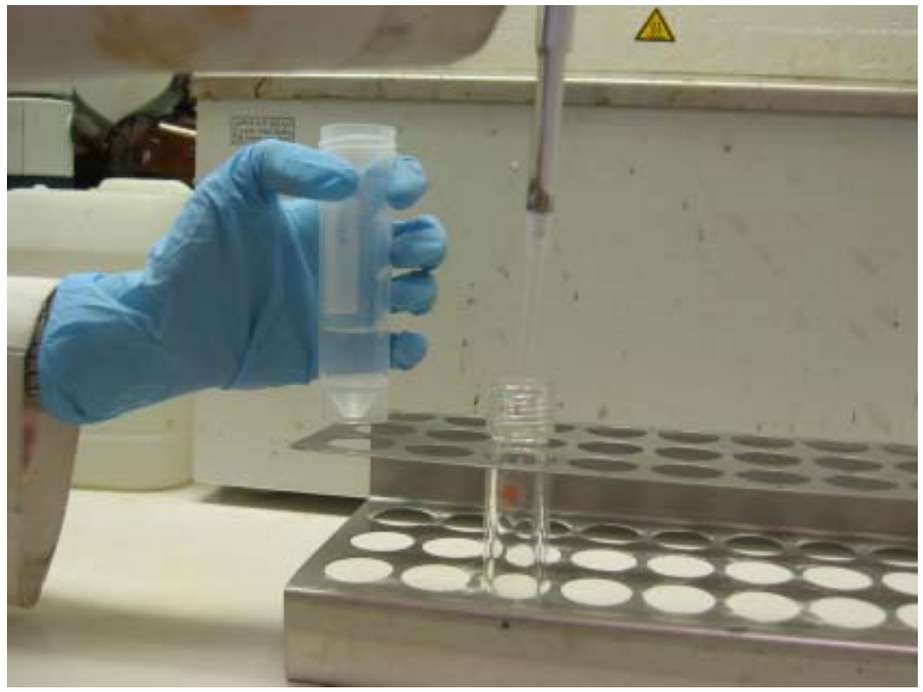
Typical analysis

Methylation

Add 7% BF_3 reagent and toluene.



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Typical analysis

Methylation

Seal vials with screwcap and heat them at 100 ^{o}C.

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Typical analysis

Methylation

Allow vials to cool to room temperature.

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Typical analysis

Methylation

Add water (5 mL),
hexane (1 mL),
Na_2SO_4 (1 g).
Shake

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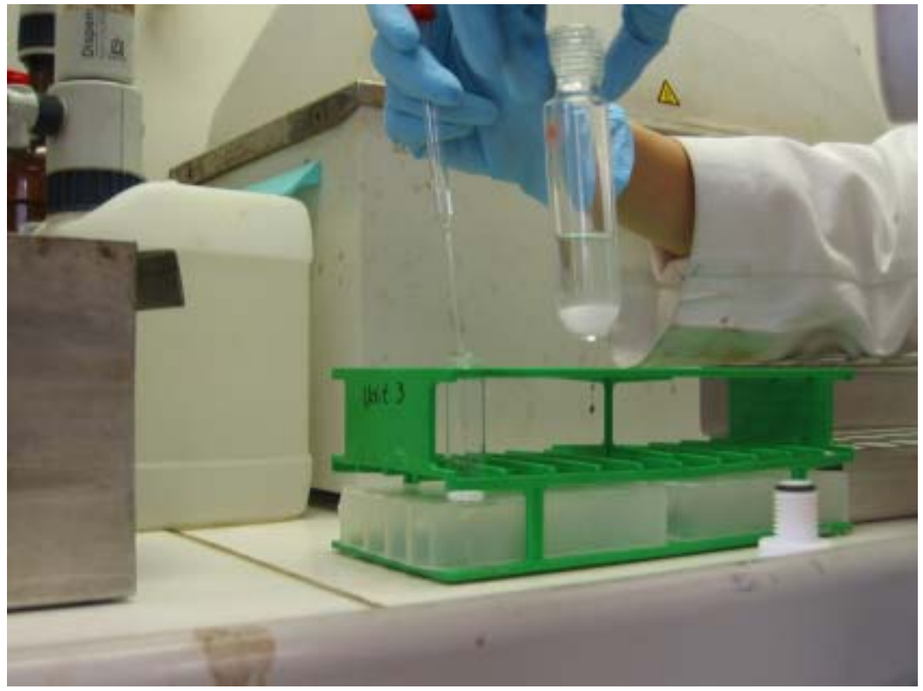
Typical analysis

Methylation

Allow layers to separate and transfer top layer to another vial containing Na_2SO_4 (1 g).



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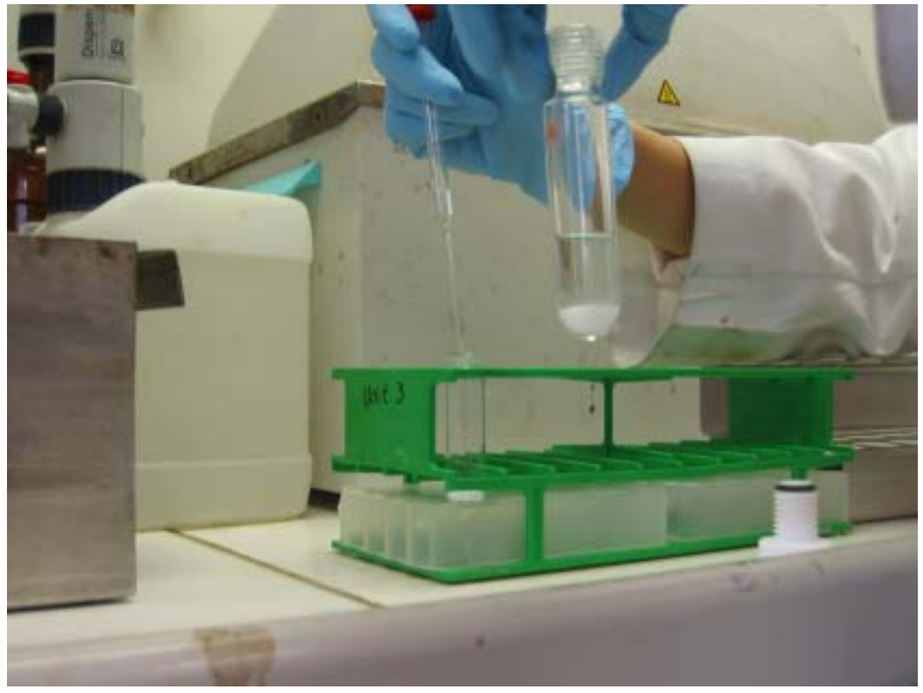
Typical analysis

Methylation

Top layer should contain FAMEs and internal standard.



