Nutrient Test Methods (Part 2)

1. Sugars
2. Sodium
3. Fatty acids (sat fat & trans fat)
4. Cholesterol

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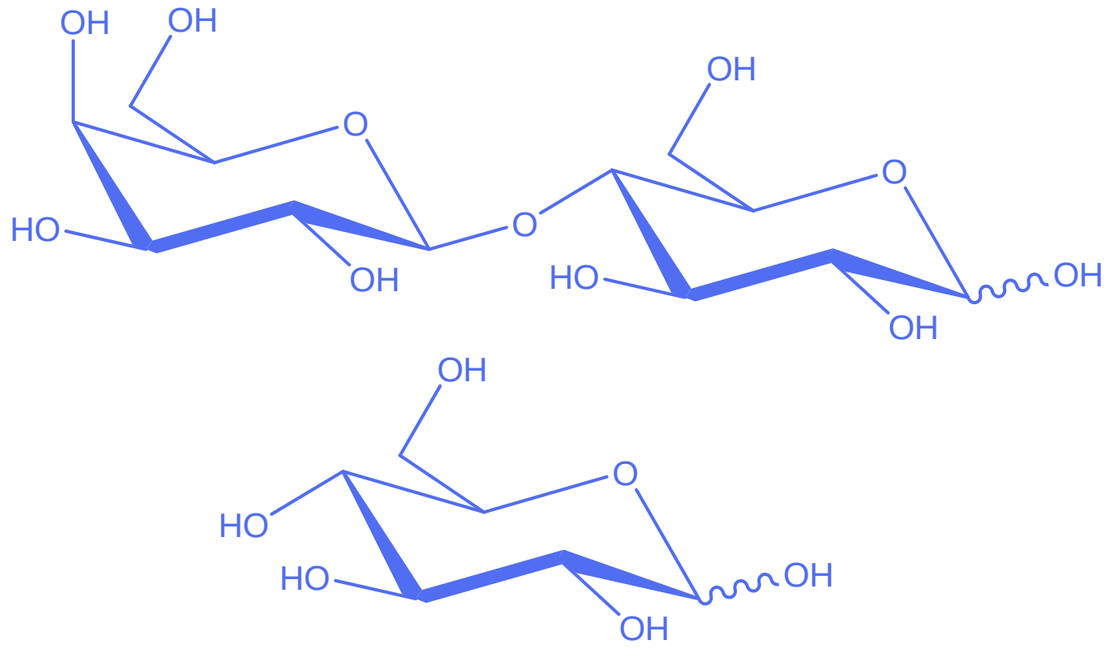
1. Analysis of Sugars in Foods



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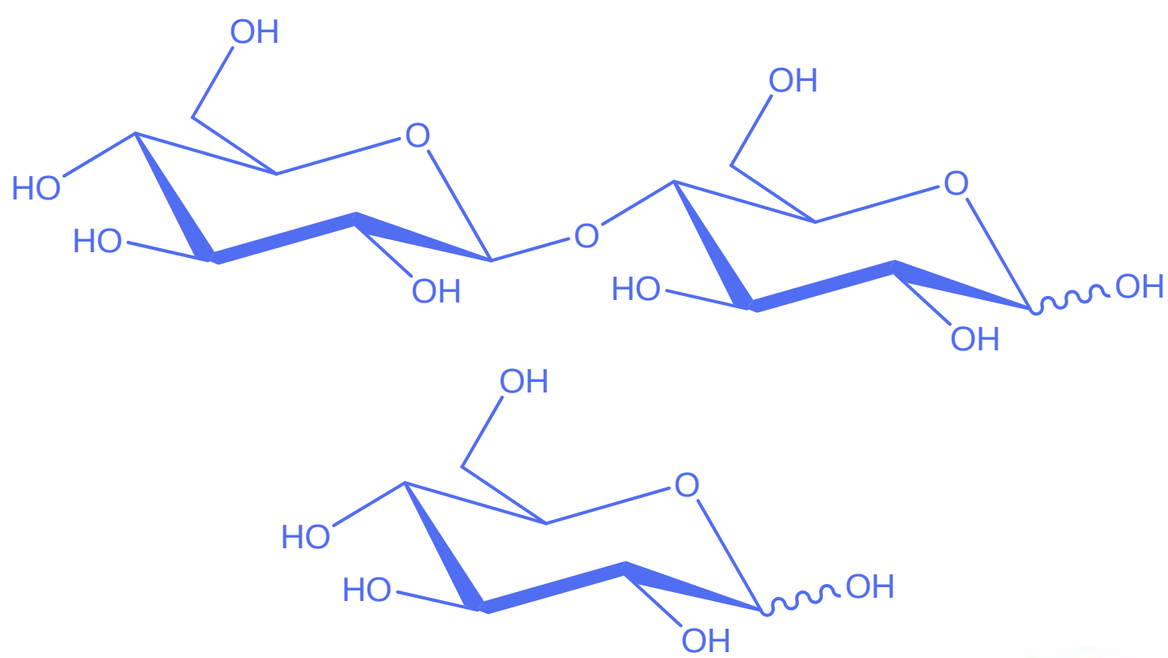
Definition of Sugars (Codex)

All monosaccharides and disaccharides in food



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Common sugars in foods

Monosaccharides:

Fructose, Glucose, Galactose

Disaccharides:

Lactose, Maltose, Sucrose

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Common Properties

1. Water soluble

2. Low thermal stability

3. High chemical reactivity

4. Do not have properties of fluorescence emission and only absorb in UV at very short wavelenghts

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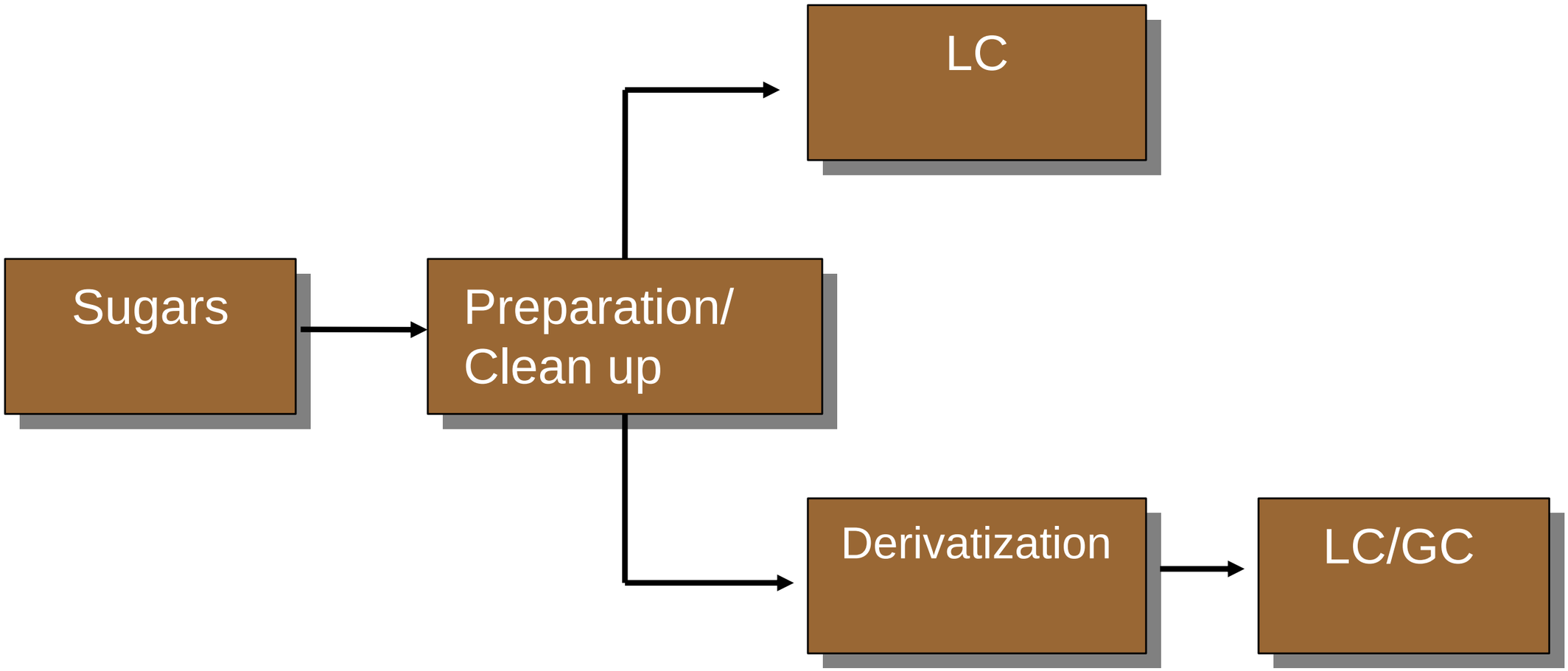
Typical analysis



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Preparation/Clean up

Extraction:

Water or water/ethanol mixture

Clean up:

Solid phase extraction (e.g. C18, charcoal)

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LC Analysis

After the clean up step, carbohydrates can be analysed by HPLC without derivatization, using

1. Refractive index detector

2. Light scattering detector

3. UV detector at low wavelengths

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LC analysis with derivatization

Introduce chromogenic or fluorogenic group to the saccharide molecules.

Common reagents:

p-Aminobenzoic acid (*p*-AMBA)

4-(3-methyl-5-oxo-2-pyrazolin-1-yl) benzoic acid (PMPA).

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GC analysis with derivatization

Convert carbohydrates into volatile derivatives before GC analysis.

Popular derivatives are methyl, trifluoroacetyl, trimethylsilyl and tert-butyldimethylsilyl ethers.

