

# Analysis of Fatty Acids

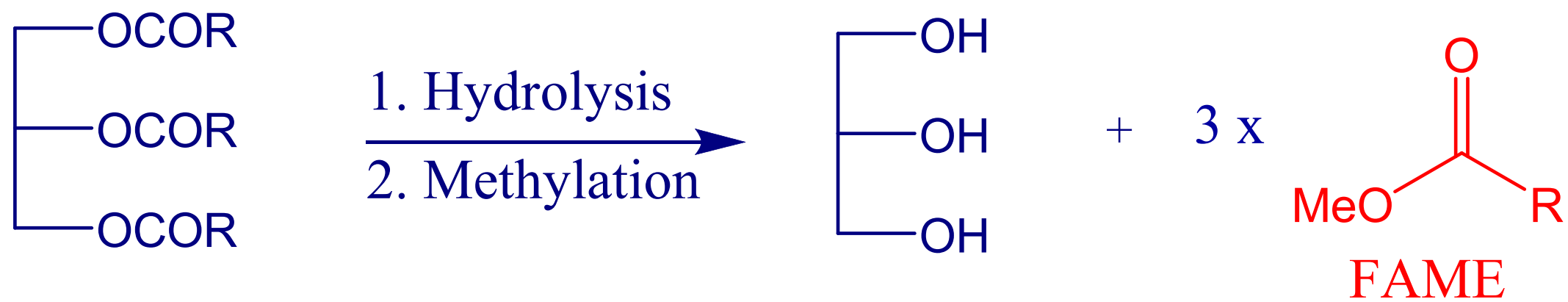
# AOAC Official Methods

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	<i>cis</i> - and <i>trans</i> -Octadecenoic isomers and general fatty acid composition	GC-IR
985.21 etc	<i>trans</i> -Fatty acid in margarines	GC-FID

# 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.

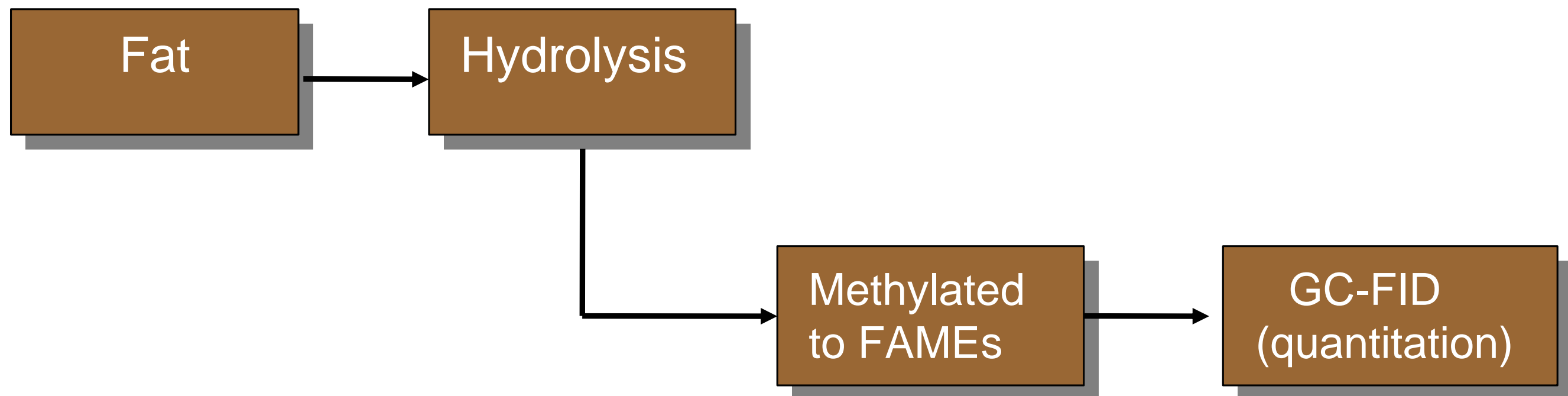
# Typical analysis



R = hydrocarbon chain

GC analysis

# Typical analysis



# Typical analysis

## Hydrolysis

Weigh sample  
and pyrogalllic  
acid into the  
Mojonnier flask



# Typical analysis

## Hydrolysis

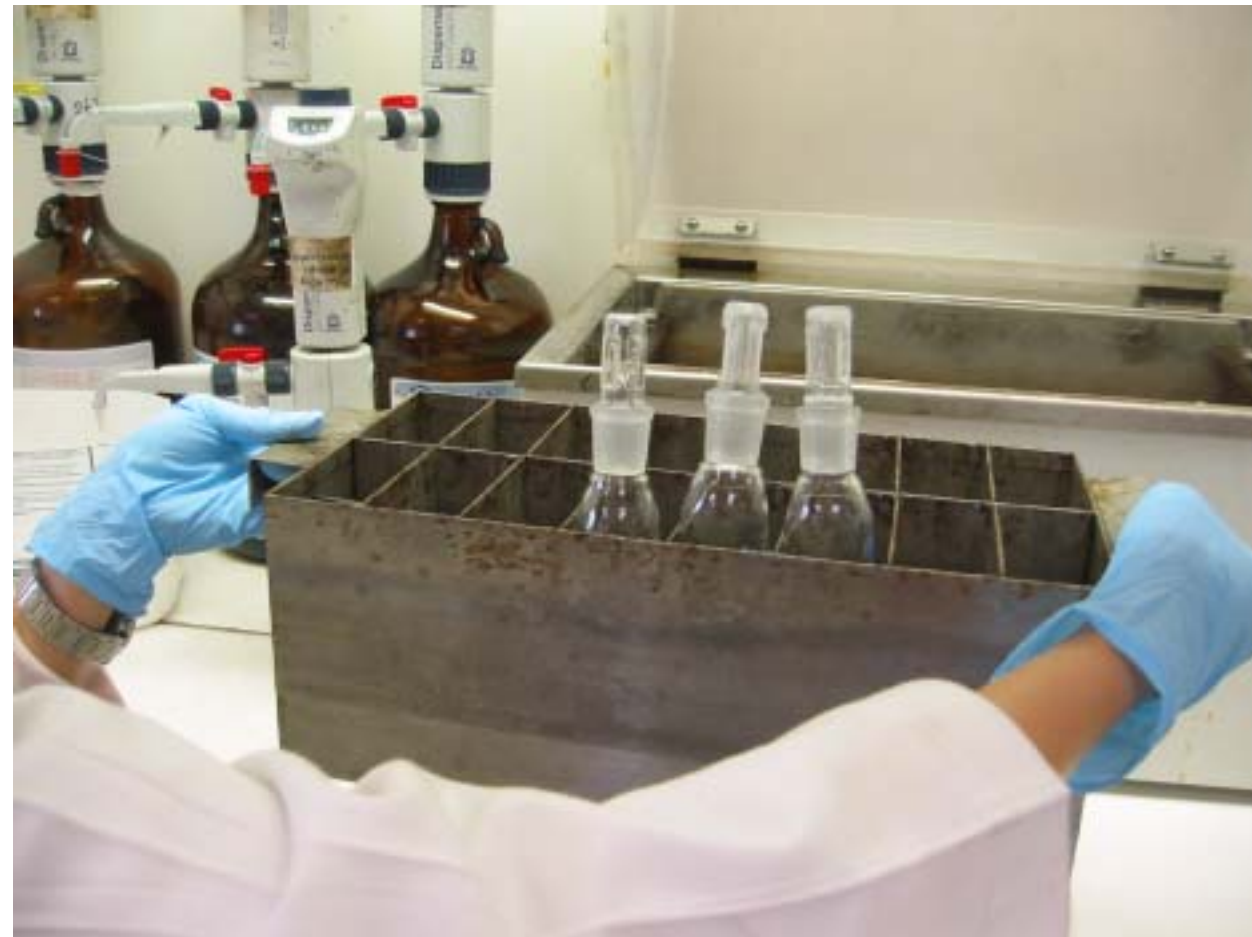
Add internal standard, ethanol and 8.3 M HCl.



# Typical analysis

## Hydrolysis

Hydrolyse the  
sample at 70 –  
80 °C





# Typical analysis

## Hydrolysis

Remove flasks  
from water bath.  
Cool to room  
temperature.



# Typical analysis

## Extraction

Add ethanol,  
Ether and shake.