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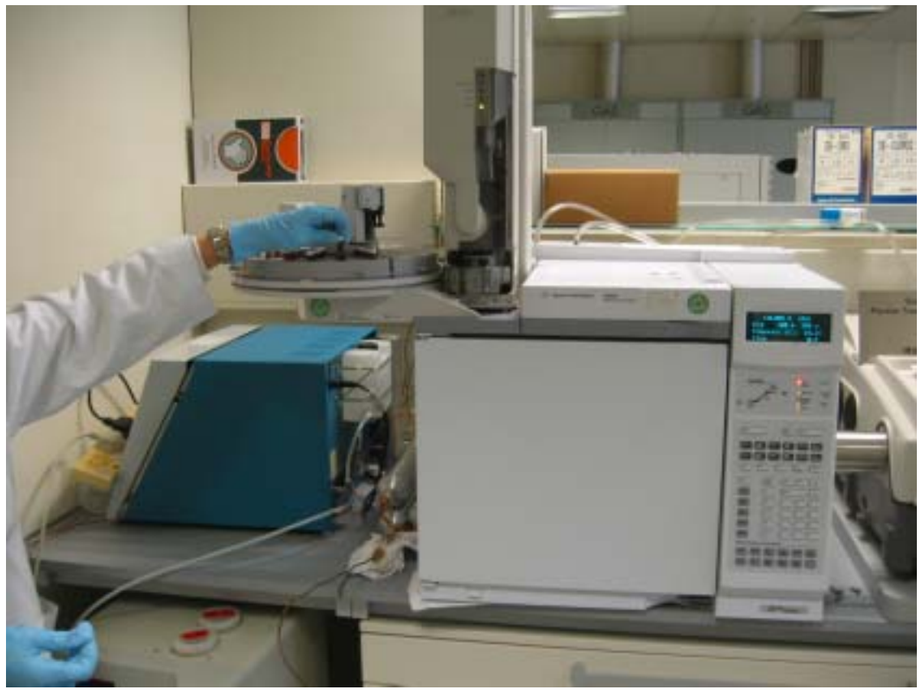
Typical analysis

GC Determination

Transfer to autosampler vial for GC analysis.



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Fatty acids (saturated)

4:0 Butyric

6:0 Caproic

8:0 Caprylic

10:0 Capric

11:0 Undecanoic

12:0 Lauric

14:0 Myristic

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Fatty acids (saturated)

15:0 Pentadecanoic

16:0 Palmitic

17:0 Margaric

18:0 Stearic

20:0 Arachidic

22:0 Behenic

24:0 Lignoceric

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Definition

- ***Trans fatty acids*** means the sum of all unsaturated fatty acids which contains at least one nonconjugated and *trans* double bond.

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trans-Fatty acids

14:1 *trans*-Myristelaidic

16:1 *trans-Palmitelaidic*

18:1 *trans 6-Petroselenic*

18:1 *trans-Elaidic*

18:1 *trans 11-Vaccenic*

18:2 *trans-Linolelaidic*

18:2 *trans 9-Linolelaidic*

18:2 *trans 12-Linolelaidic*

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trans-Fatty acids

18:2 *cis*-9, *trans*-11-Octadecadienic

18:2 Linoleic – conjugated*

20:1 Eicosenic *trans* 11

22:1 13-*trans* Docosenic

*Three 18:2 linoleic – conjugated fatty acids were listed in method 996.06

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trans-Fatty acids (18:3 *trans*)

18:3 *trans*-9, *trans*-12, *trans*-15-octadecatrienoic

18:3 *trans*-9, *trans*-12, *cis*-15-octadecatrienoic

18:3 *trans*-9, *cis*-12, *trans*-15-octadecatrienoic

18:3 *cis*-9, *trans*-12, *trans*-15-octadecatrienoic

18:3 *cis*-9, *cis*-12, *trans*-15-octadecatrienoic

18:3 *cis*-9, *trans*-12, *cis*-15-octadecatrienoic

18:3 *trans*-9, *cis*-12, *cis*-15-octadecatrienoic

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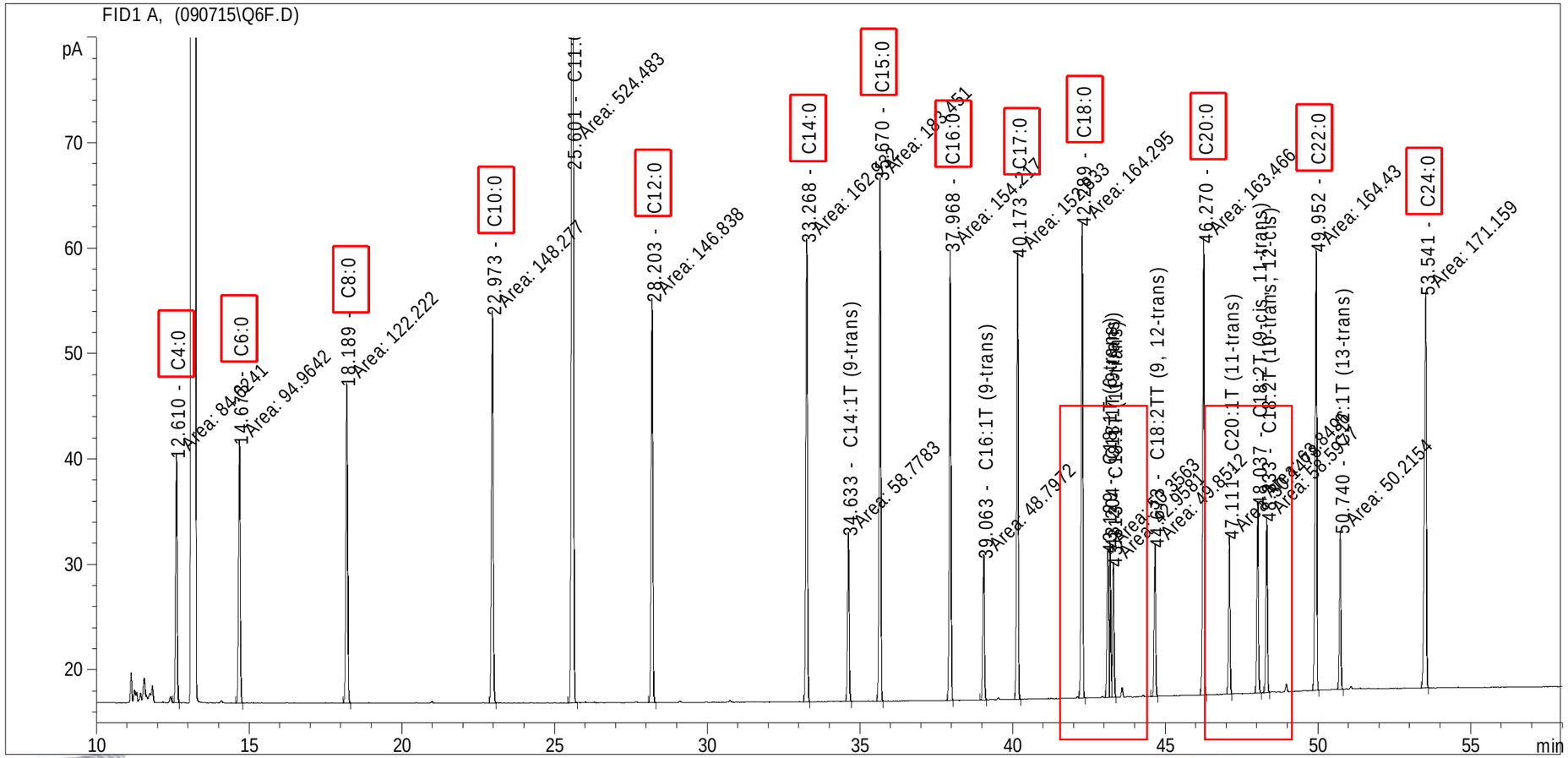
FAME (saturated and *trans*)