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AOAC methods

AOAC 980.13	Fructose, glucose, lactose, maltose and sucrose in milk
AOAC 977.20	Separation of sugars in honey
AOAC 982.14	Glucose, fructose, sucrose and maltose in presweetened cereals
AOAC 995.13	Carbohydrates in soluble (instant) coffee

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AOAC 980.13 with modification

Extraction:	Mixture of ethanol and water
Separation:	Centrifuge
Equipment:	LC with refractive index detector
Column:	Amine & sugar column
Mobile phase:	Acetonitrile and water

No derivatization required

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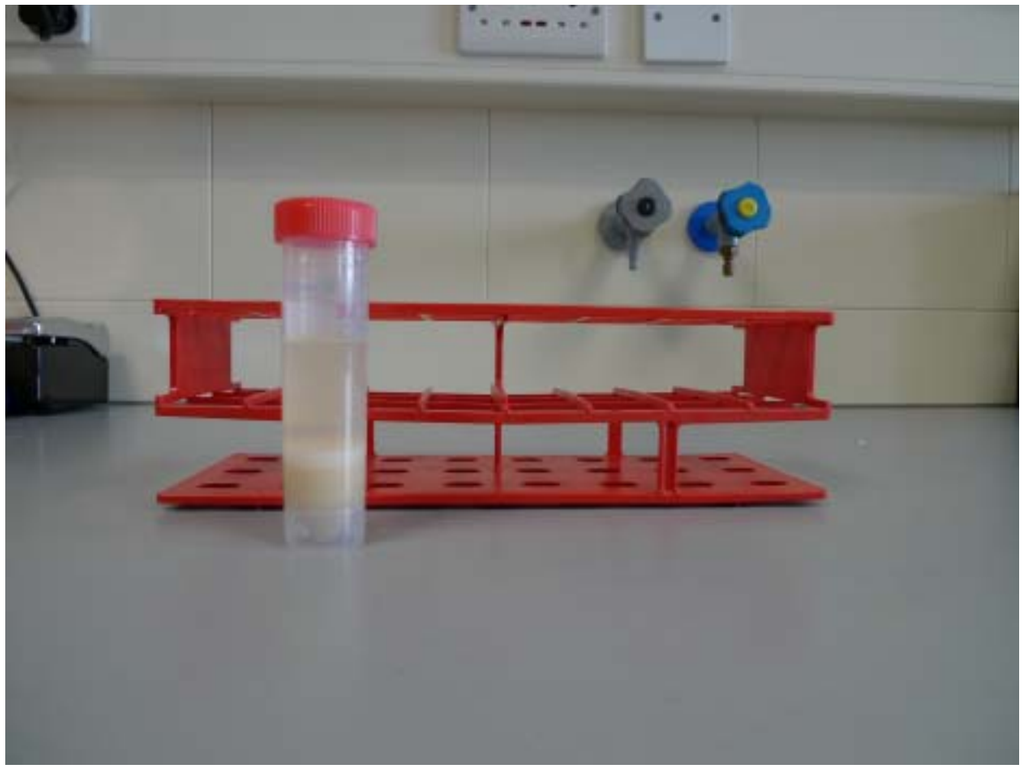
AOAC 980.13 with modification

Extract the sugars from sample using a mixture of ethanol and water



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AOAC 980.13 with modification

Centrifuge the mixture after the extraction

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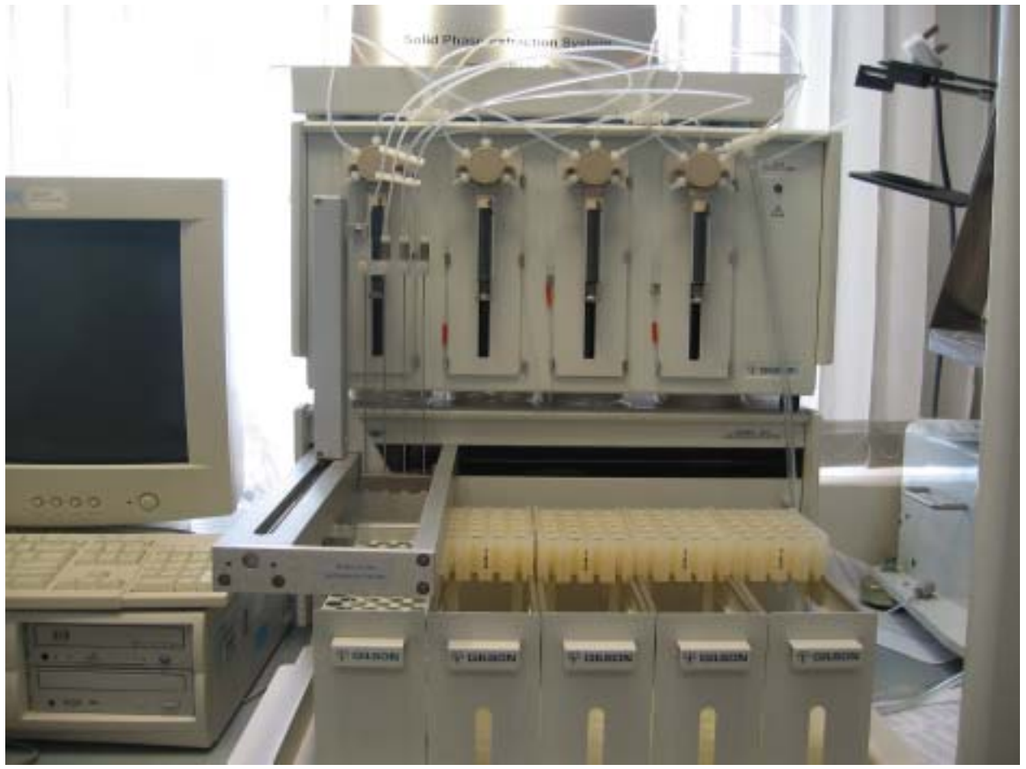
AOAC 980.13 with modification

Clean up the extract by SPE



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AOAC 980.13 with modification

Analyse the extract using LC with refractive index detector (No derivatisation)

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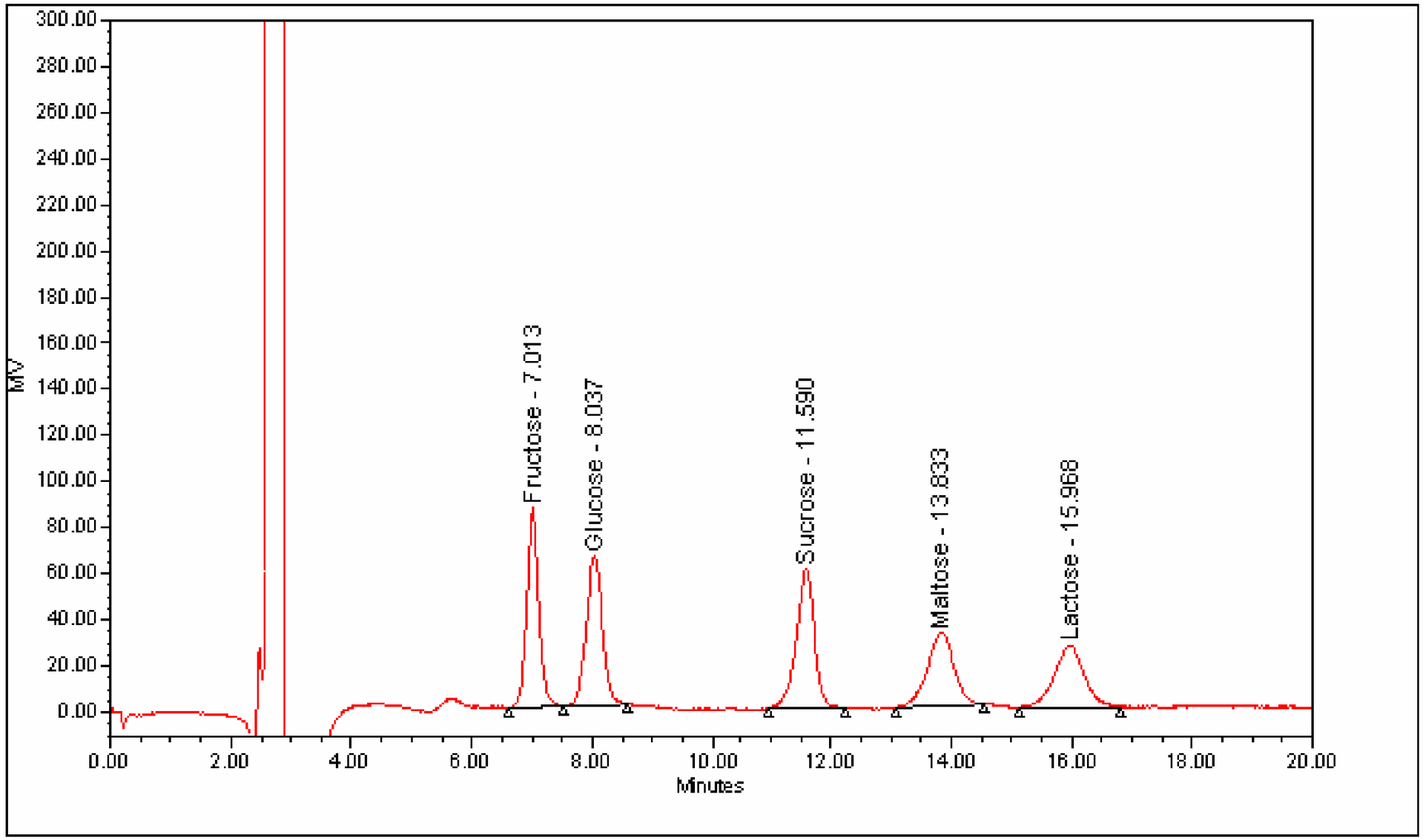

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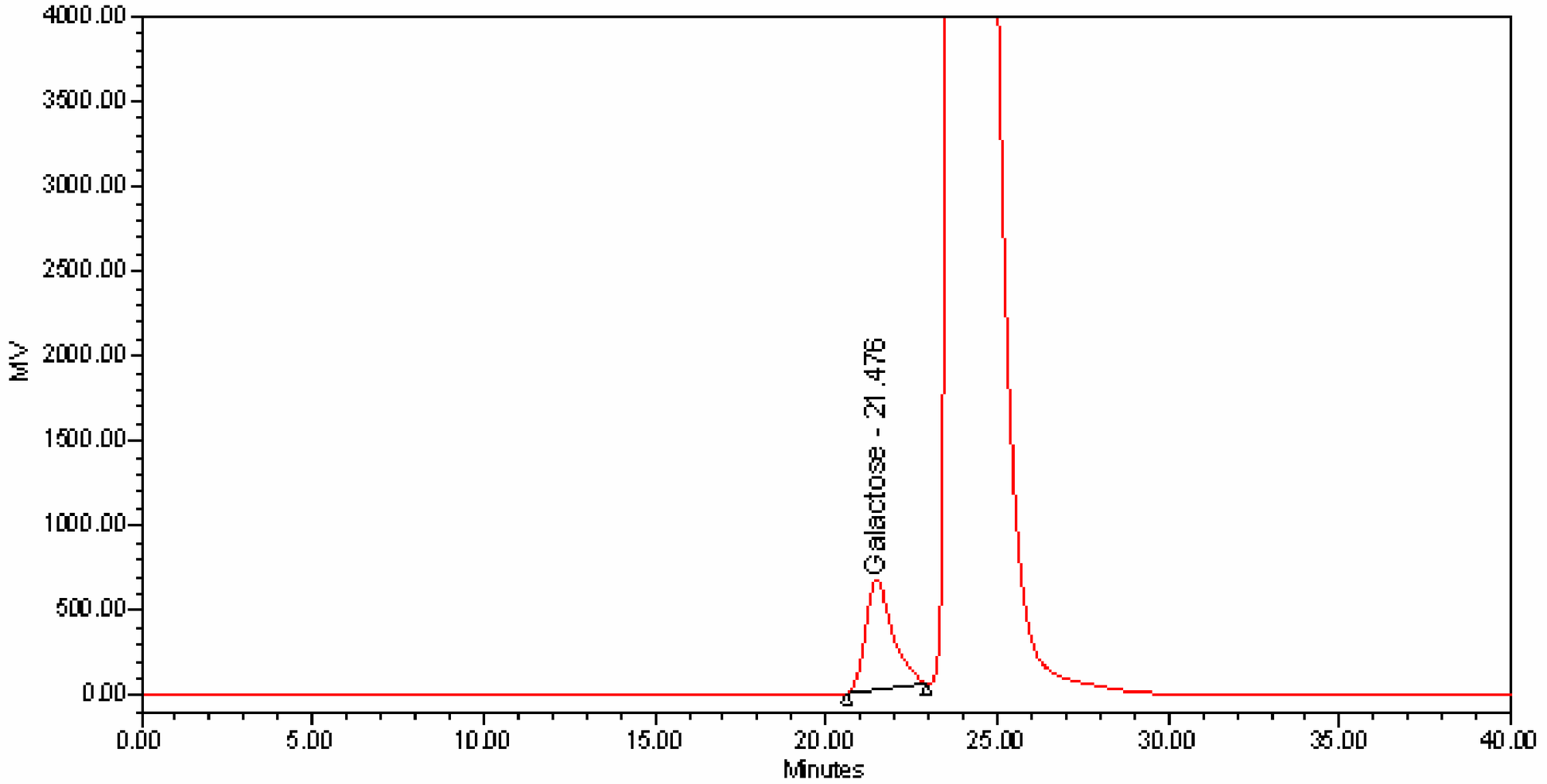
AOAC 980.13 with modification



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Proficiency test

FAPAS

LGC

AOAC

Points to note

Sugar alcohols (polyols) are not “sugars”

Not all monosaccharides and disaccharides are reducing sugars. Test method for reducing sugar may not be suitable for the analysis of sugars.