# Typical analysis

## Extraction

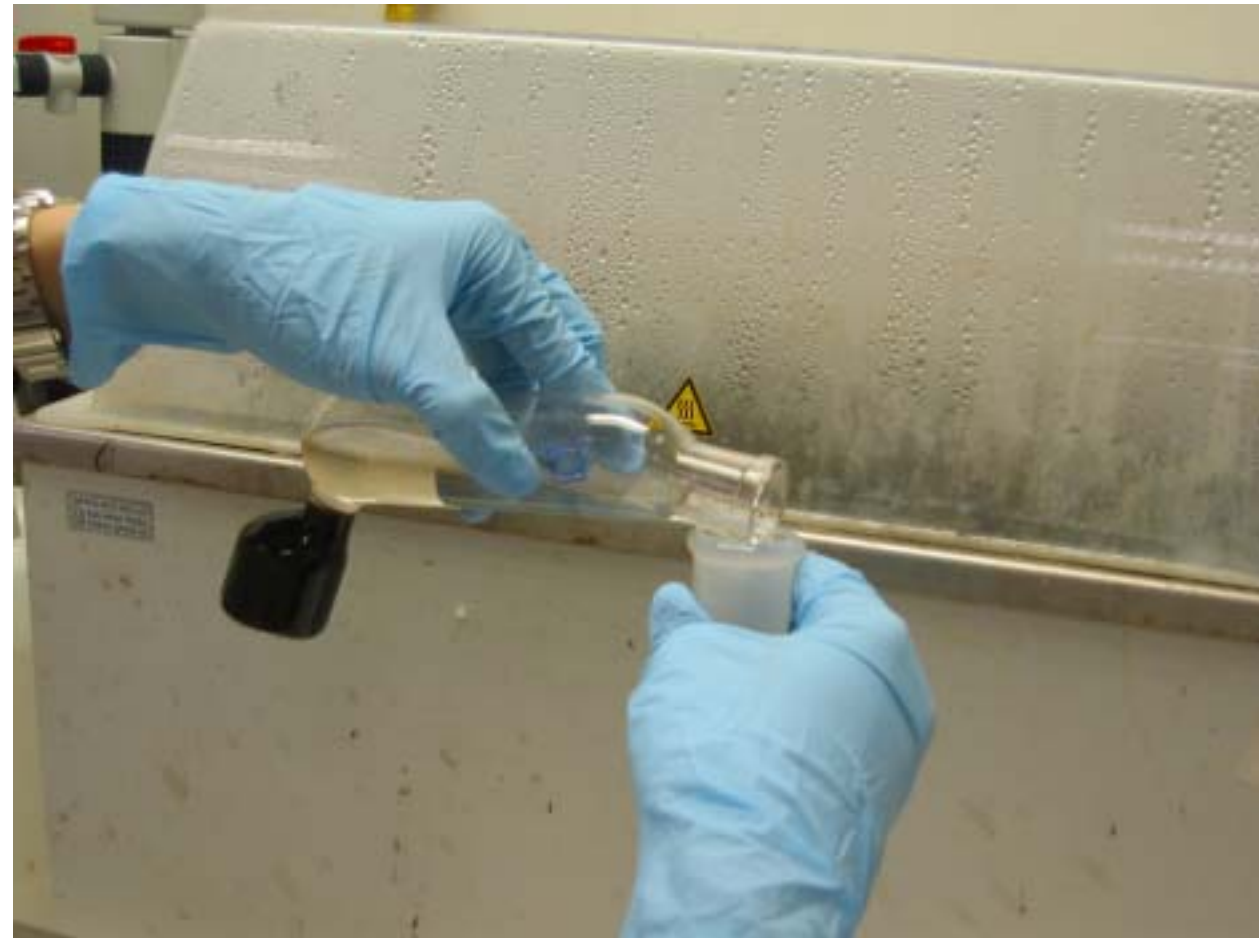
Centrifuge sample.  
(allow contents to  
sep. at least 1 h if  
centrifuge is not  
available)



# Typical analysis

## Extraction

Decant the ether layer into a tube.



# Typical analysis

Extraction

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



# Typical analysis

## Extraction

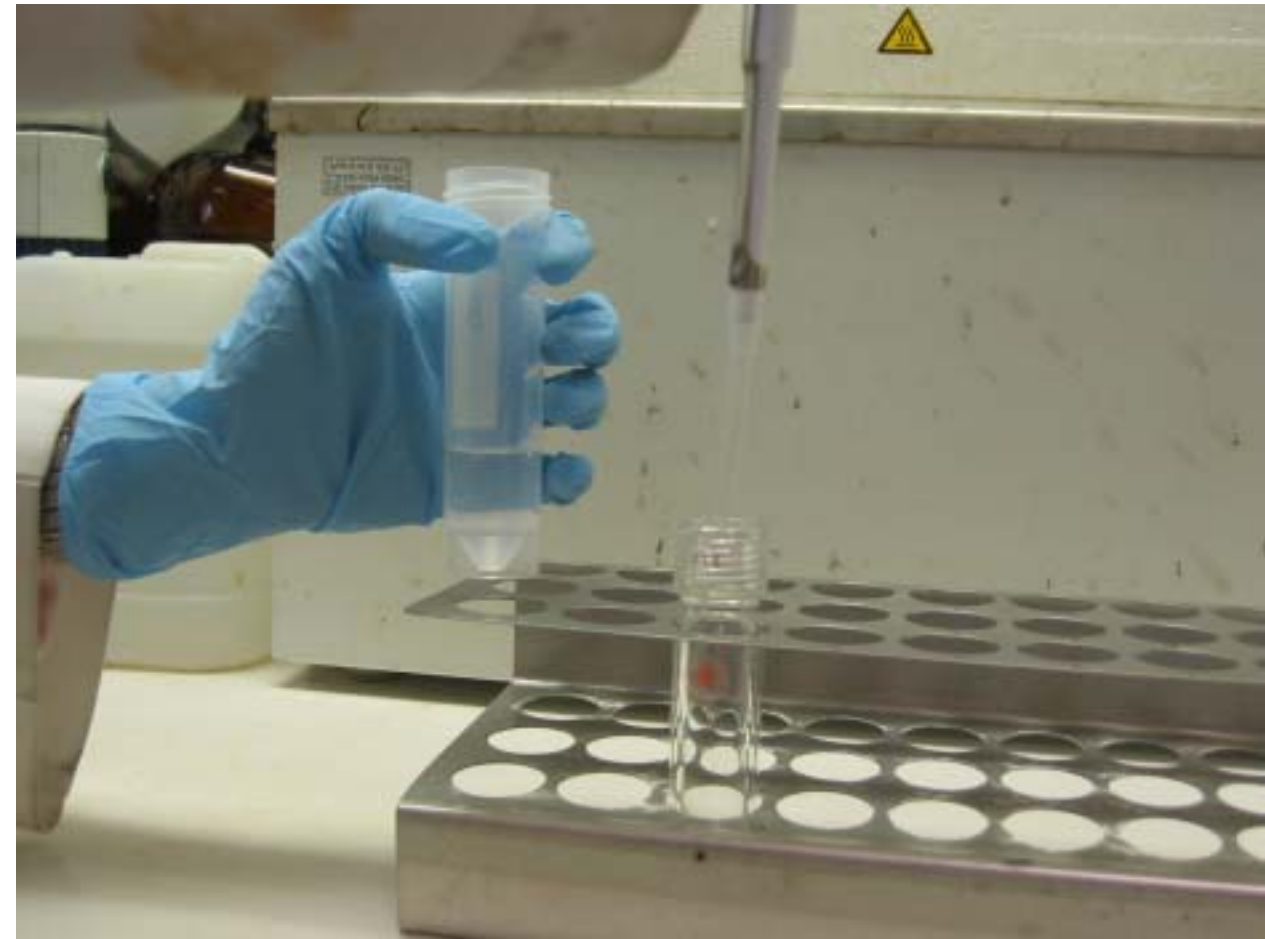
Dissolve the residue in chloroform.



# Typical analysis

## Methylation

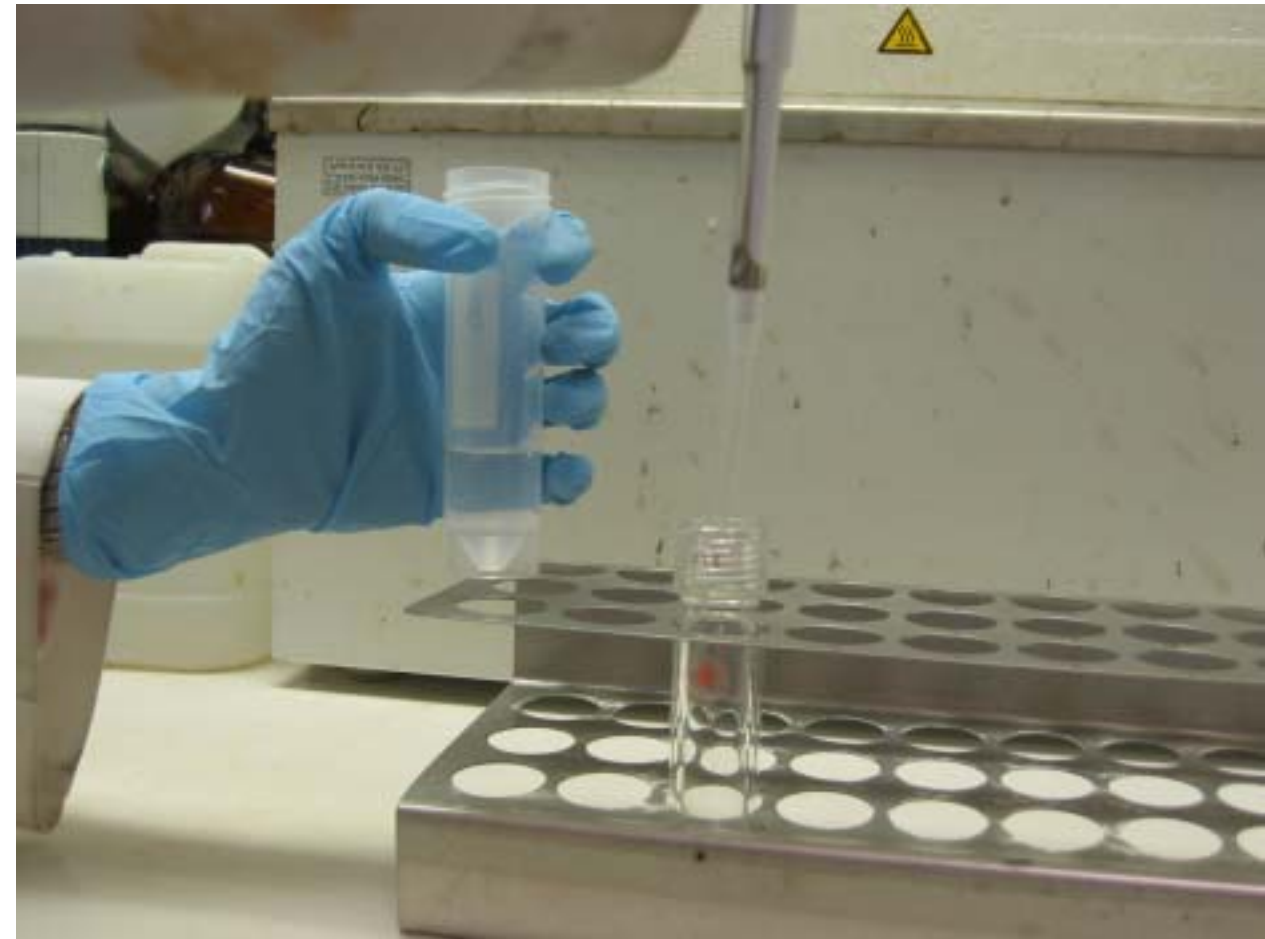
Transfer mixture  
to a glass vial.  
Evaporate to  
dryness.