SP-2560, 100m x 0.25mm ID, 0.2 μm,
flow rate: 0.7 mL/min



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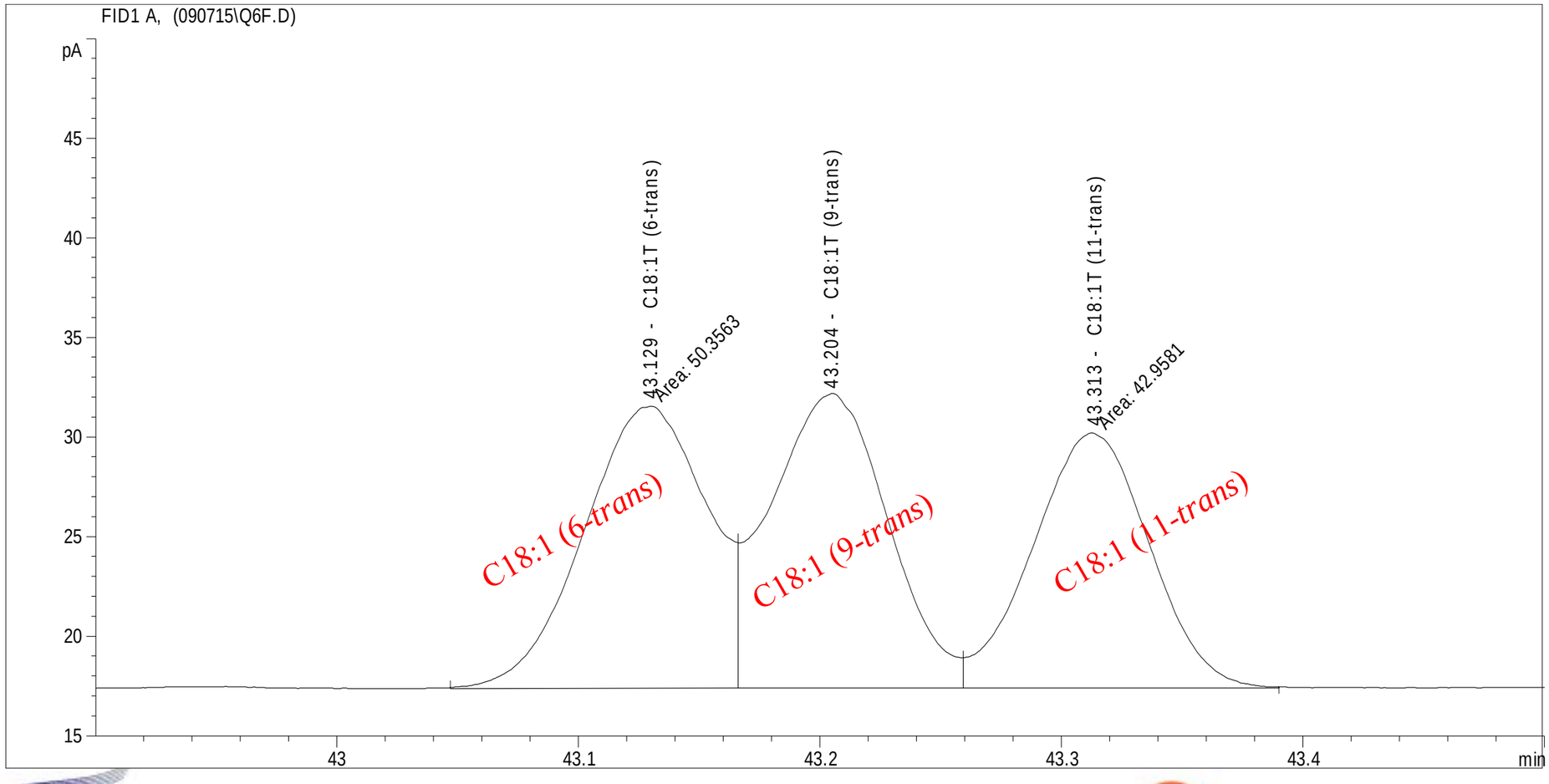
FAME (18:1 *trans*)