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Points to note

Definition of “0”

Sugars ≤0.5 g/100 g

Limit of detection of total sugars should be less than or equal to 0.5 g/100 g.

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2. Analysis of Sodium in Foods

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Examples in AOAC

AOAC 969.23	Sodium and Potassium in Seafood
AOAC 985.35	Minerals in Infant Formula, Enteral Products and Pet Foods
AOAC 984.27	Ca, Cu, Fe, Mg, Mn, P, K, Na and Zn in Infant Formula

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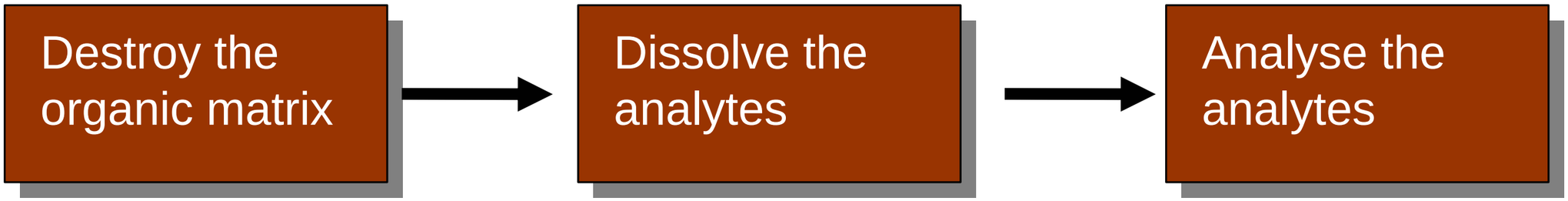
Examples in AOAC



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Destroy the organic matrix

Organic matrix is destroyed by:

1. Dry ashing
2. Wet digestion

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Analyse the analytes

Sodium in the residue of dry ashing/wet digestion are dissolved in diluted acid and analysed by:

1. Flame atomic absorption spectrometry (FAAS)
2. Inductively coupled plasma atomic emission spectrometry (ICP-AES)

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Analyse the analytes

Dissolve the residue of digestion in dilute acid

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Analyse the analytes

Analyse the elements using ICP-AES

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Flame atomic absorption spectrometry

Flame is used to produce ground-state atoms.

Sodium atoms formed during the atomization may be lost by ionization.

$$Na \longrightarrow Na^{+} + e^{-}$$

Depression of signal.

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Flame atomic absorption spectrometry

Ionization suppressor

Large excess of easily ionisable element (e.g., Cs) can be used to suppress the ionization.

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Flame atomic absorption spectrometry

Excess Cs in the flame suppresses the Ionization (e.g. Na) by mass action effect

$$Cs \longrightarrow Cs^+ + e^-$$

$$Na \longleftarrow Na^+ + e^-$$

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Flame atomic absorption spectrometry

Typical limits of detection: 0.01 – 0.1 mg/L

Linear dynamic range is generally no more than three orders of magnitude (e.g. from 0.01 to 10 mg/L).

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Inductively coupled plasma atomic emission spectrometry

Plasma instead of flame is the excitation source.

Temperature in plasma is much higher than that in flame (7,000 K compared with 2000 to 3000 K).

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Inductively coupled plasma atomic emission spectrometry

Because of the high temperature most samples are completely atomized and this technique suffers from few chemical interferences compared with the flame.

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Inductively coupled plasma atomic emission spectrometry

Characterized by low detection limits of the order of 1 – 100 ng/mL, because of its high excitation temperature compared with the flame.

It has a larger linear dynamic range compared with FAS.

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Proficiency test

FAPAS

AOAC

LGC

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Points to note

Addition of Cs can produce higher results especially in the presence of high level of potassium in food.

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Points to note

Definition of “0”

Sodium ≤5 mg/100 g.

Limit of detection should be better than 5 mg/100g

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3. Analysis of Fatty Acids (saturated and trans) in Foods

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Fat (Total, Saturated, and Unsaturated) in Foods

Hydrolytic Extraction Gas Chromatographic Method

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The method is supported by interlaboratory study for the determination of total, saturated fat and monounsaturated fat in different food stuffs

Wheat-based cereal
Peanut butter
Fish sticks
Ground beef, etc

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

Triglyceride, triundecanoin ($C_{11:0}$), is added as internal standard.

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Fat is extracted into ether, then methylated to fatty acid methyl esters (FAMEs) using BF_3 in methanol.

FAMEs are quantitatively measured by capillary gas chromatography against $C_{11:0}$ internal standard.

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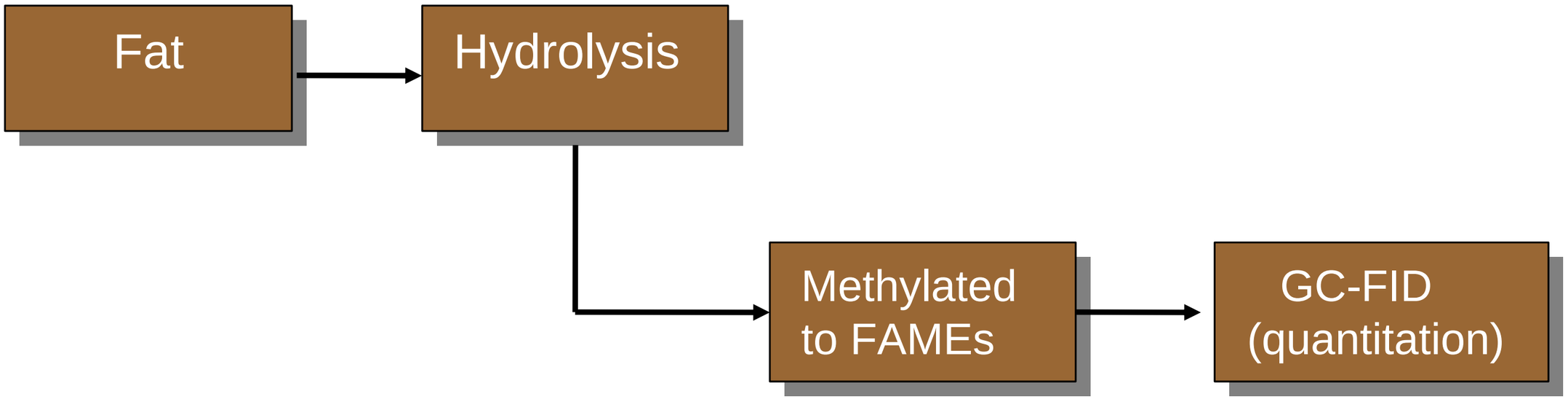

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Apparatus

Gas chromatograph equipped with flame ionisation detector (GC-FID).

Capillary column (SP2560 100 m x 0.25 mm with 0.25 μm film is suitable).

Mojonnier flasks.

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Mojonnier flask

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Hydrolysis

Weigh sample and pyrogallic acid into the Mojonnier flask

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Hydrolysis

Add internal standard, ethanol and 8.3 M HCl.

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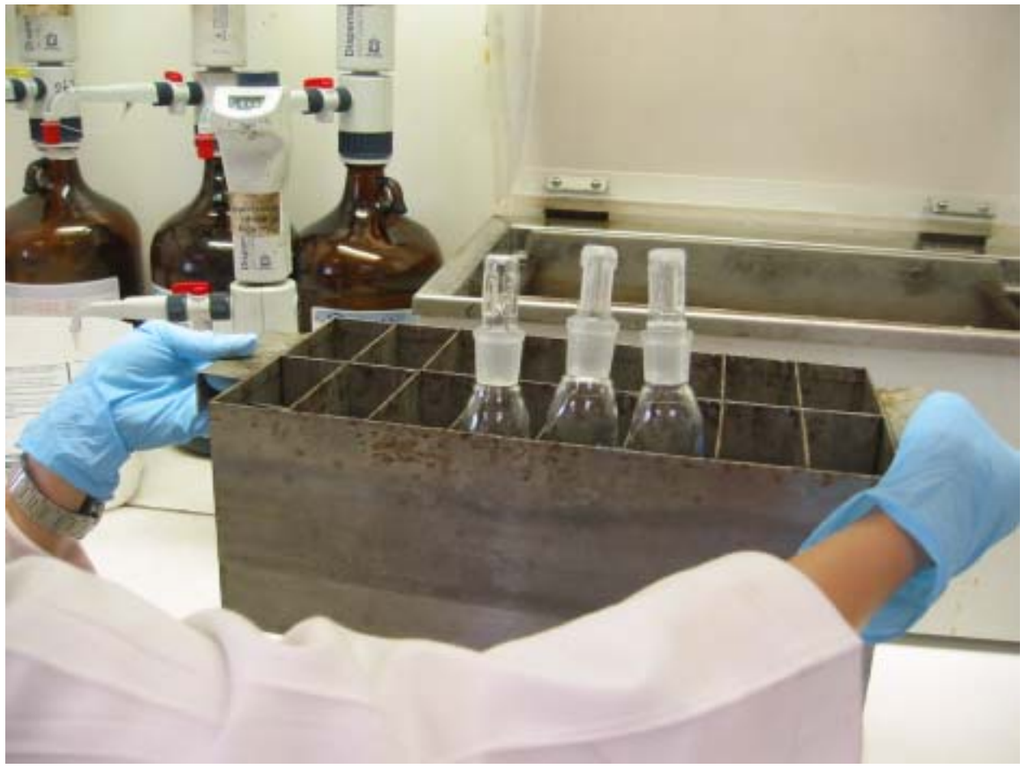
Hydrolysis