# Typical analysis

## Methylation

Add 7%  $\text{BF}_3$   
reagent and  
toluene.





# Typical analysis

## Methylation

Seal vials with  
screwcap and  
heat them at  
100 °C.



# Typical analysis

## Methylation

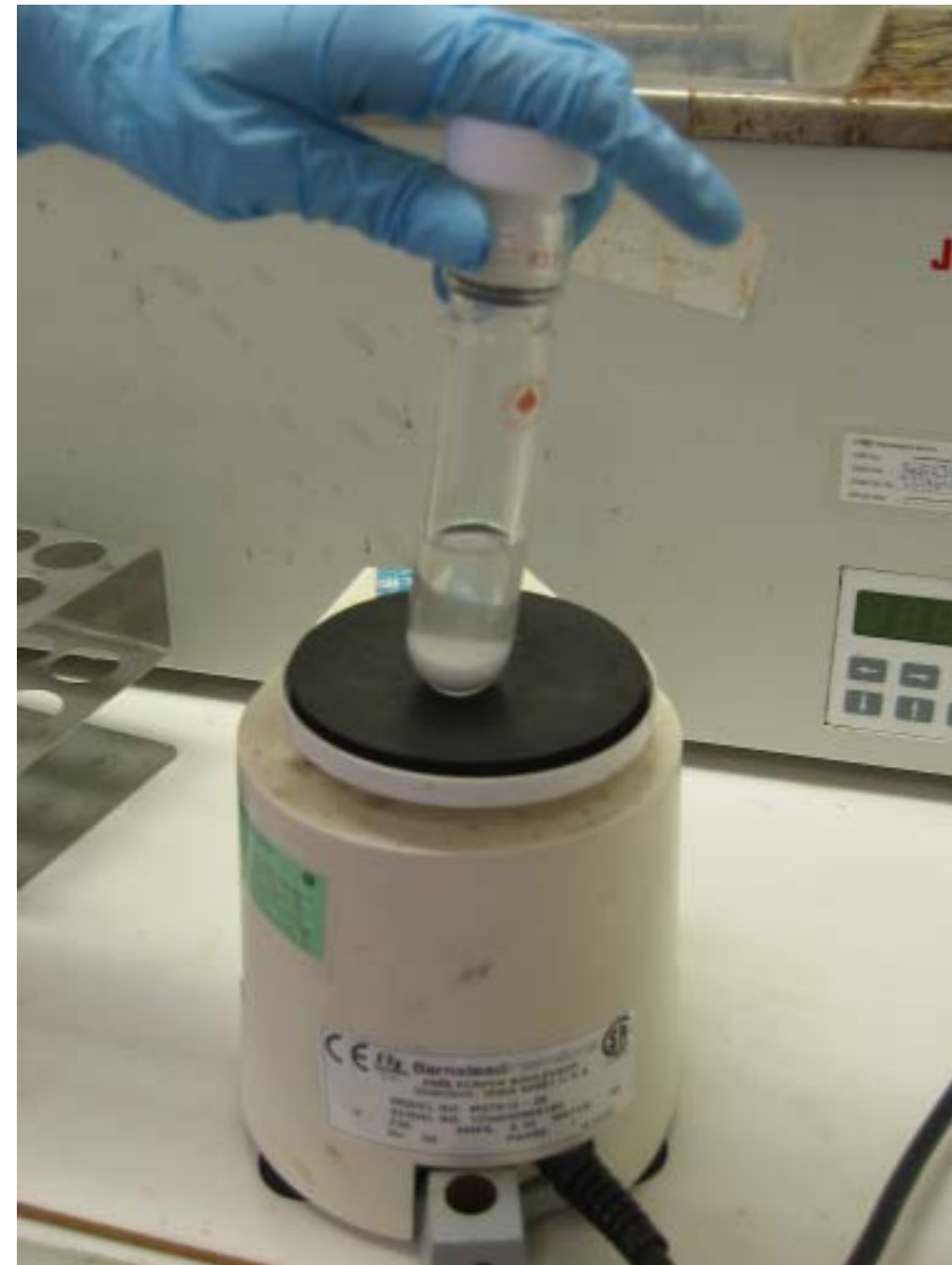
Allow vials to cool  
to room  
temperature.



# Typical analysis

## Methylation

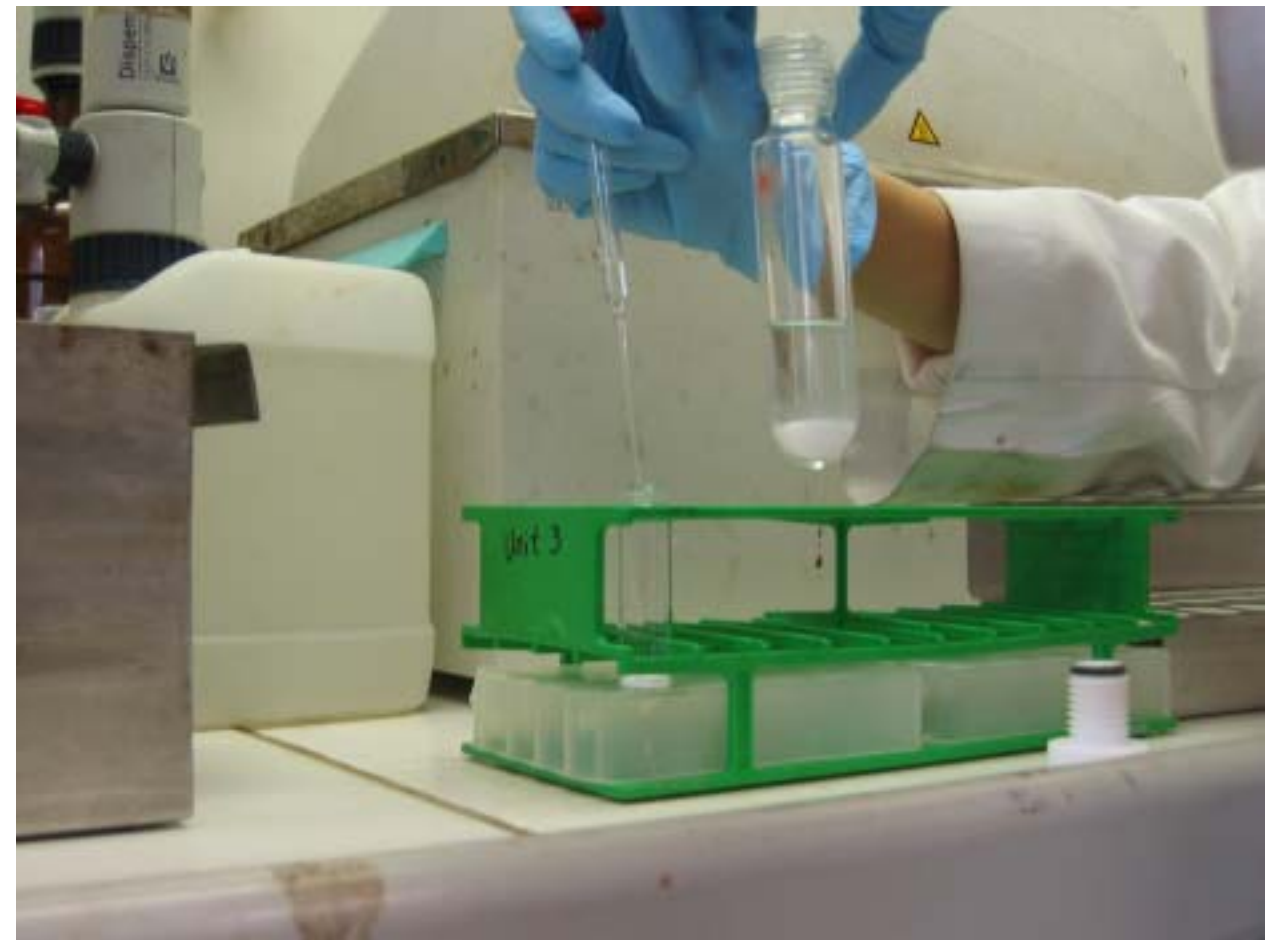
Add water (5 mL),  
hexane (1 mL),  
 $\text{Na}_2\text{SO}_4$  (1 g).  
Shake



# Typical analysis

## Methylation

Allow layers to separate and transfer top layer to another vial containing  $\text{Na}_2\text{SO}_4$  (1 g).