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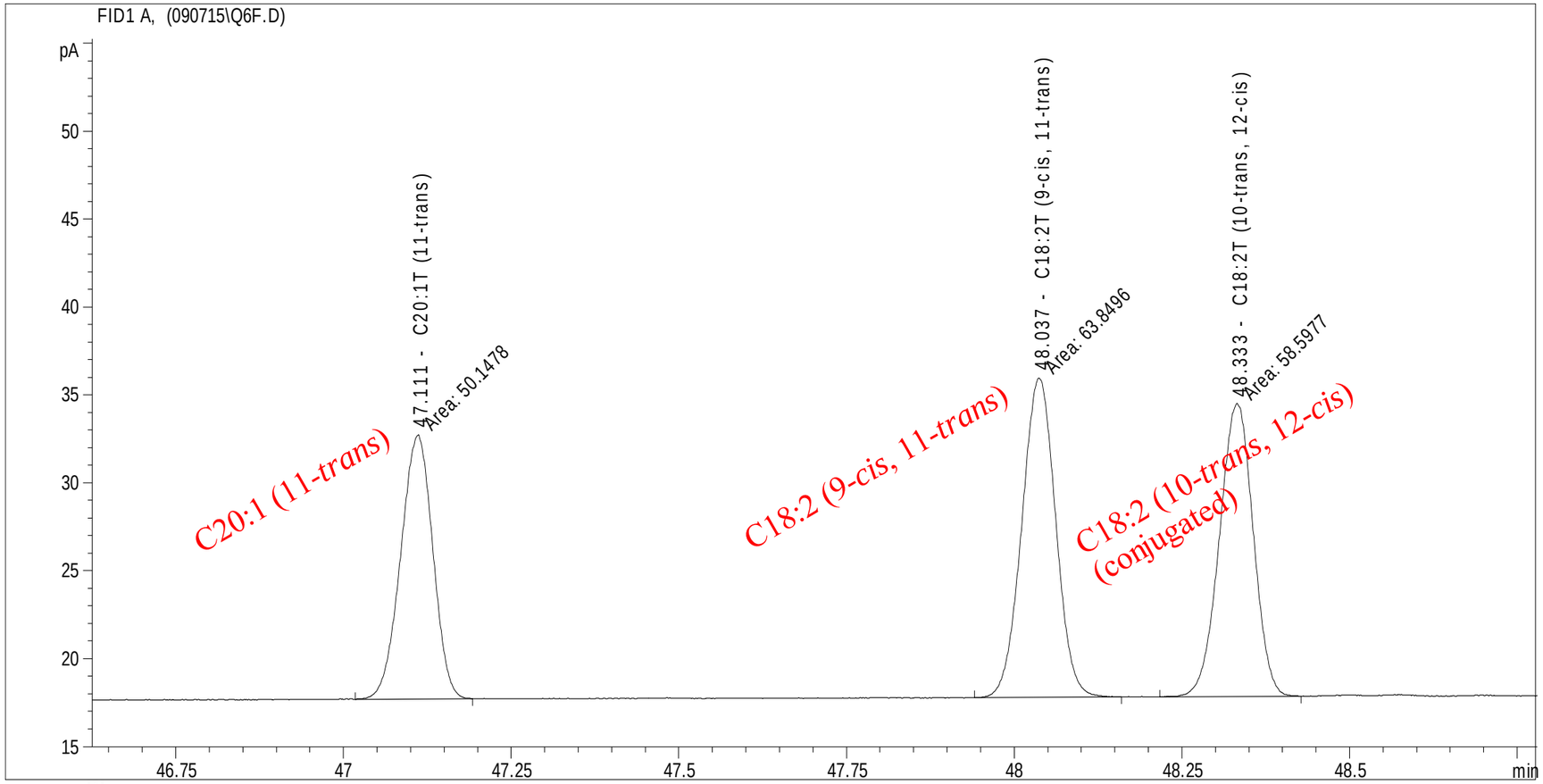
FAME (20:1 *trans* and 18:2 *trans*)



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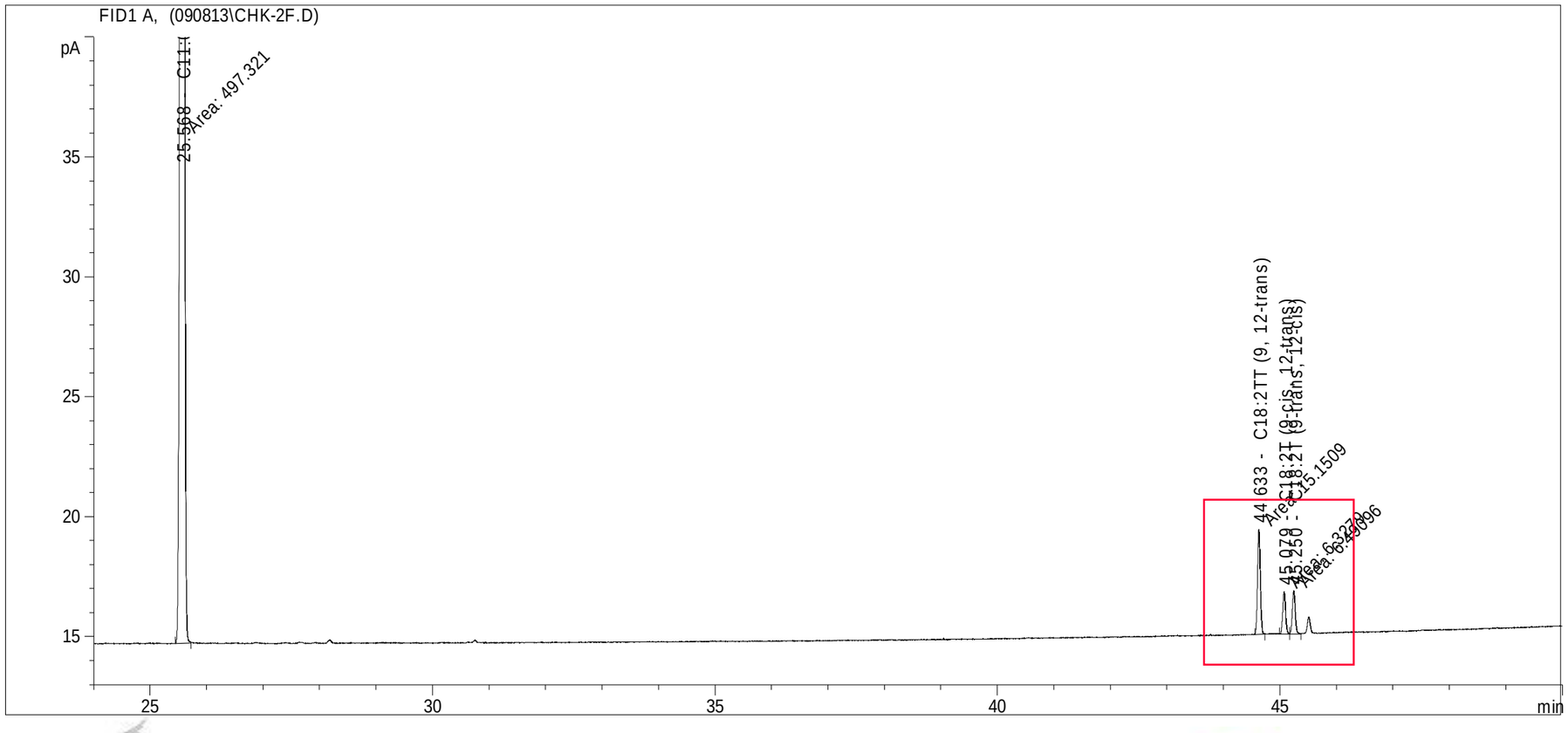
FAME (18:2 *trans*)