Hydrolyse the sample at 70 – 80 ºC



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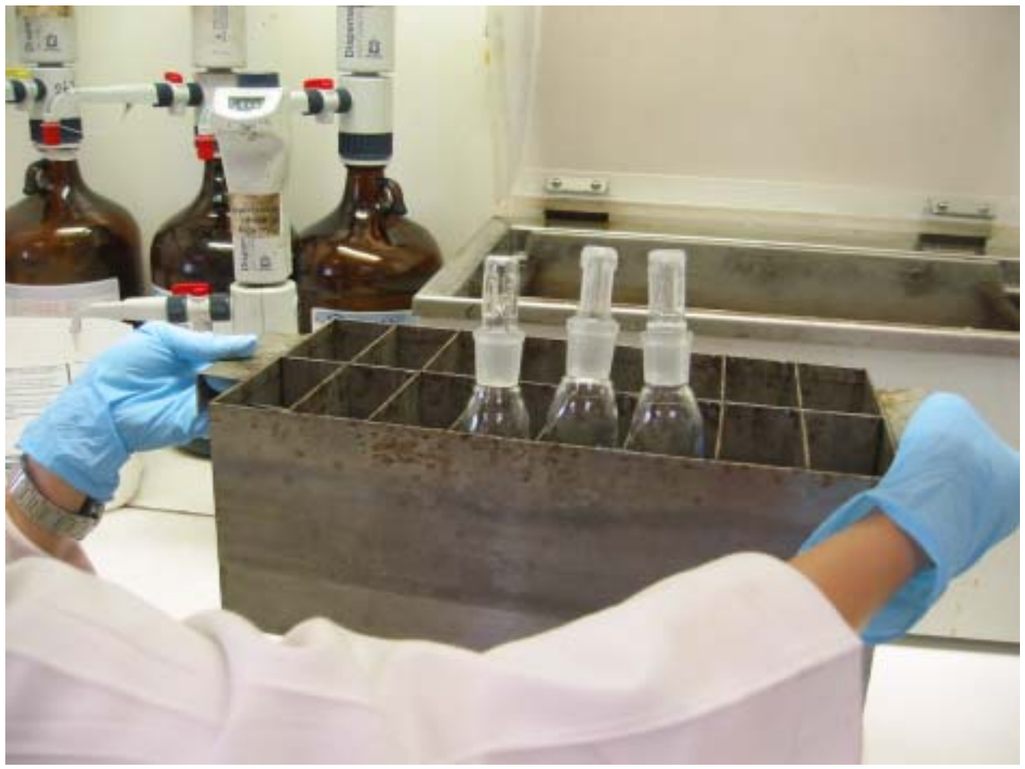
Hydrolysis

Remove flasks from water bath. Cool to room temperature.



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Extraction

Add ethanol and diethyl ether. Shake.

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Extraction

Add petroleum ether.

Shake.

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Extraction

Centrifuge sample at 600 rpm. (allow contents to sep. at least 1 h if centrifuge is not available)

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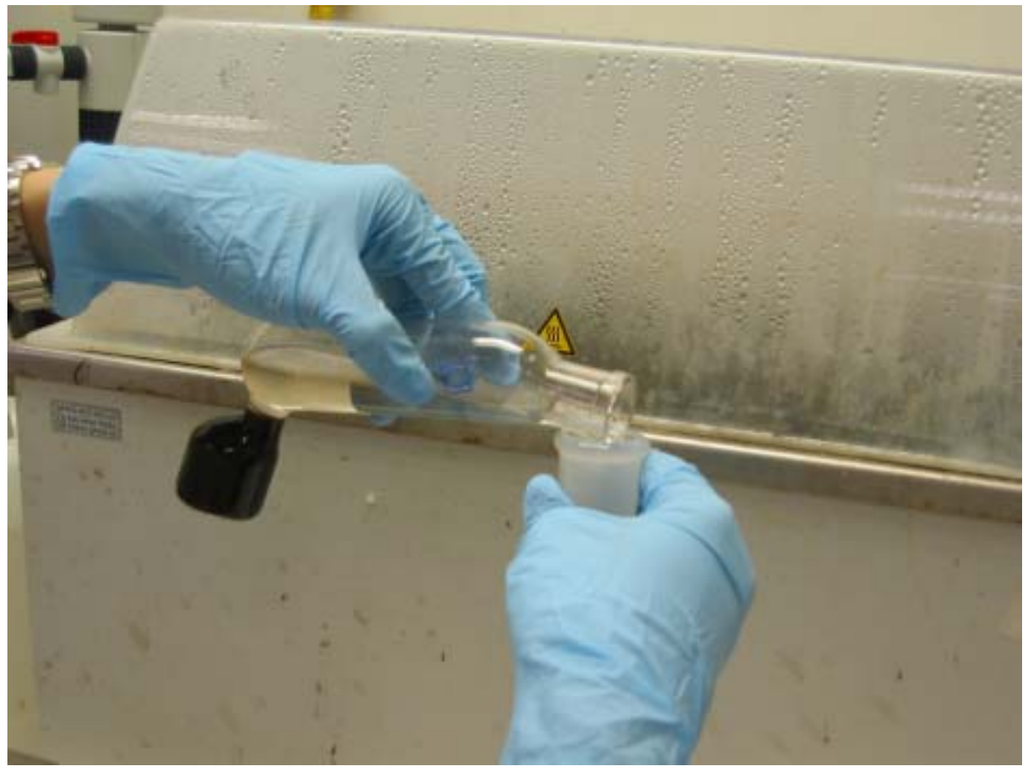
Extraction

Decant the ether layer into a tube.



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Extraction

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

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Extraction

Dissolve the residue in chloroform.

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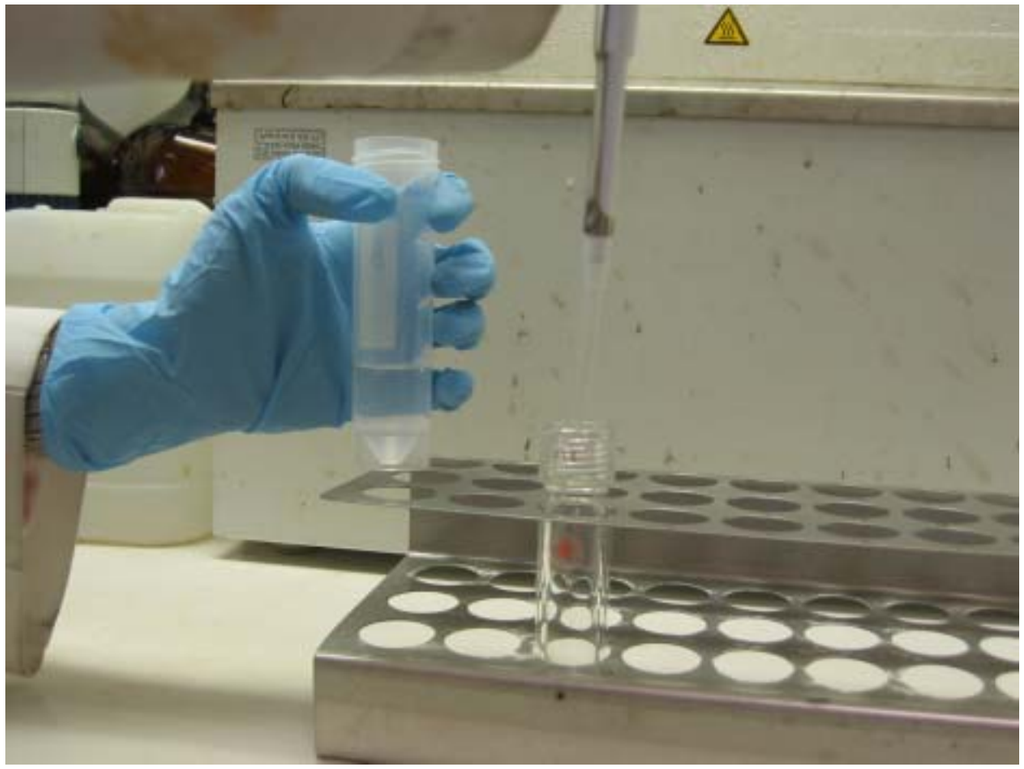
Methylation

Transfer mixture to a glass vial. Evaporate to dryness.



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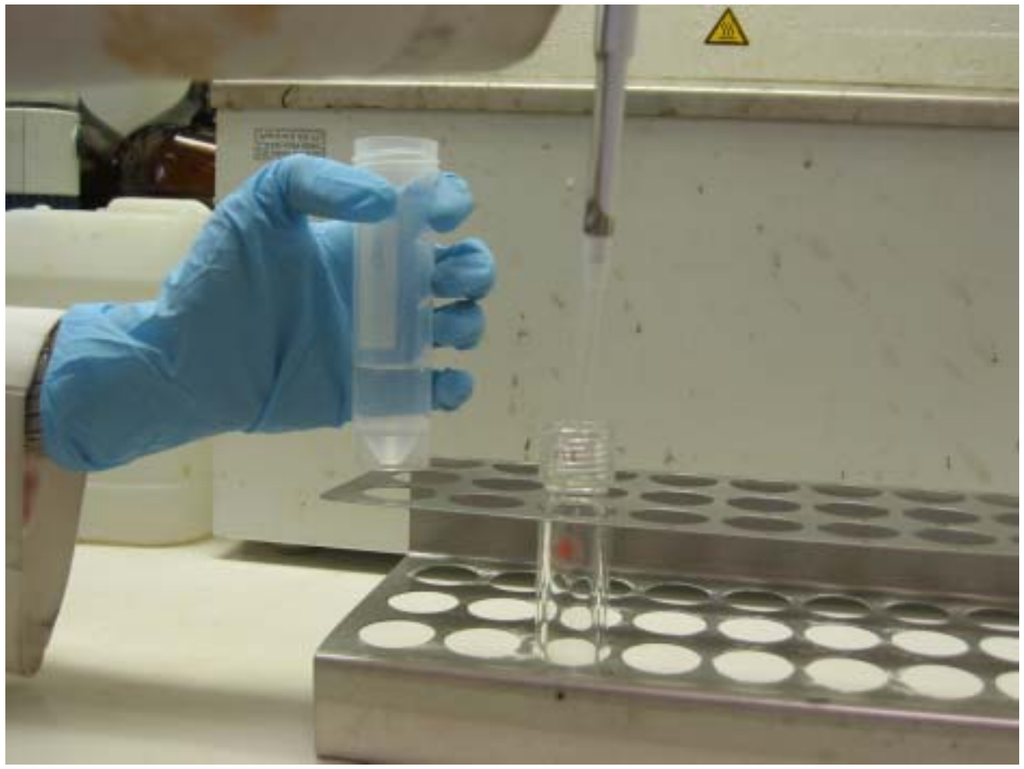
Methylation

Add 7% BF_3 reagent and toluene.



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Methylation

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

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Methylation

Allow vials to cool to room temperature.