# Typical analysis

## Methylation

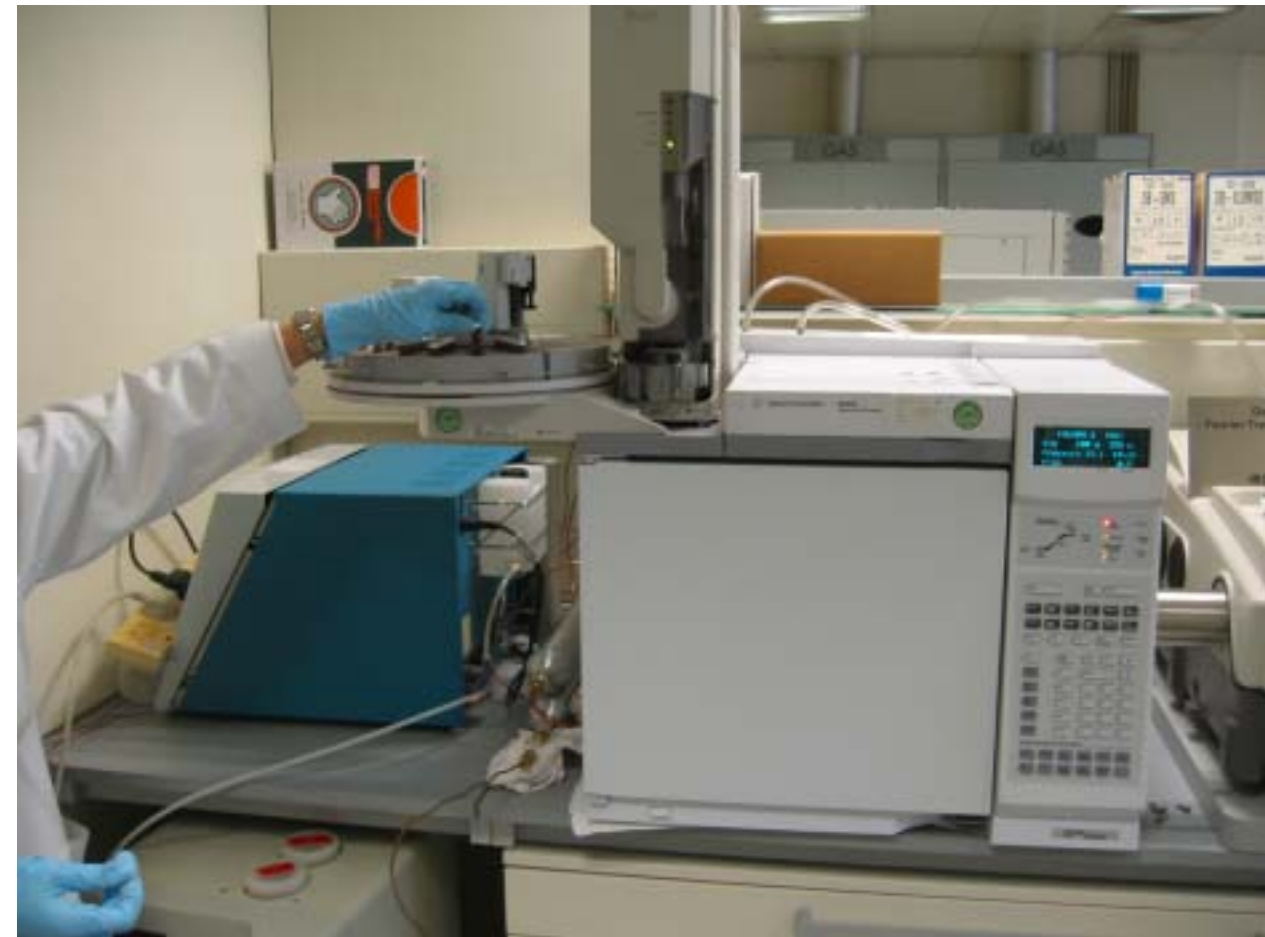
Top layer should contain FAMES and internal standard.



# Typical analysis

## GC Determination

Transfer to  
autosampler vial  
for GC analysis.



# Fatty acids (saturated)

4:0 Butyric

6:0 Caproic

8:0 Caprylic

10:0 Capric

11:0 Undecanoic

12:0 Lauric

14:0 Myristic

# Fatty acids (saturated)

15:0 Pentadecanoic

16:0 Palmitic

17:0 Margaric

18:0 Stearic

20:0 Arachidic

22:0 Behenic

24:0 Lignoceric



# Definition

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

# *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

# *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

## *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

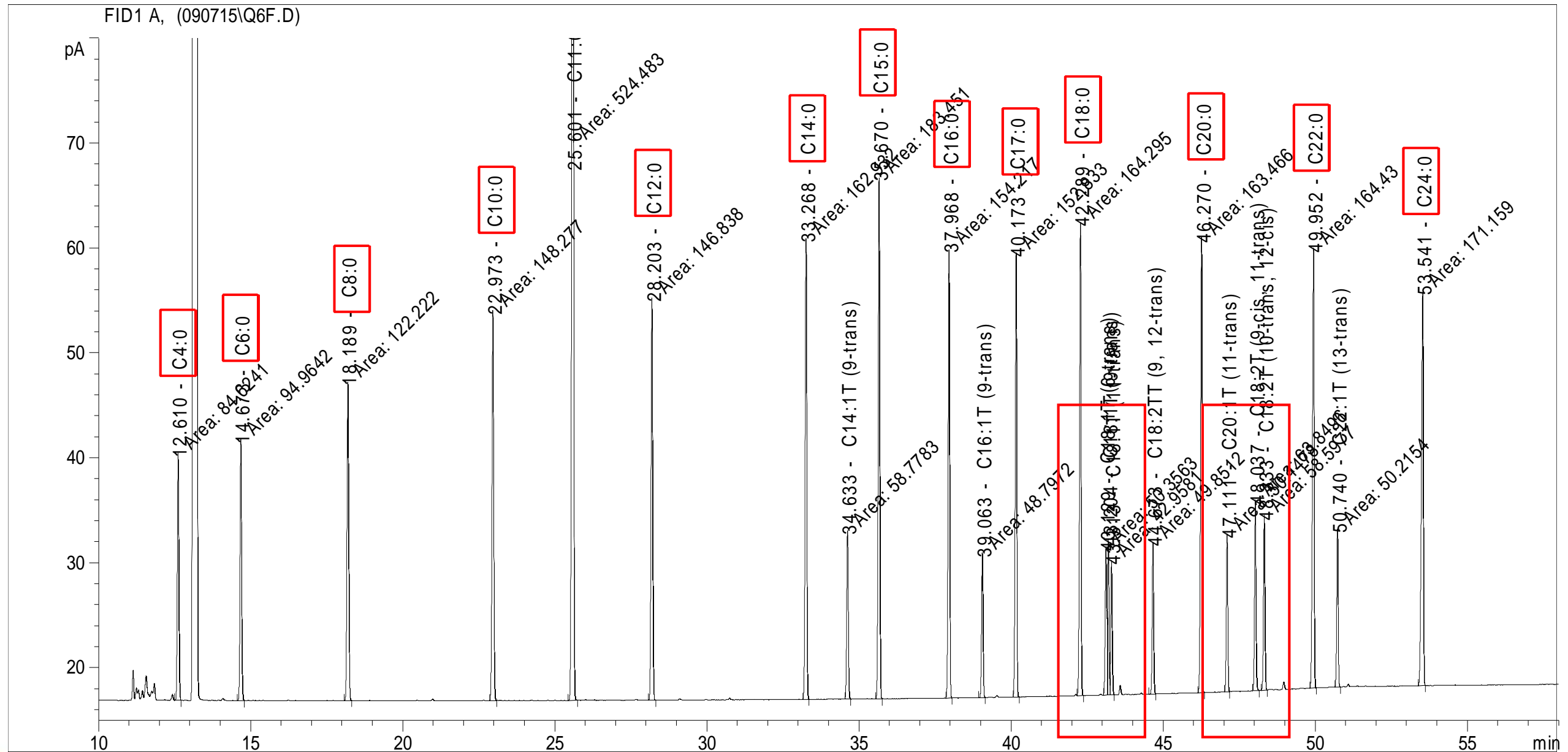
18:3 *cis*-9, *cis*-12, *trans*-15-octadecatrienoic