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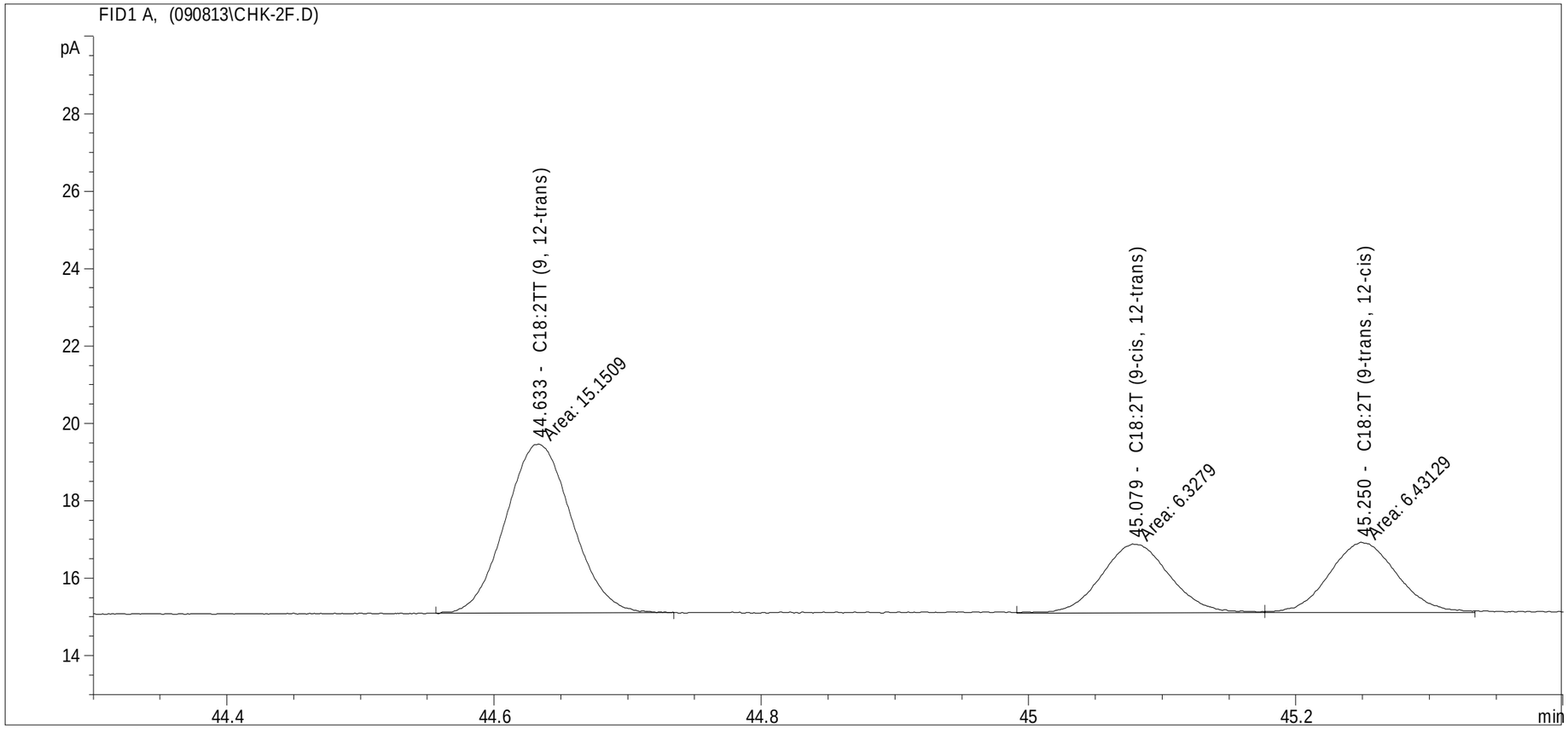
FAME (18:2 *trans*)



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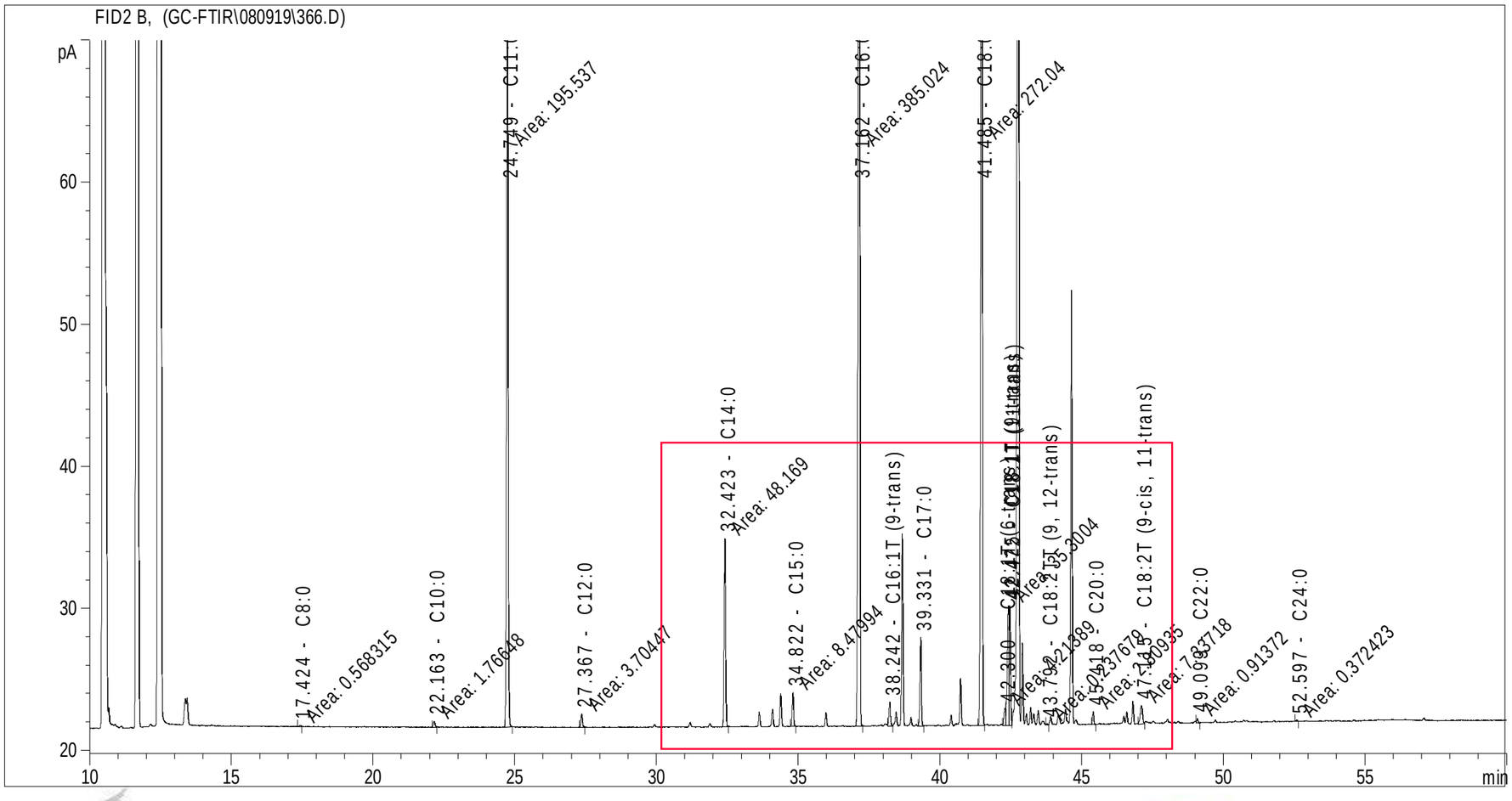
Hard margarine