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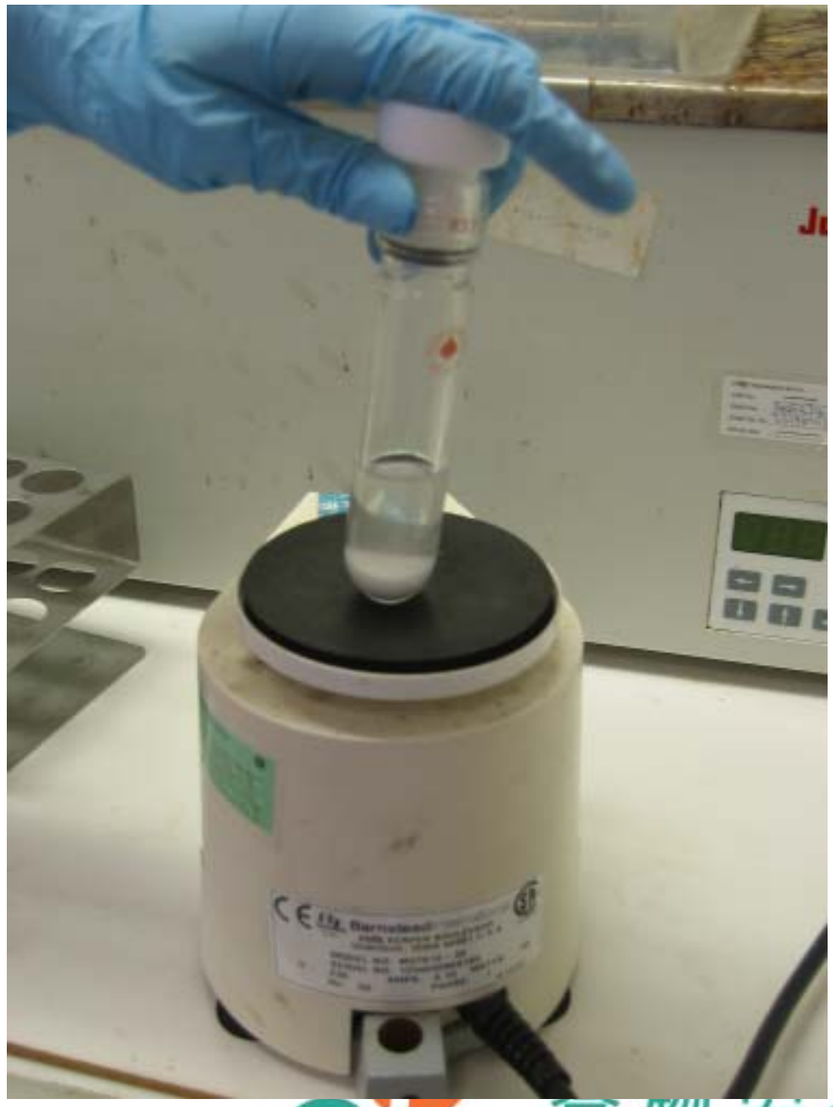
Methylation

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



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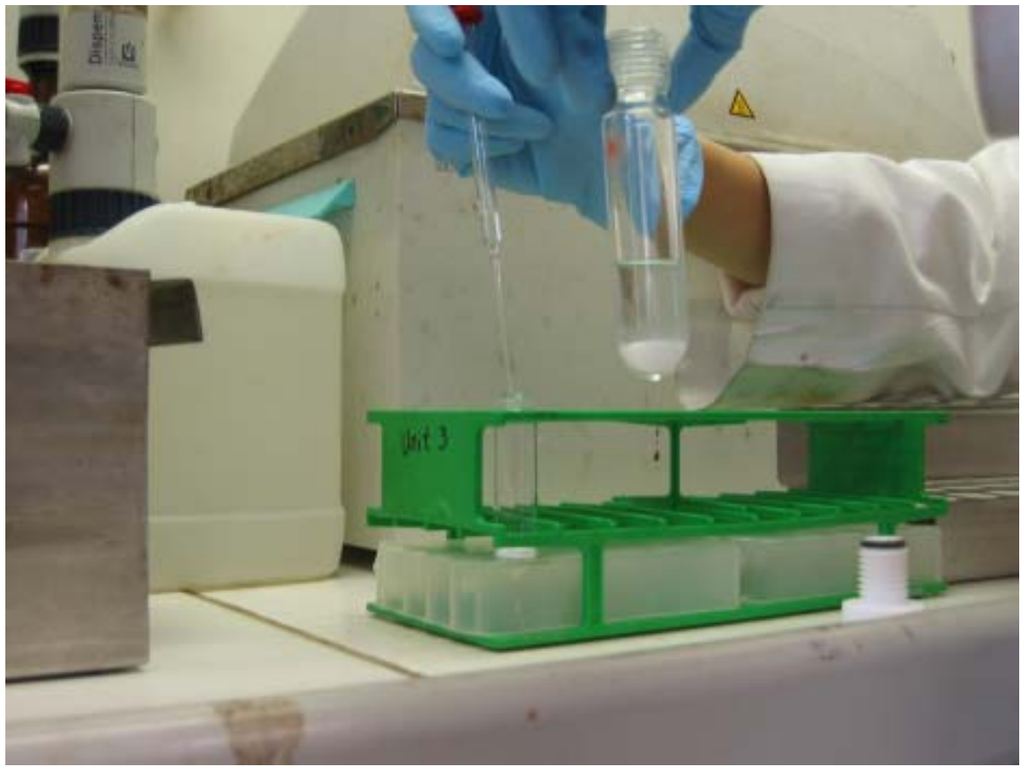
Methylation

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



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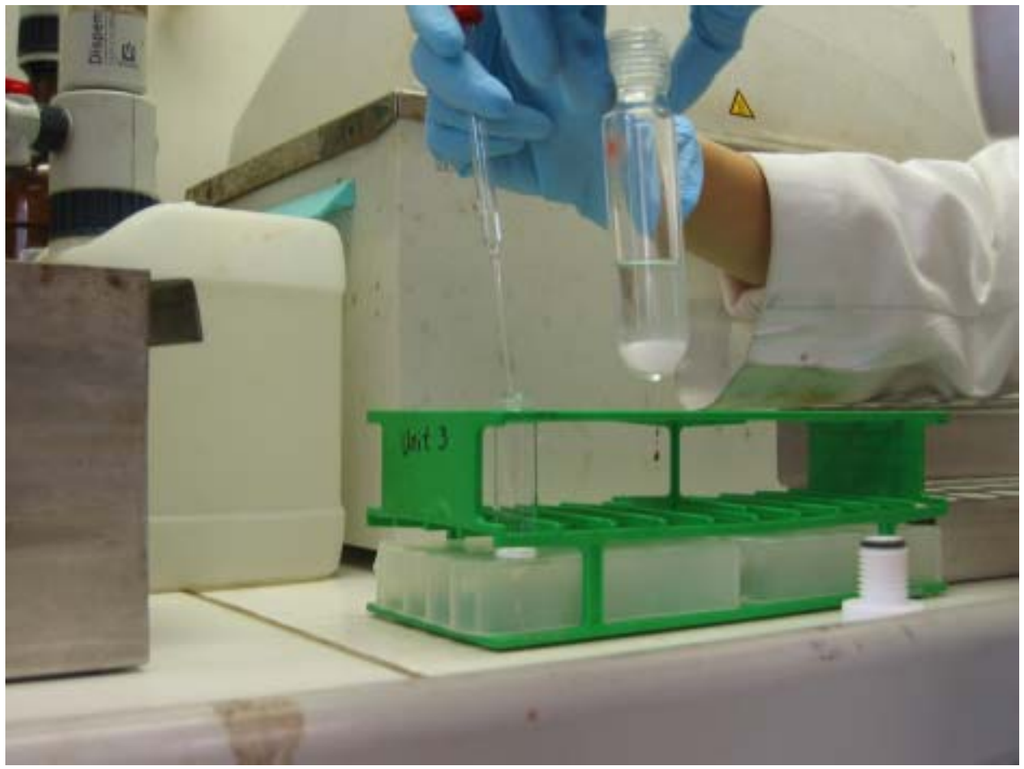
Methylation

Top layer should contain FAMEs and internal standard.



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GC Determination

Transfer to autosampler vial for GC analysis.

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Peaks of unknown identity should not be included in the summation when quantifying fat in the test portion.

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Peaks of known identity with relative retention times are given the method (more than 50 FAMEs).

The FAME are ranging from butyric acid ($C_{4:0}$) To docosahexaenoic acid ($C_{22:6}$).

Relative retention time of several *trans* and *cis*-FAMEs are also provided in the method.

FAME standards (saturated fat)

C4:0 Methyl butyrate

C6:0 Methyl hexanoate

C8:0 Methyl octanoate

C10:0 Methyl decanoate

C12:0 Methyl laurate

C14:0 Methyl myristate

C15:0 Methyl pentadecanoate

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FAME standards (saturated fat)

C16:0 Methyl palmitate

C17:0 Methyl heptadecanotate

C18:0 Methyl stearate

C20:0 Methyl eicosanoate

C22:0 Methyl docosanoate

C24:0 Methyl tetracosanoate

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FAME standards (trans fat)

C14:1T(9-*trans*) Methyl *trans*-9-tetradecanoate

C16:1T (9-*trans*) Methyl *trans*-9-hexadecenoate

C18:1T (6-*trans*) Methyl *trans*-6-octadecenoate

C18:1T (9-*trans*) Methyl *trans*-9-octadecenoate

C18:1T (11-*trans*) Methyl *trans*-11-octadecenoate

C18:2TT (9,12-*trans*) Methyl *trans*-9,12-octadecadienoate

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FAME standards (trans fat)