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

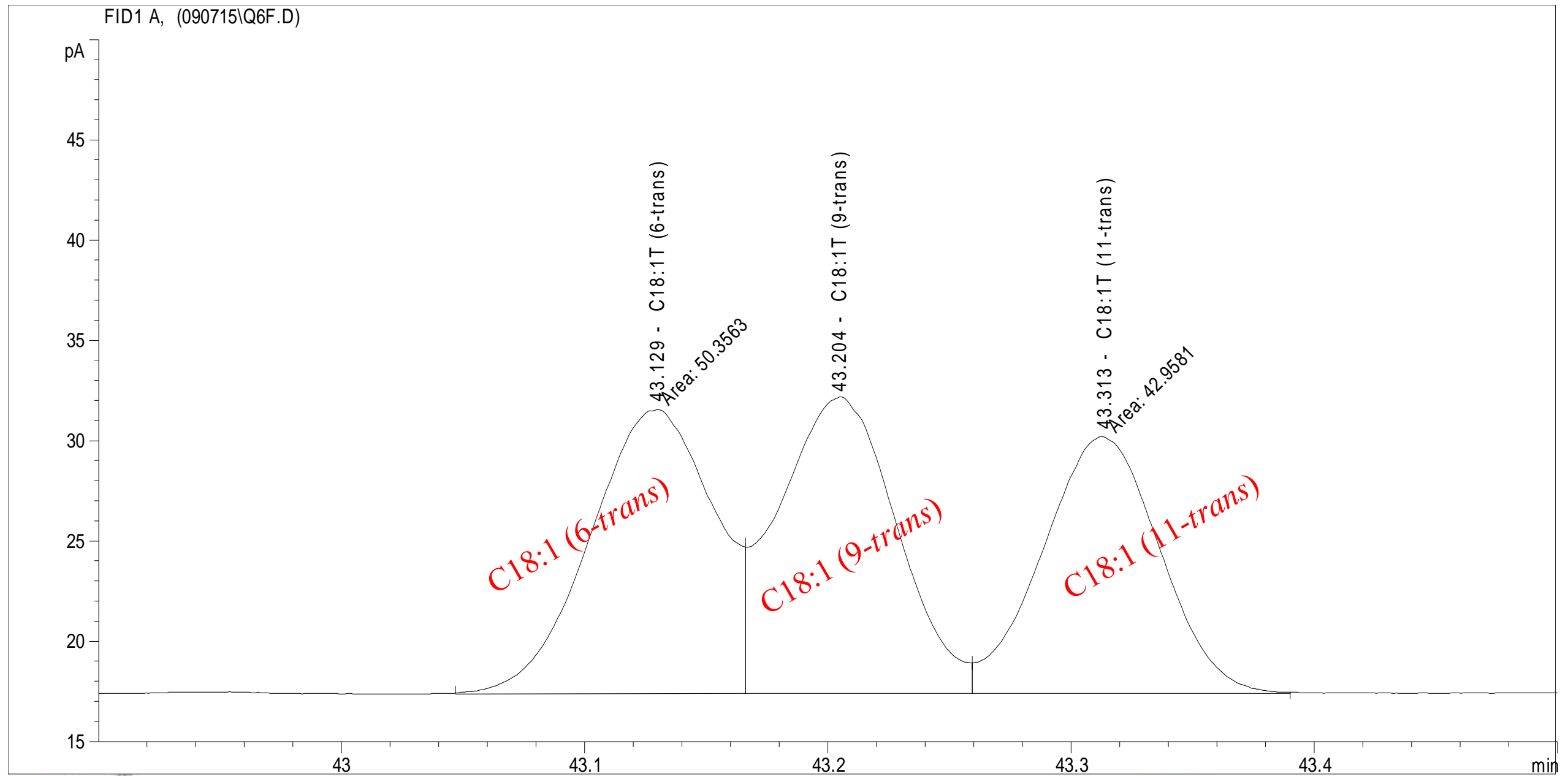
18:3 *trans*-9, *cis*-12, *cis*-15-octadecatrienoic

# FAME (saturated and *trans*)

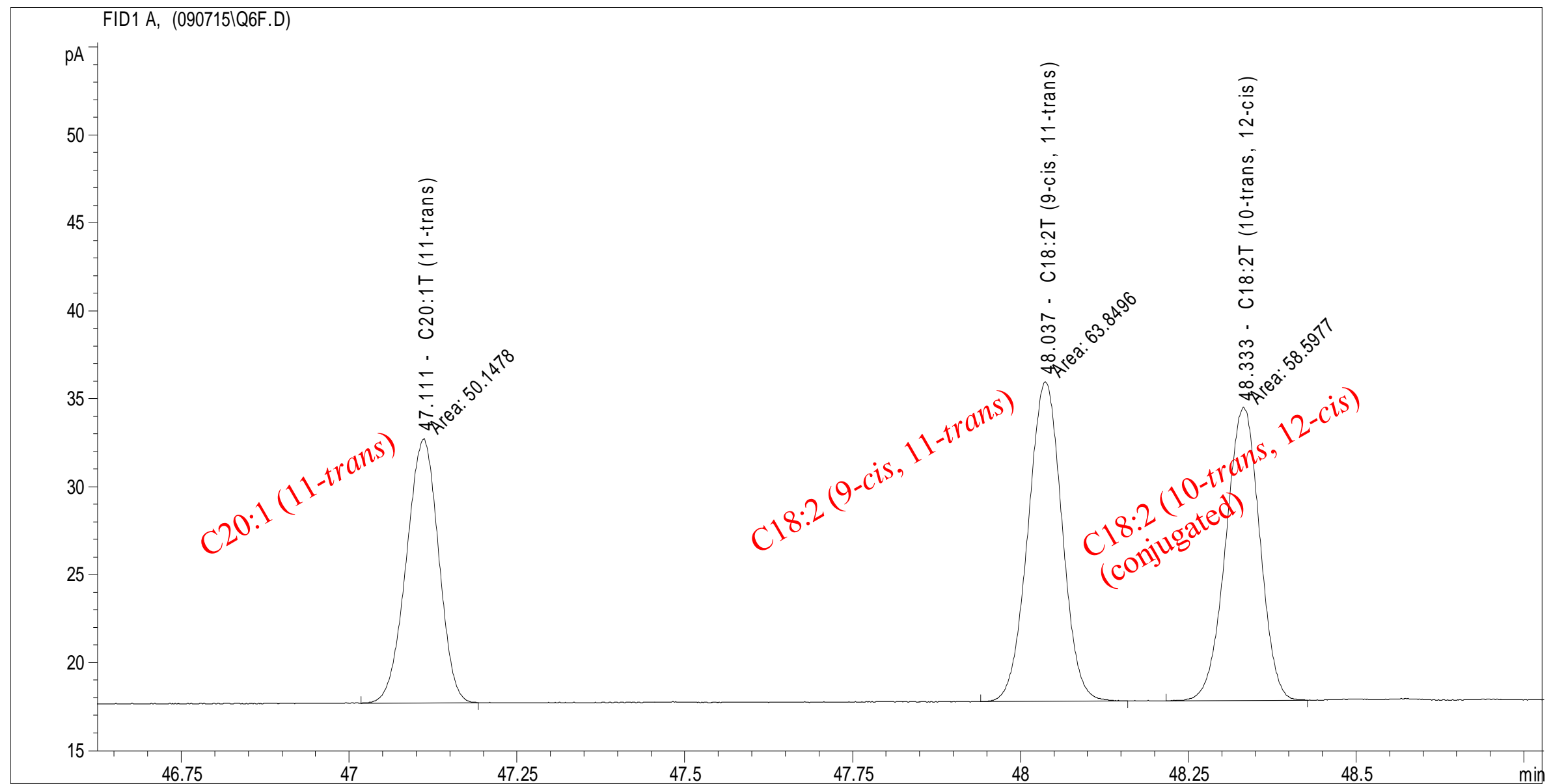
SP-2560, 100m x 0.25mm ID, 0.2  $\mu$ m,  
flow rate: 0.7 mL/min



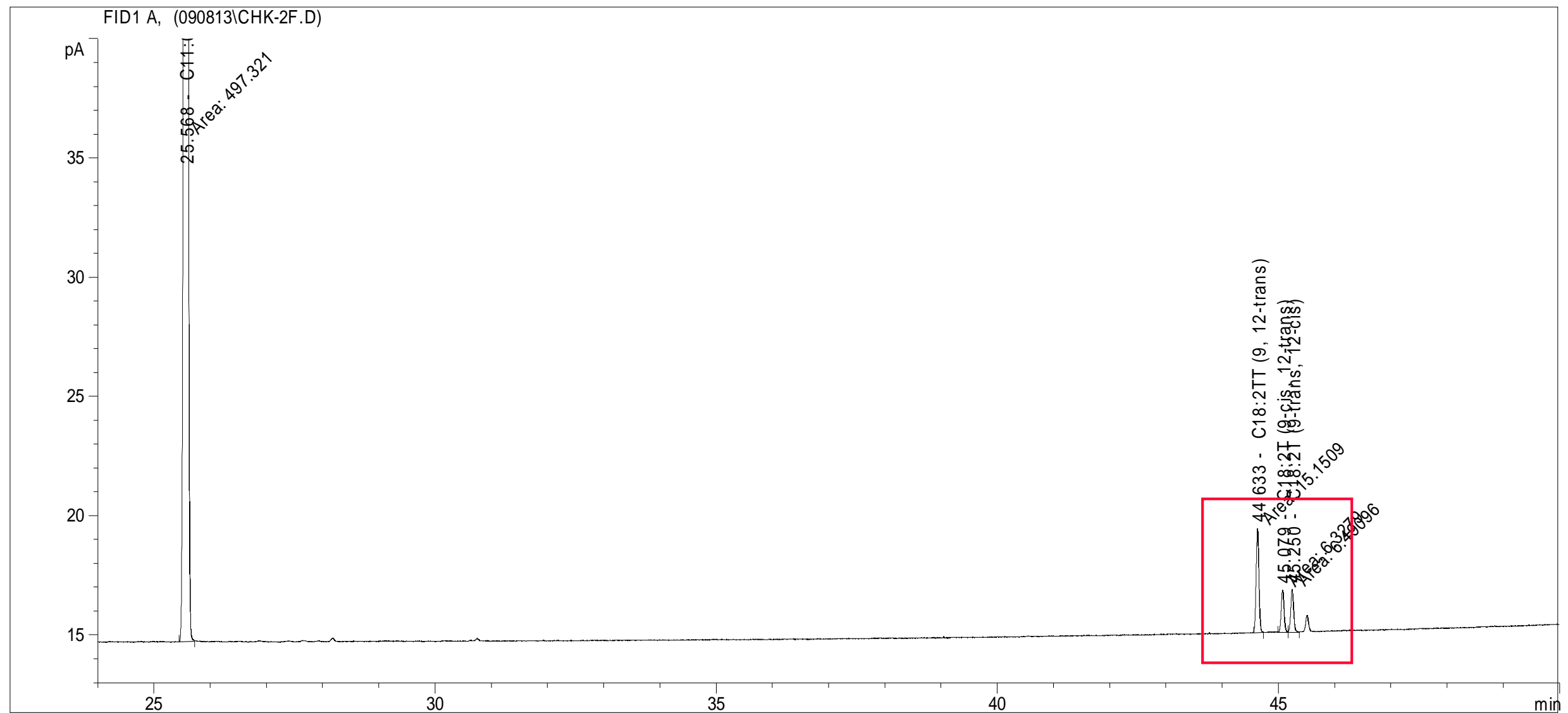
# FAME (18:1 *trans*)