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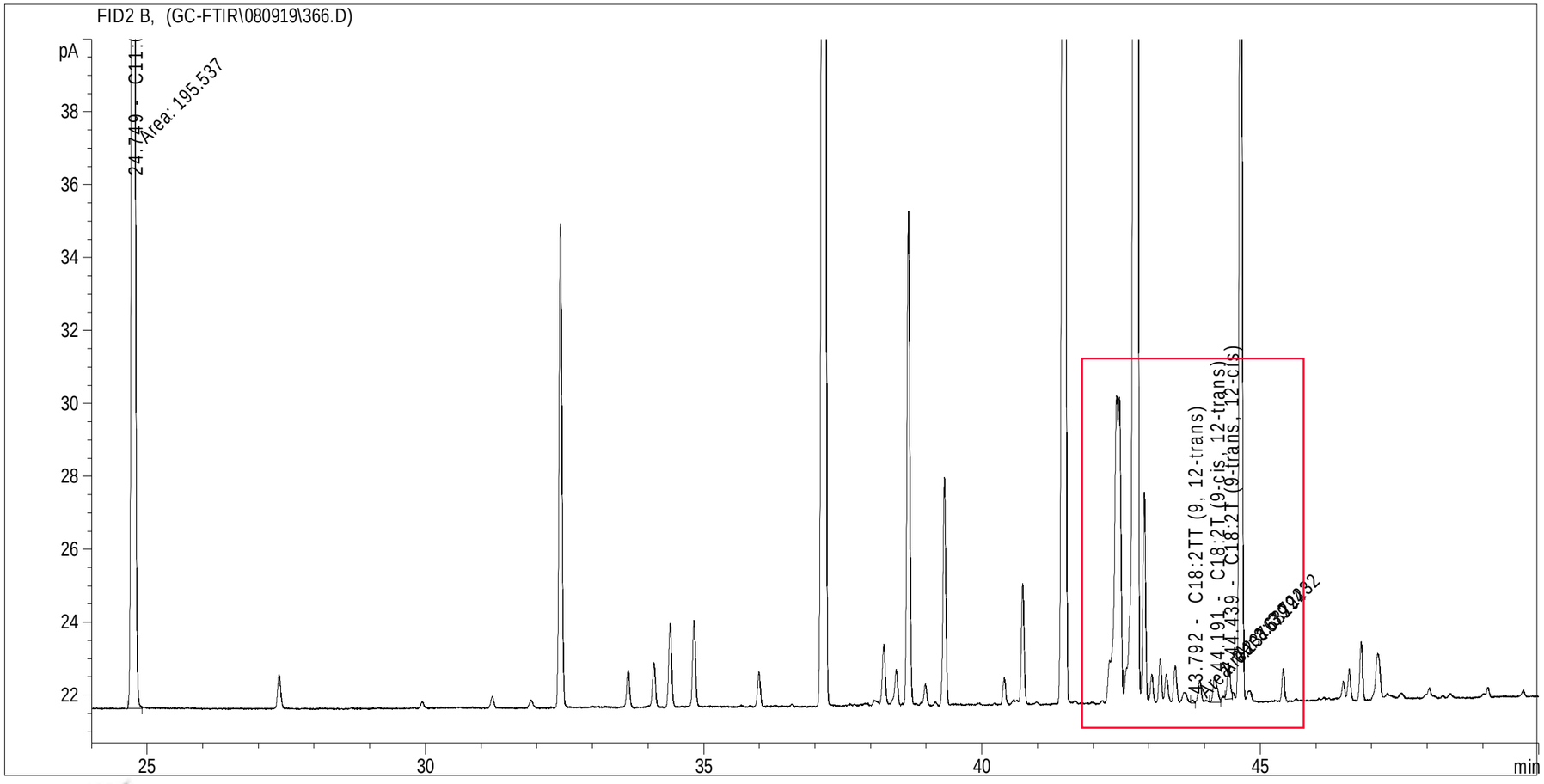
Hard margarine



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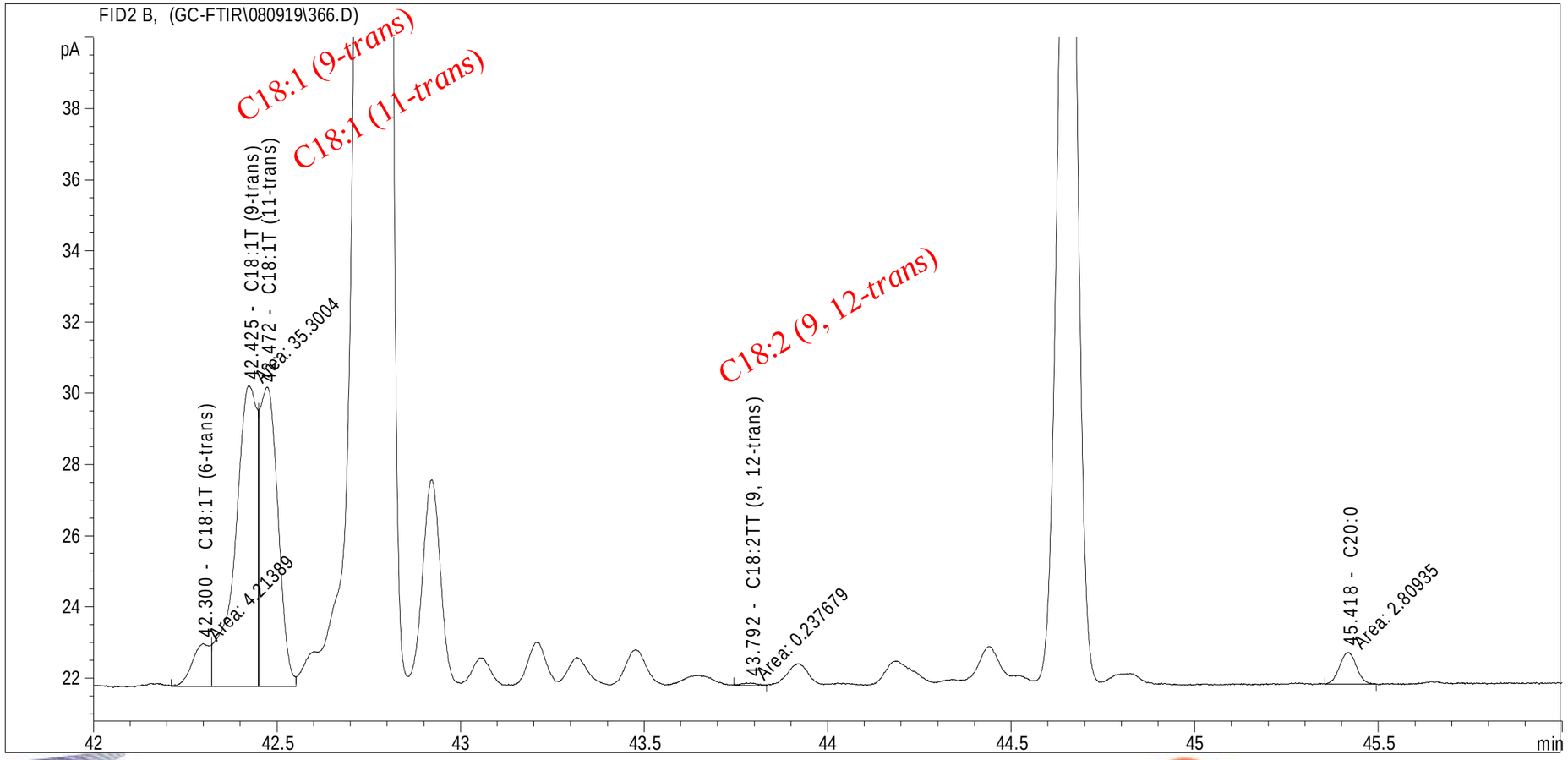
Hard margarine