C18:2T (9-*cis*,12-*trans*) Methyl *cis*-9, *trans*-12-octadecadienoate

C18:2T (9-*trans*,12-*cis*) Methyl *trans*-9, *cis*-12-octadecadienoate

C20:1T (11-*trans*) Methyl *trans*-11-eicosenoate

C18:2T (9-*cis*,11-*trans*) Methyl *cis*-9, *trans*-11-octadecadienoate

C22:1T (13-*trans*) Methyl *trans*-13-docosenoate

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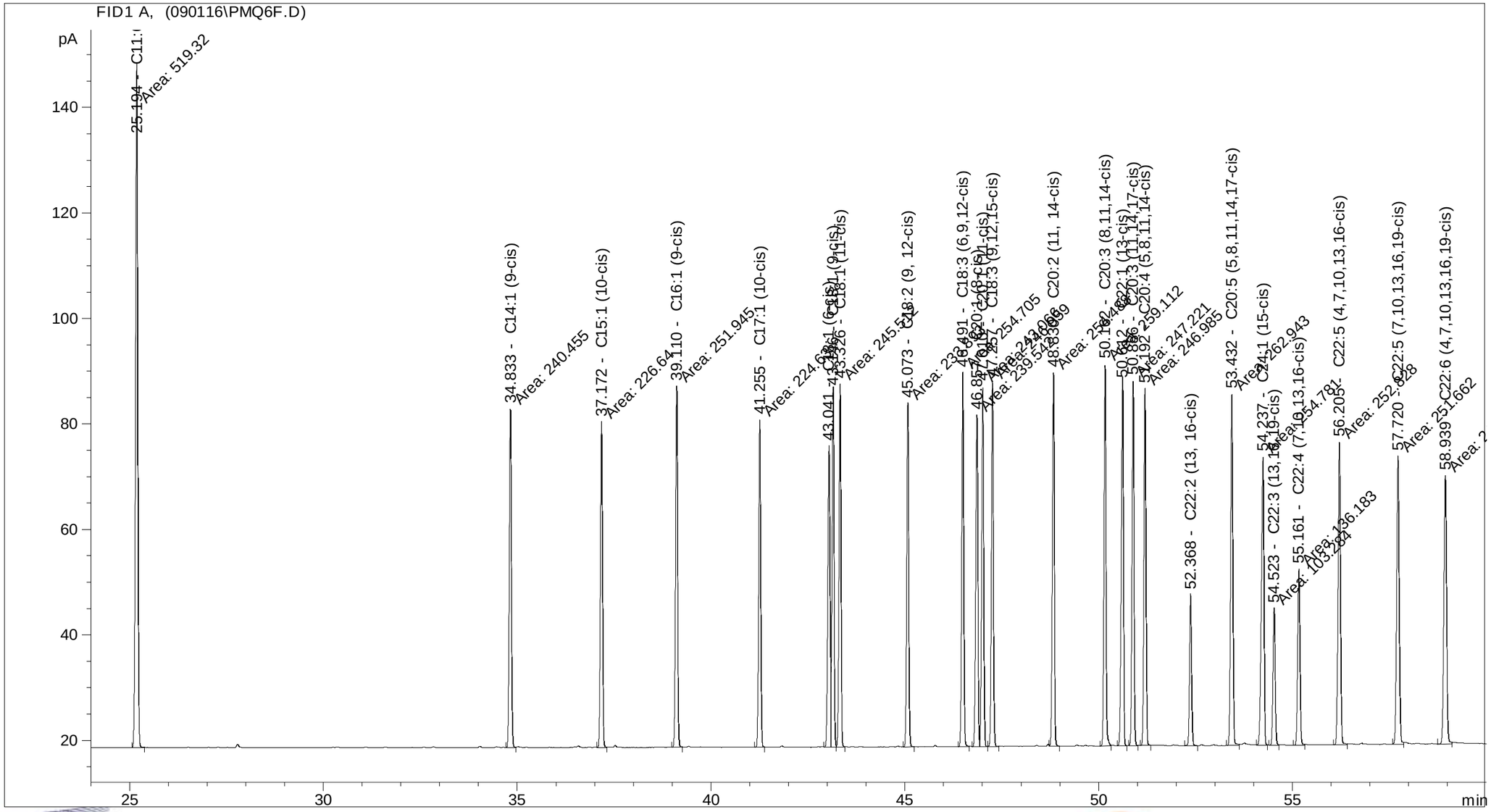
Standard FAMEs



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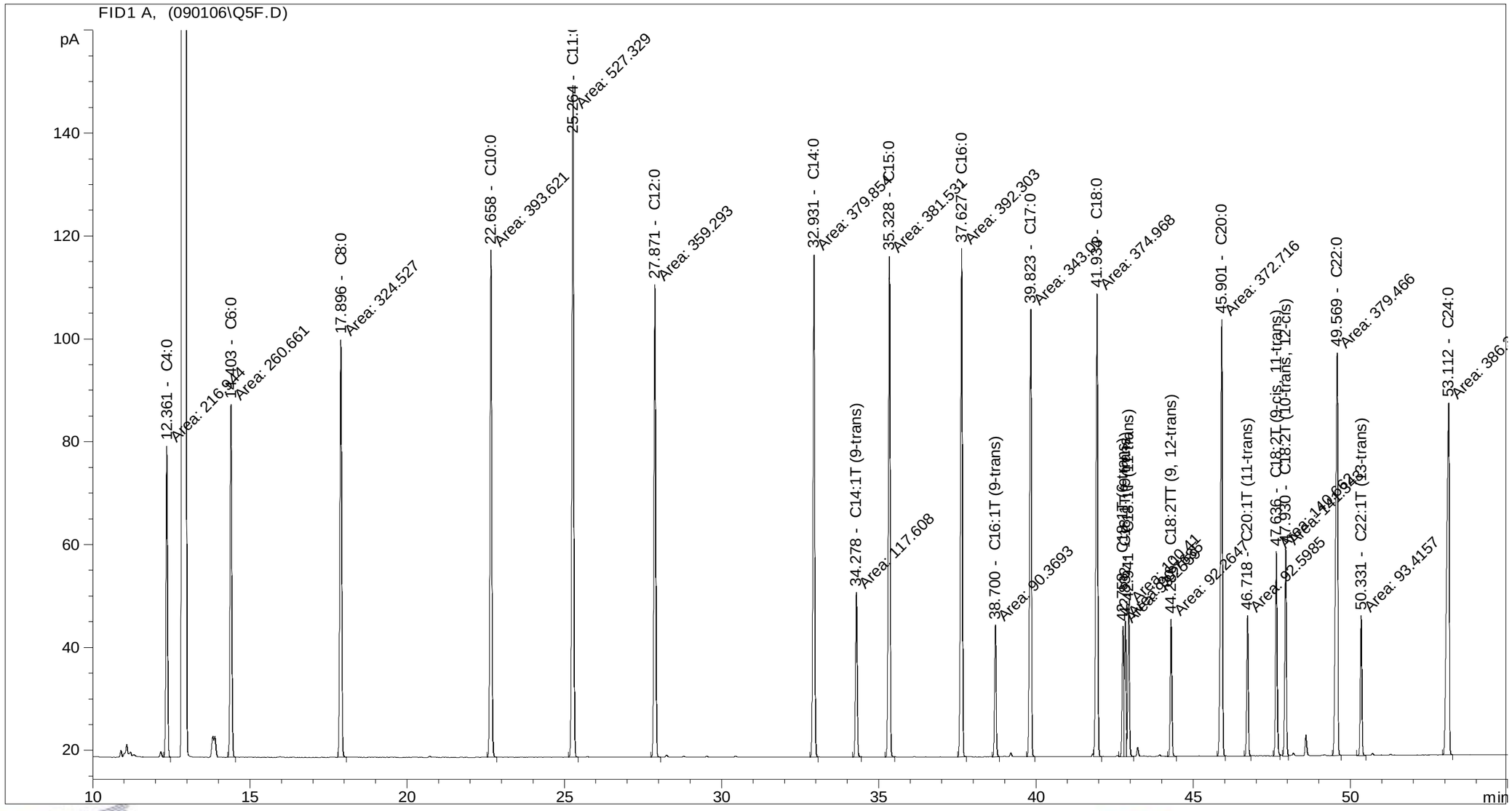
Standard FAMEs



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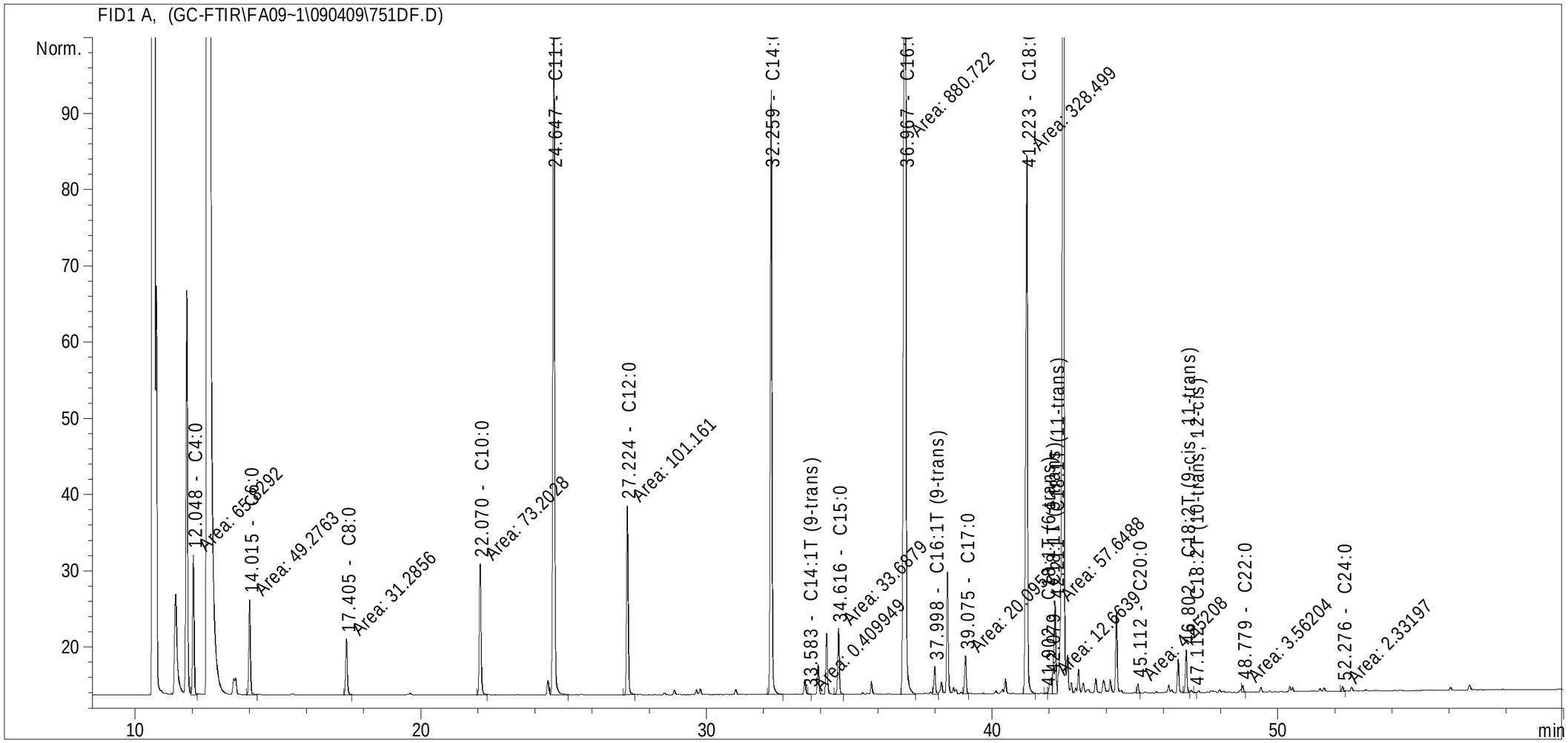
Real sample



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Proficiency test

FAPAS

AOAC

LGC

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Points to note

Availability of FAME standards is crucial to this test method.

Insufficient FAME standards would underestimate the level of sat or trans fat.