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

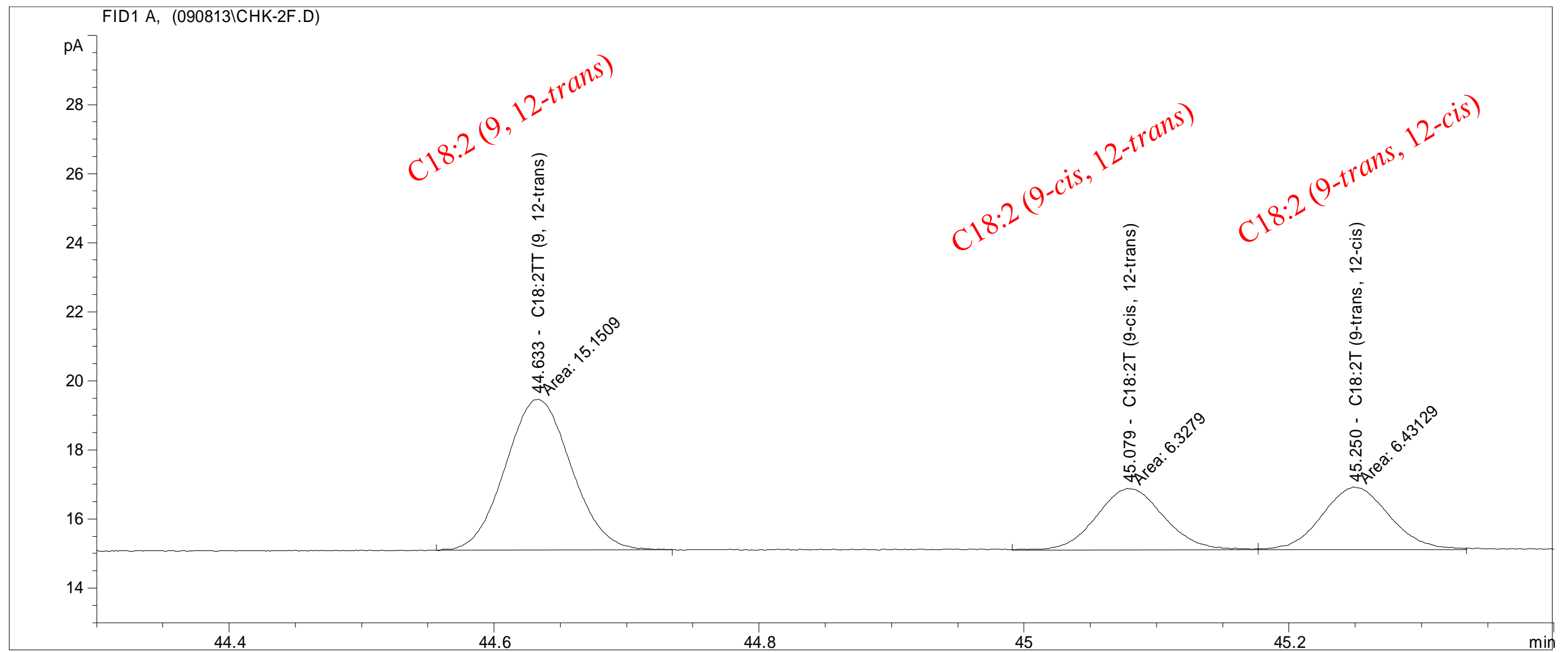


# FAME (18:2 *trans*)

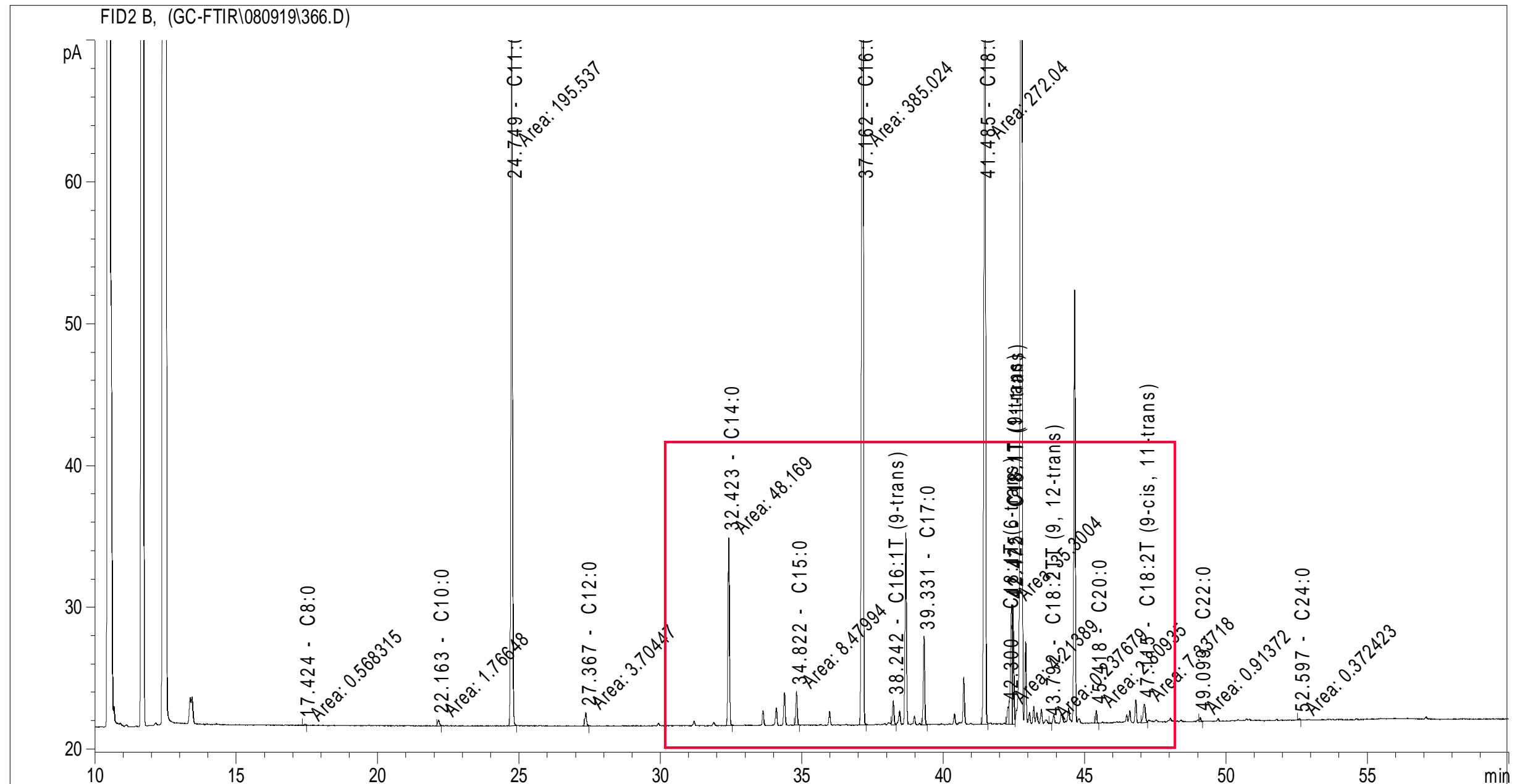




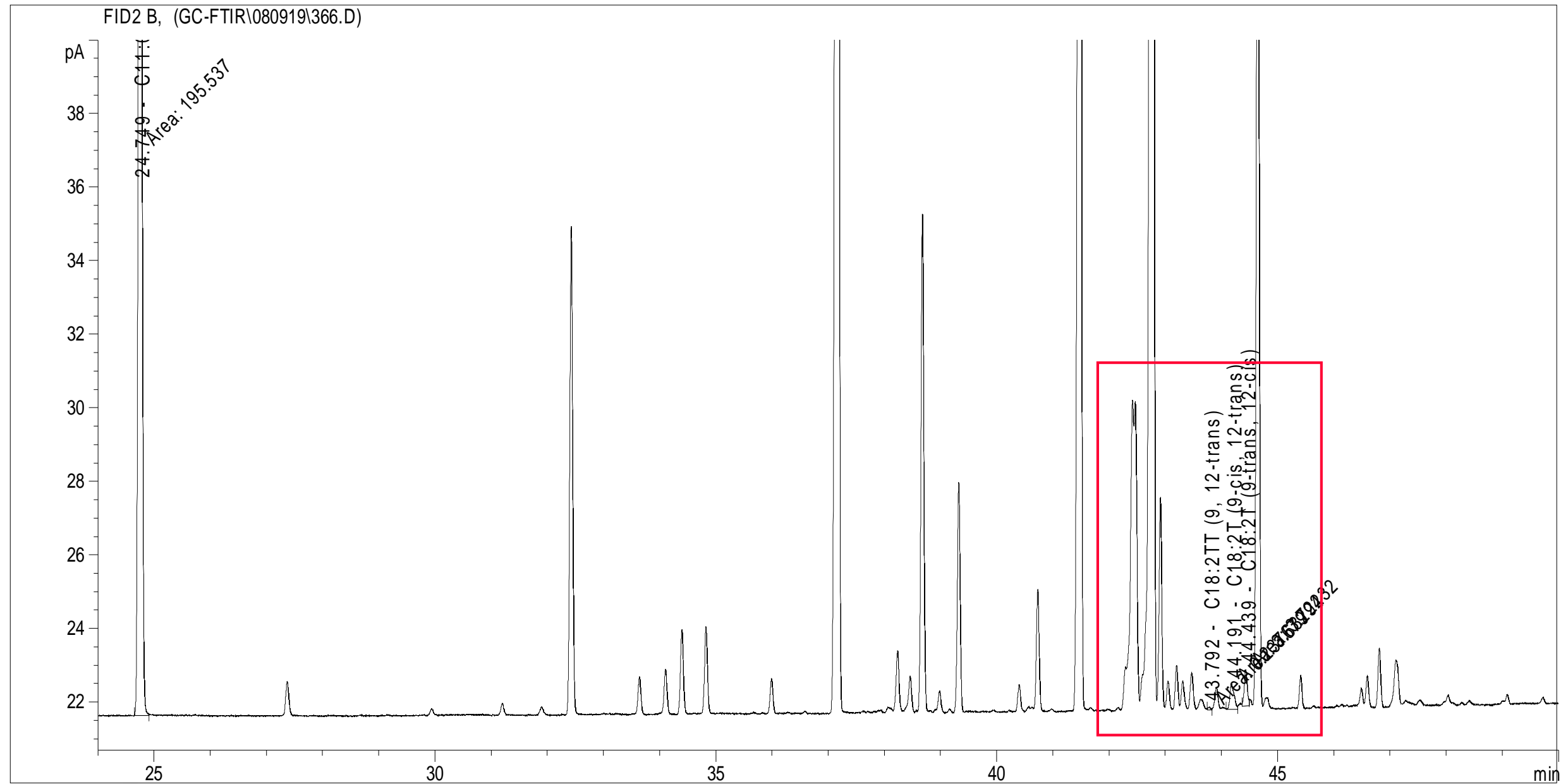
# FAME (18:2 *trans*)