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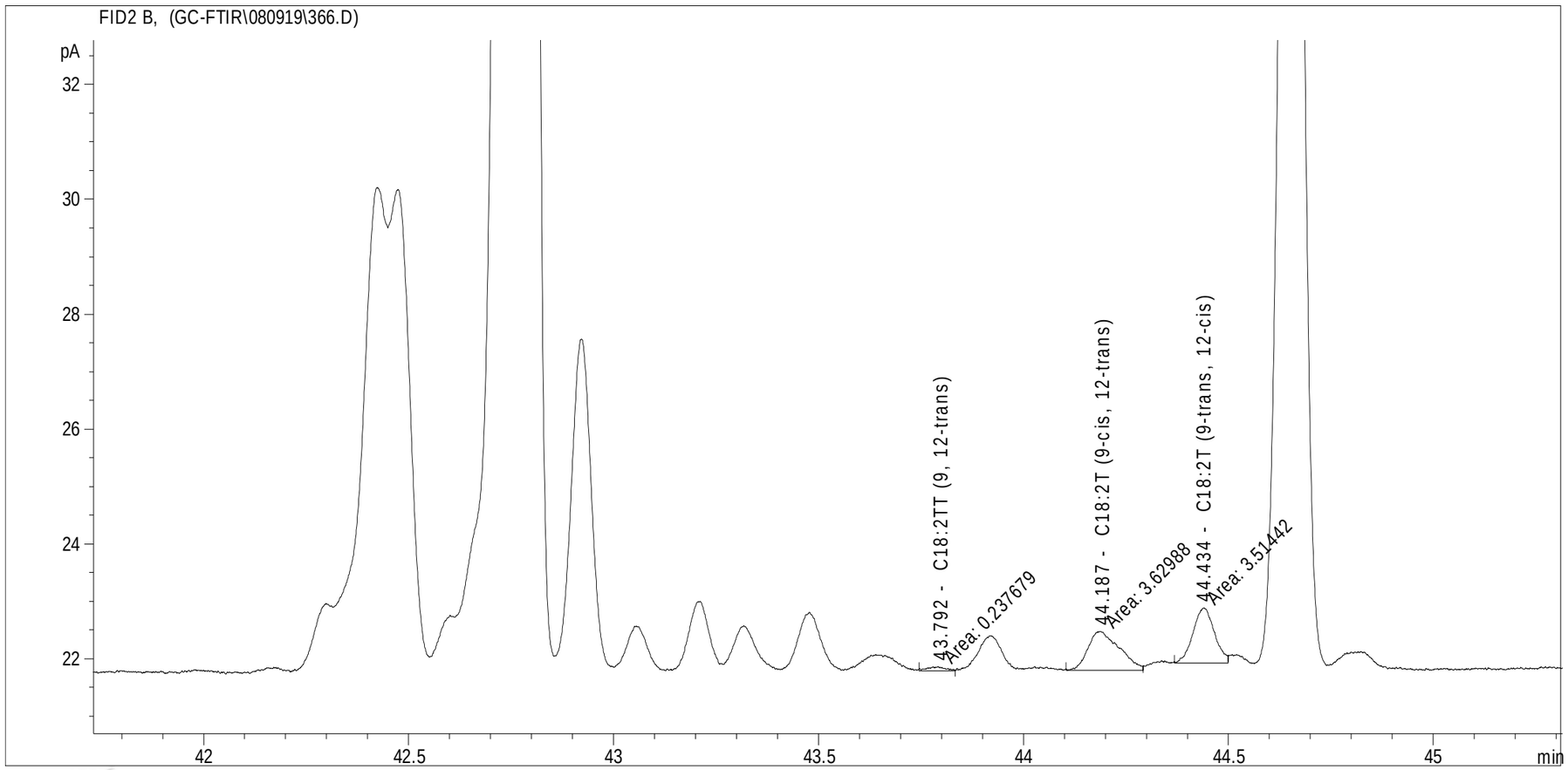
Hard margarine



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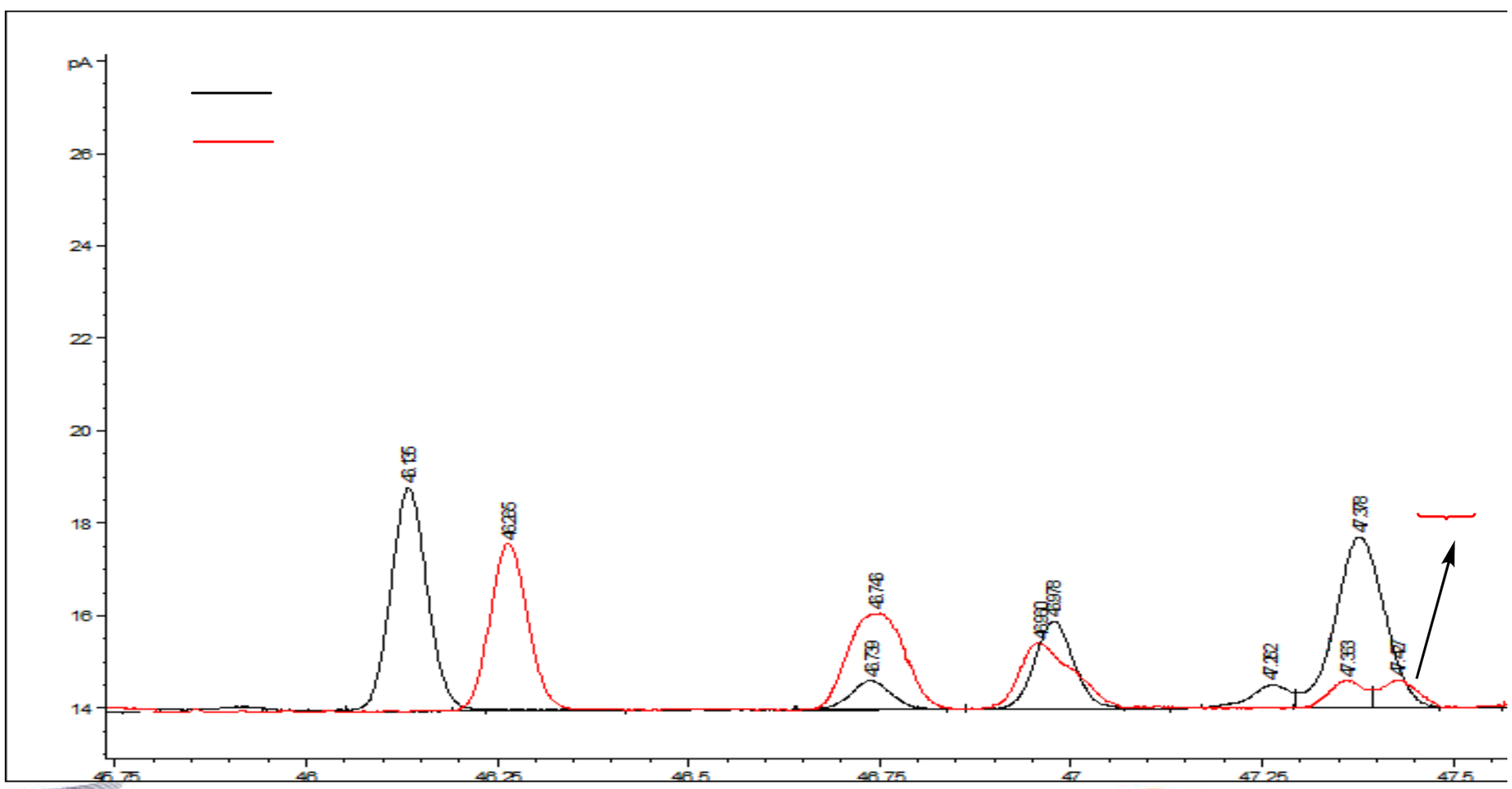
Corn oil and 18:3 *trans*