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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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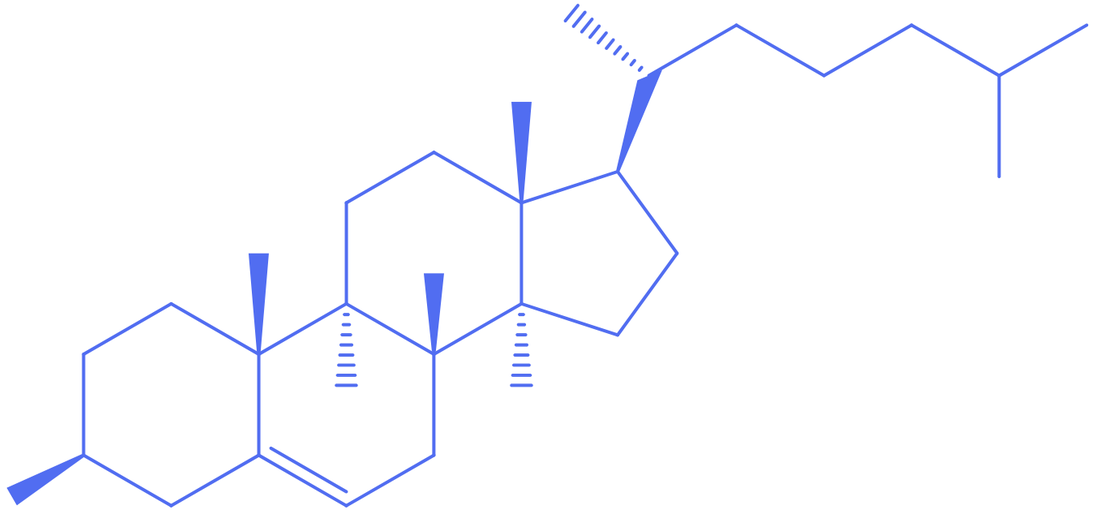
4. Analysis of Cholesterol in Foods



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Properties

Waxy alcohol

Low solubility in water

High boiling point (360 ^{o}C)

High chemical reactivity

Absorb in UV at very short wavelength

Examples

AOAC 994.10	Cholesterol in Foods
AOAC 976.26	Cholesterol in Multicomponent Foods

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AOAC 976.26

Cholesterol in Multicomponent Foods
Gas Chromatographic Method

Similar to method 944.10

Benzene instead of toluene is used to extract the cholesterol.

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AOAC 994.10

The method has been used in interlaboratory for the determination of cholesterol in different a variety of foods, e.g.,

butter cookies, vegetable bacon baby food, vegetable chicken baby food, commercial powdered eggs, etc.

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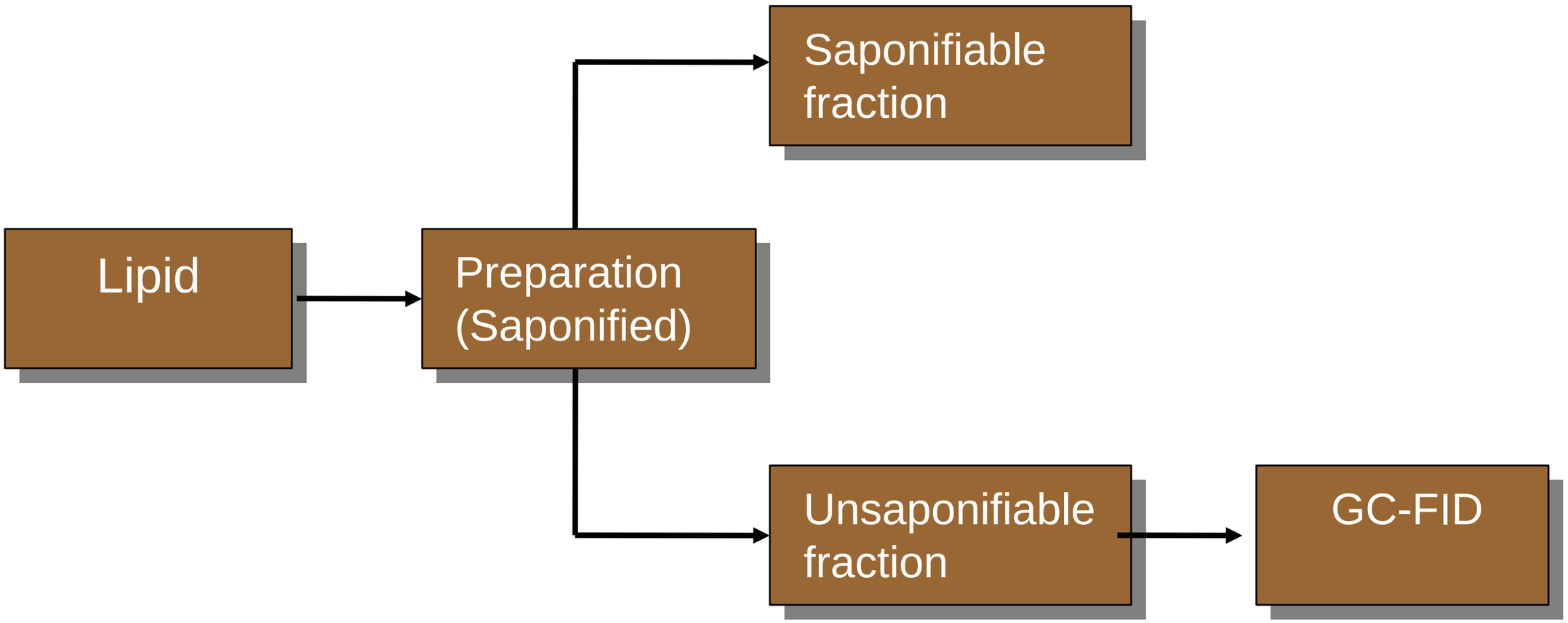
Analysis



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Reagents:

- Toluene
- KOH
- Hexamethyldisilane (HMDS)
- Trimethylchlorosilane (TMCS)
- Internal standard (5α-cholestane)

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Saponify the sample at high temperature with ethanolic KOH (70 min)

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Turn of heat.

Add ethanol.

Remove flask from condenser and allow to cool.

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Add toluene.

Pour solution into a 500-mL separatory funnel.

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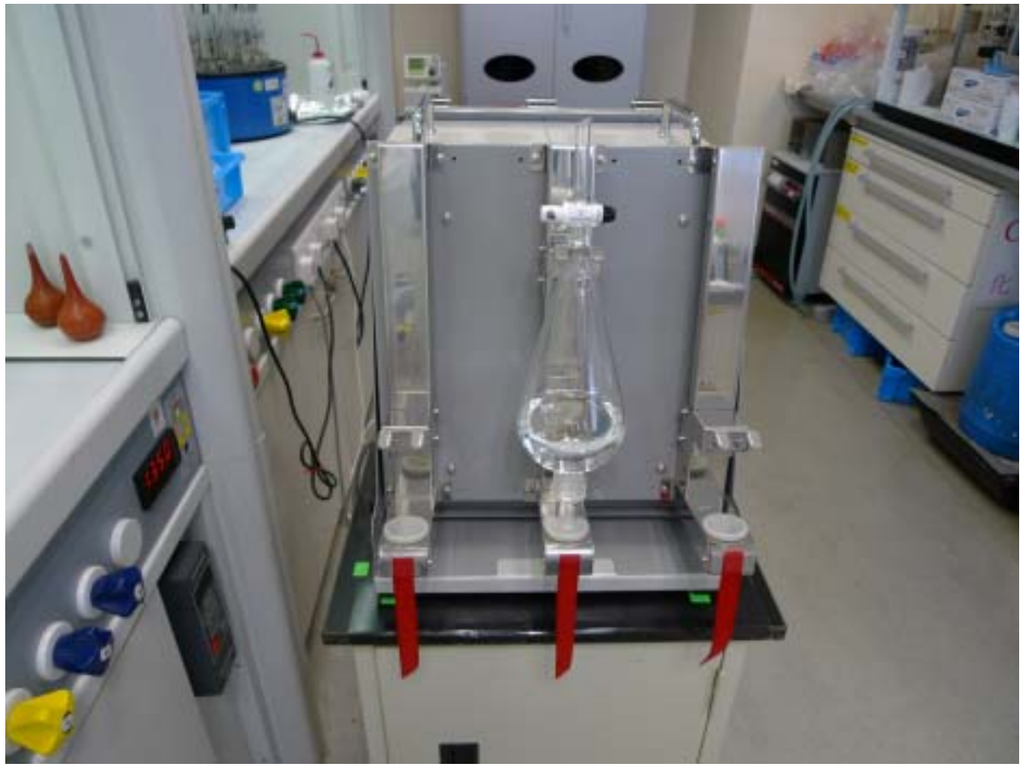
Add 1 M KOH and shake.

Discard aqueous layer.

Wash toluene layer with water.



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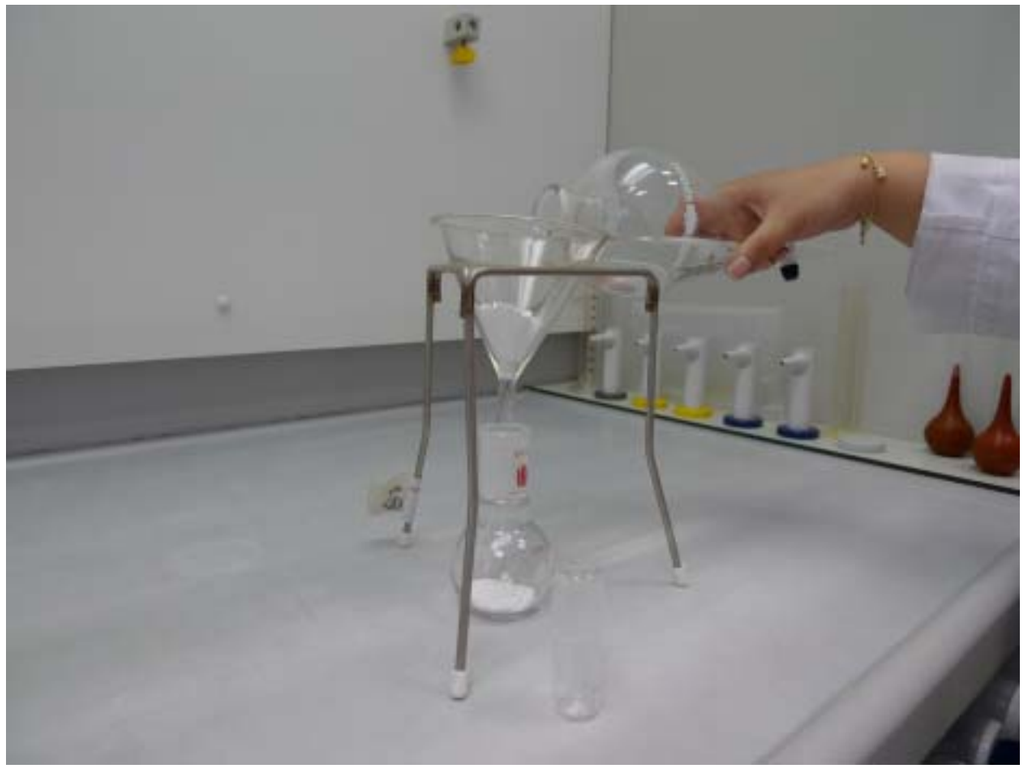
Pour toluene layer through funnel containing plug of glass wool and Na_2SO_4. (Flask contains ~2 g Na_2SO_4)



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Evaporate extract to dryness (40 ºC).

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Add acetone and evaporate to dryness again. Dissolve residue in DMF.