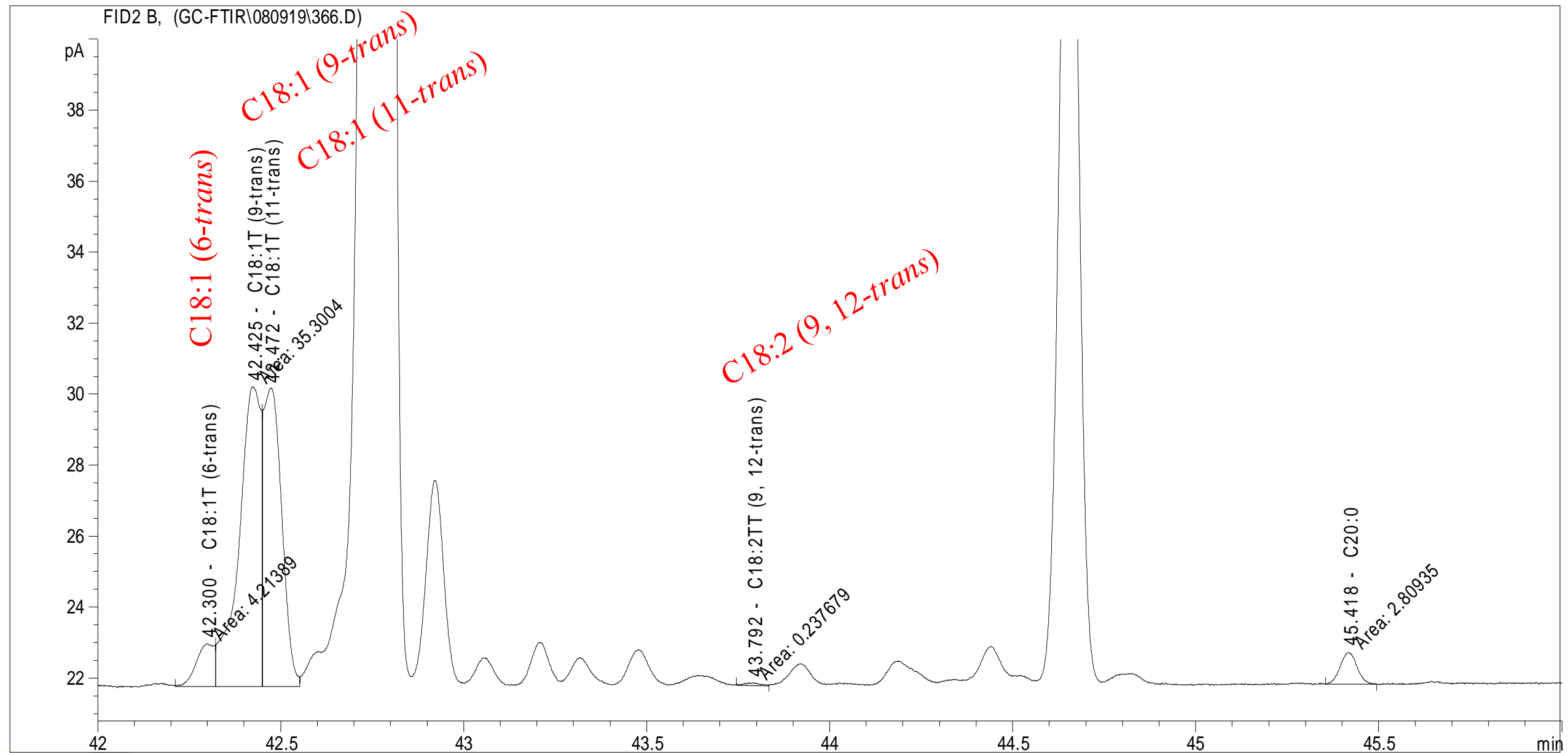
# Hard margarine



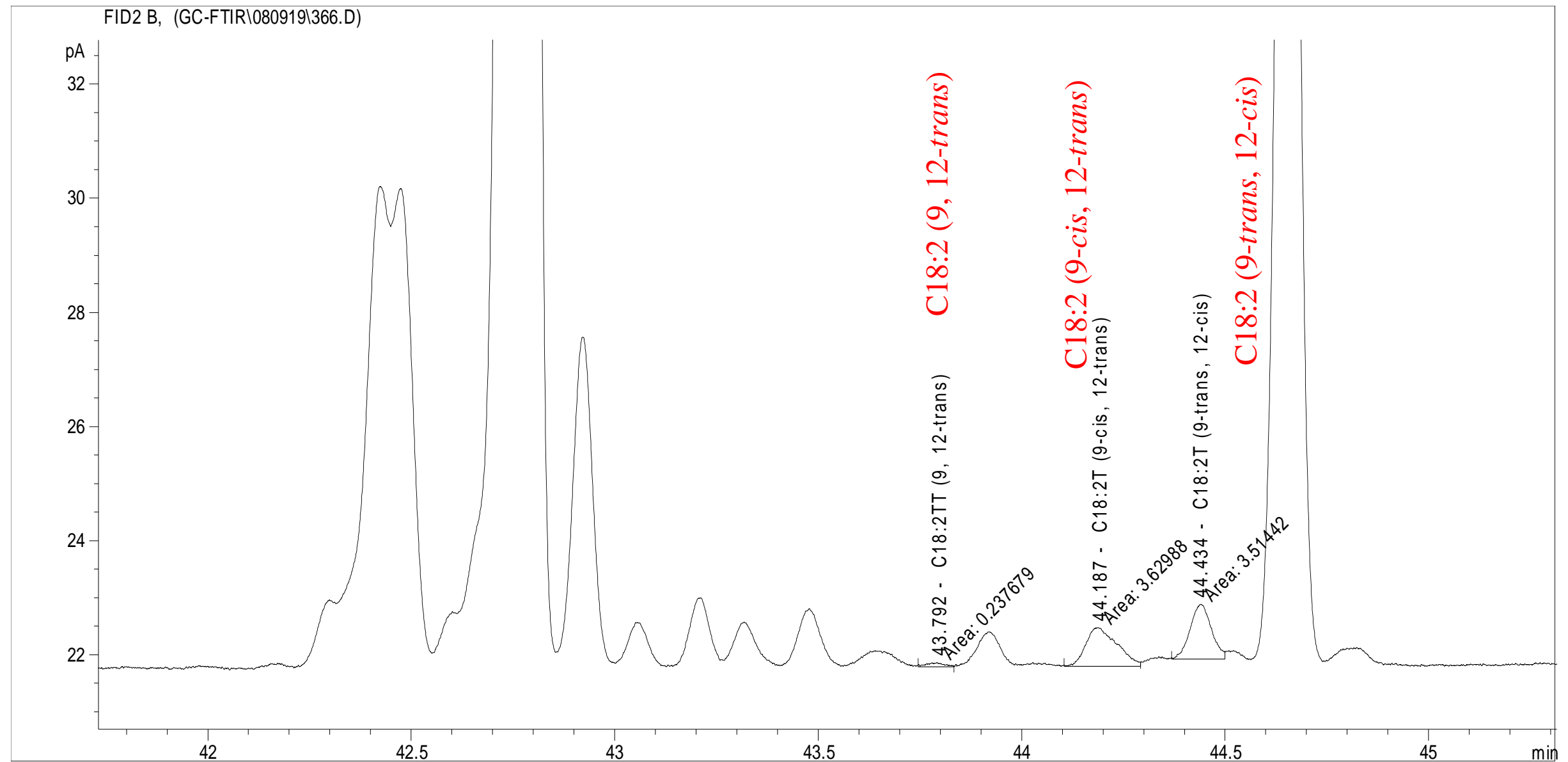
# Hard margarine



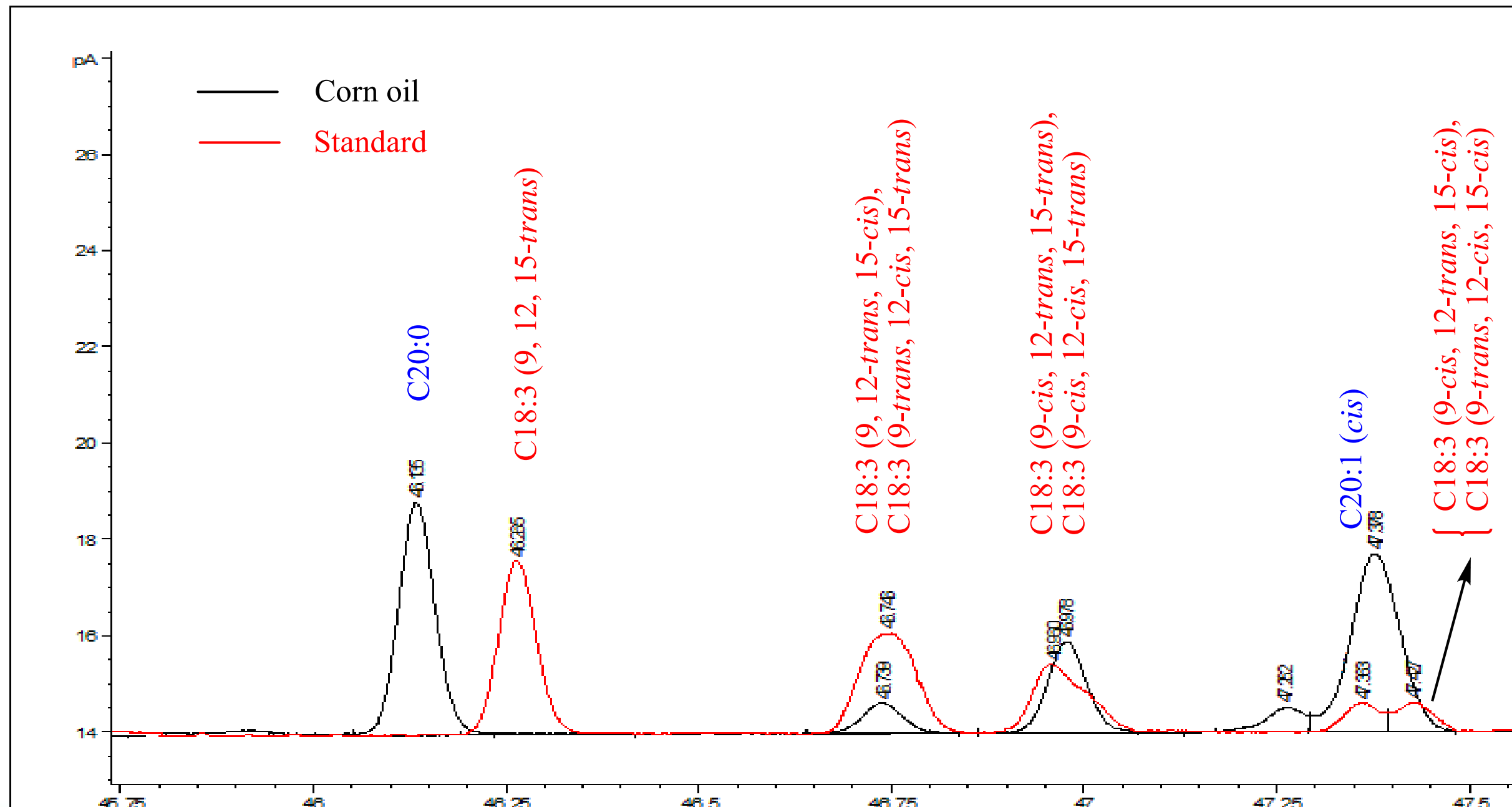
# Hard margarine



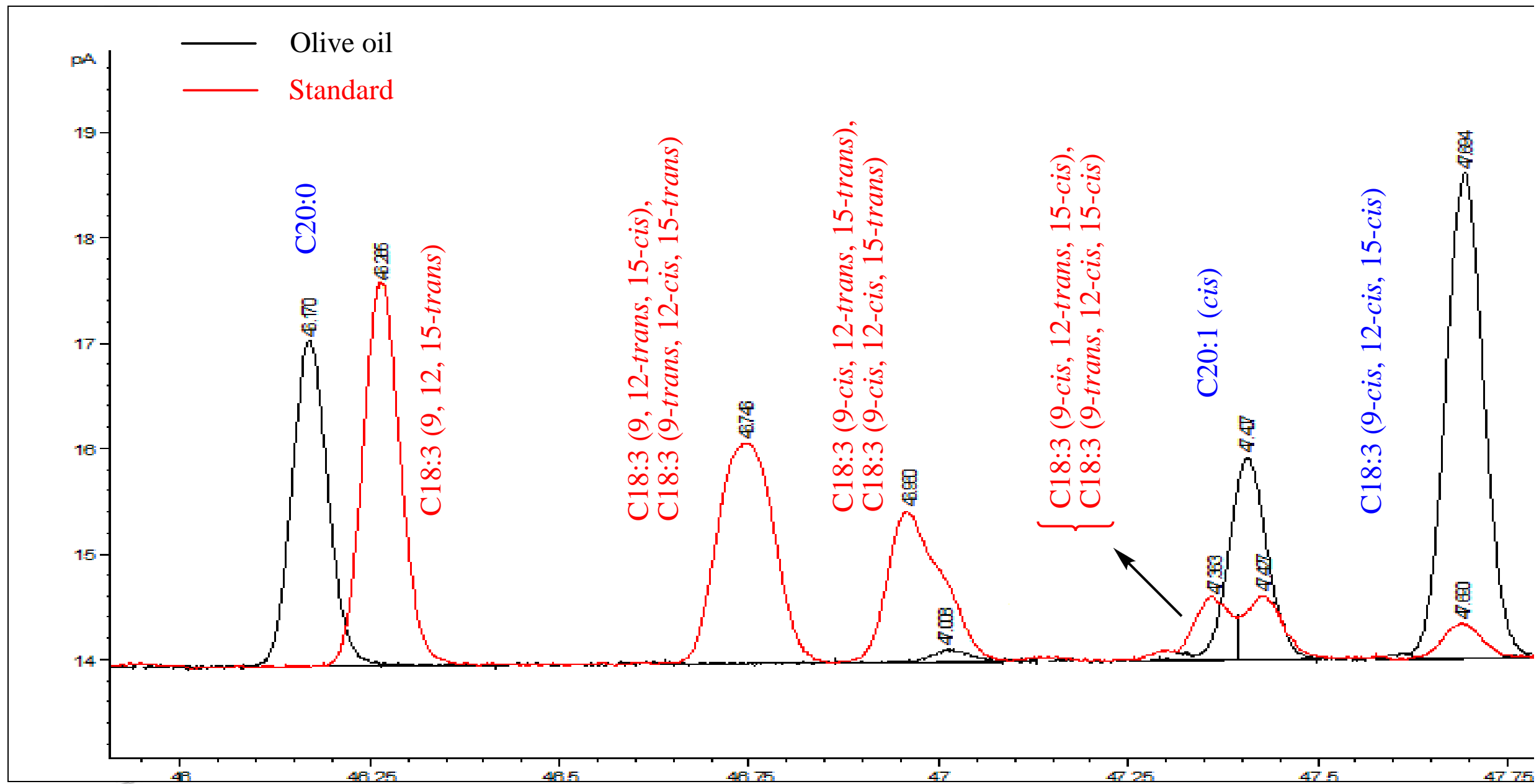
# Hard margarine



# Corn oil and 18:3 *trans*



# Olive oil and 18:3 *trans*



## Points to note (1)

- Definition of “0”  
Saturated fatty acids  $\leq 0.5$  g/100 g  
*Trans* fatty acids  $\leq 0.3$  g/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



## Points to note (2)

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

Sum of sat and *trans* fat  $\leq 0.1$  g/100 g

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

## 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.

# 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

# Proficiency test

FAPAS

AOAC

LGC

# Thank You