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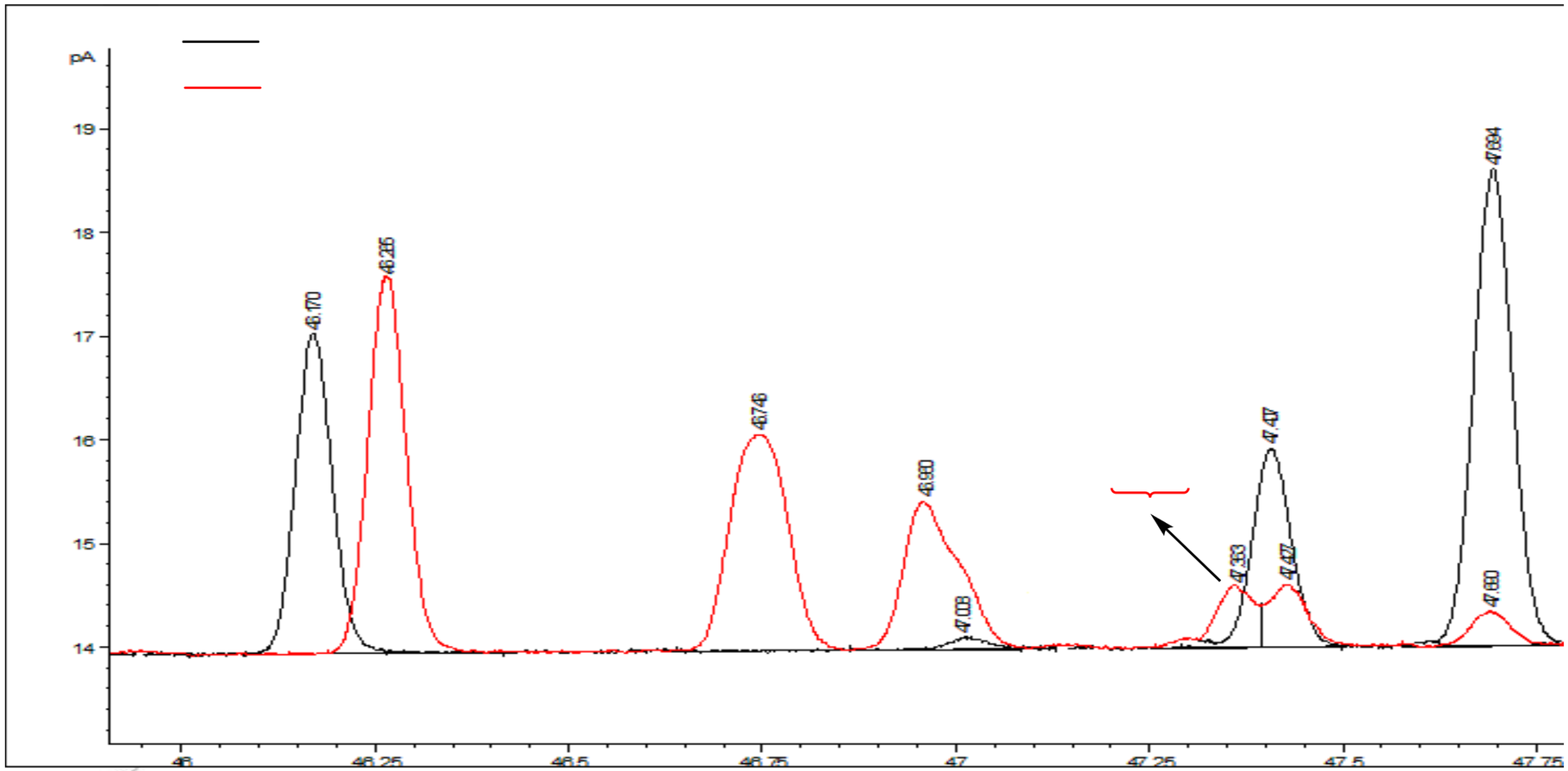
Olive oil and 18:3 *trans*



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Olive oil

Points to note (1)

- Definition of “0”
  Saturated fatty acids ≤0.5/100 g
  Trans fatty acids ≤0.3/100 g

- Limit of detection of saturated fatty acids and *trans* fatty acids should be better than 0.5 g/100 g and 0.3 g/100 g respectively

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Points to note (2)

- For prepackaged product with “Free of sat fat”claim:

  Sum of sat and *trans* fat ≤0.1/100 g

- Limit of detection of saturated fatty acids and *trans* fatty acids should be better than 0.05 g/100 g respectively.

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Points to note (3)

- Availability of FAME standards for calibration is crucial to this test method.

- Insufficient FAME standards would underestimate the level of saturated fat and *trans* fat.

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Availability of FAME standards

Single and/or mixed FAMEs are available from different suppliers, including:

1. Supelco
2. Fluka
3. Nu-check Prep
4. Chem Service
5. Sigma

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Proficiency test

FAPAS

AOAC

LGC

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Thank You

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