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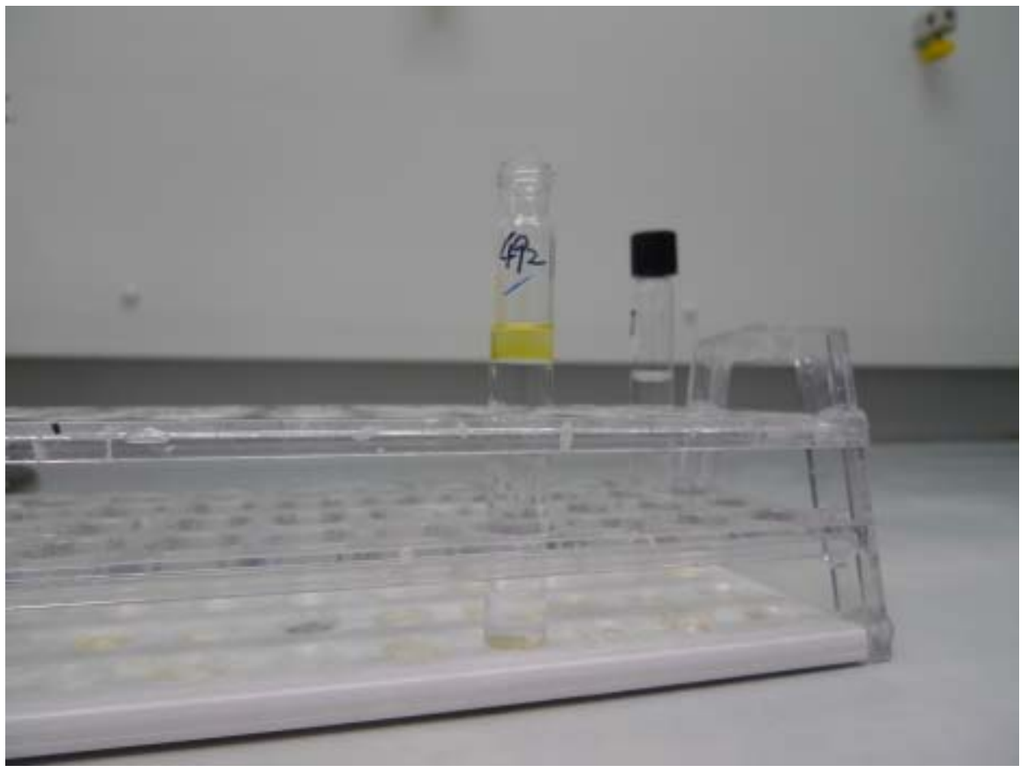
Pipet 1.0 mL test portion into a centrifuge tube. Add HMDS and TMCS.



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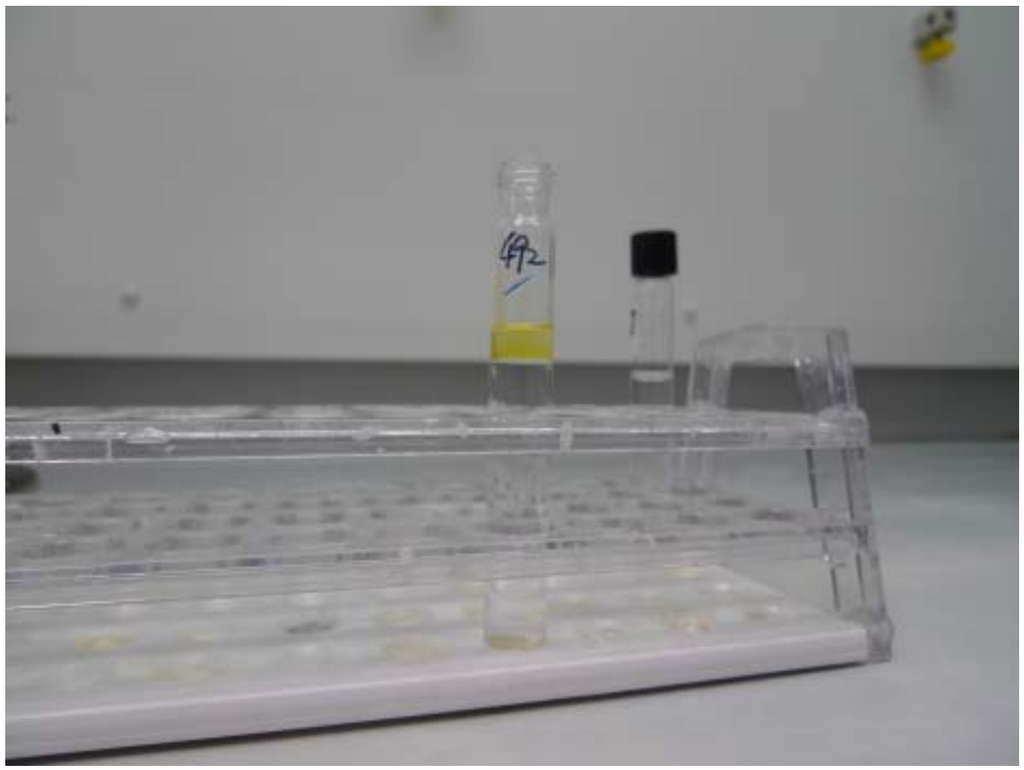
Shake and let solution stand undisturbed for 15 min.



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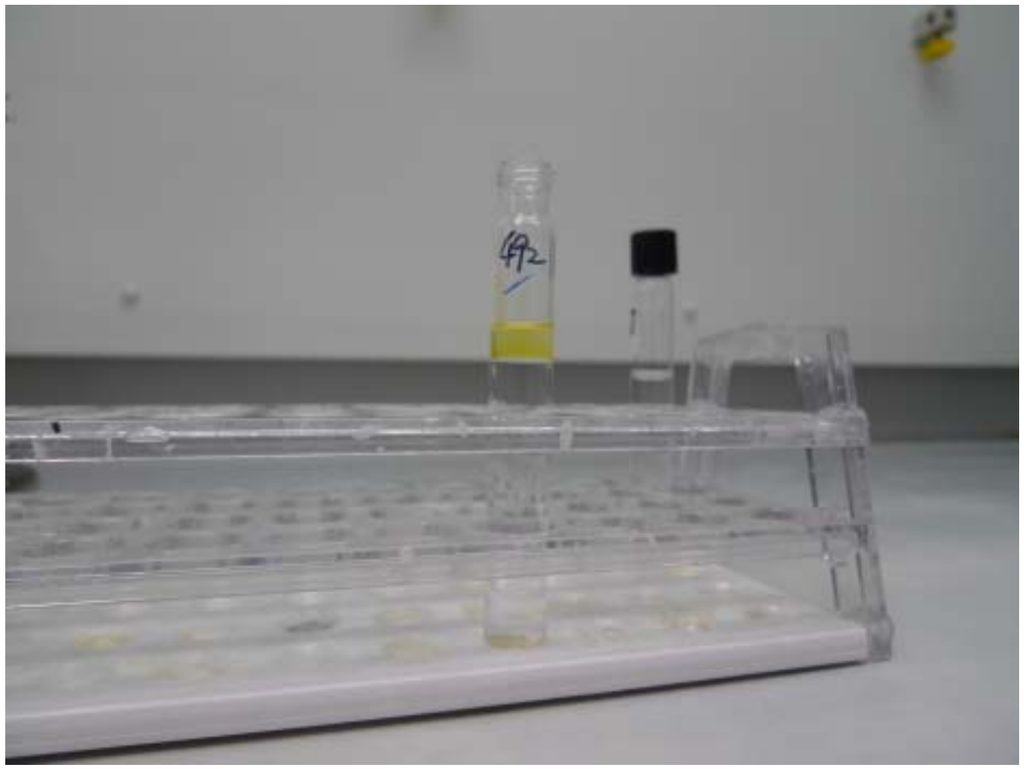
Add
5α-choletane
internal standard
and water.
Stopper tube,
shake.



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Centrifuge the tube ~2 minutes.

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Transfer upper layer to a GC injection vial.

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Analyse the upper layer by gas chromatograph

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Column:

25 m x 0.32 mm x 0.17 μm film thickness, cross-linked 5% phenyl-methyl silicone or methyl silicone gum.

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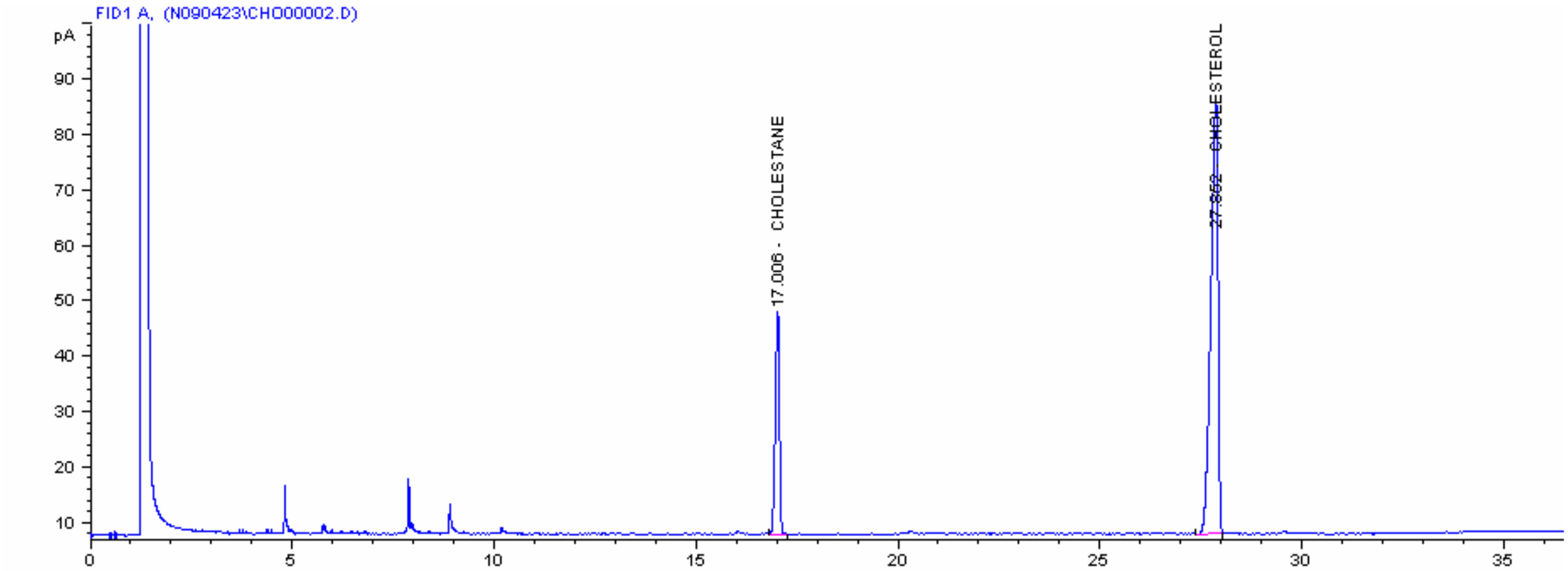

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1. Six standard solutions
2. Concentration: 0.0025 – 0.2 mg/mL
3. 5α-cholestane: 0.1 mg/mL in *n*-heptane

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Points to note

Centrifuge tubes have to be silanized.

i. Fill tubes with 10% HF (stand 10 min).
ii. Rinse with water and anhydrous methanol.
iii. Dry tube under nitrogen.
iv. Fill tubes with 10% HMDS in toluene and let stand 1 h.
v. Rinse with toluene and methanol.
vi. Dry tubes in 100 ^{o}C before use.

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Points to note

Definition of “0”

Cholesterol ≤5 mg/100 g.

Limit of detection of cholesterol should be better than 5 mg/100 g.

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Thank You

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