

DIFFERENTIATED FATTY ACIDS IN PLASMA BY DETECTOR GC/MS

Code GC75010

BIOCHEMISTRY

The term fatty acids (abbreviation FA, English Fatty Acids) refers to aliphatic monocarboxylic acids. They are, with few exceptions, long chains, with an even number of carbon atoms, without ramifications and acyclic (molecules which do not have closed ring chains); they can be saturated (molecules which have only single bonds C-C) or unsaturated (molecules containing double bonds C = C as well). They are the constituent ingredients of almost all complex lipids and plant and animal fats.

Fatty acids can be classified according to the length of the carbon chain:

short-chain fatty acids: with 1 to 5 carbon atoms

medium-chain fatty acids: with 6 to 12 carbon atoms

long-chain fatty acids: with 13 to 21 carbon atoms

very long-chain fatty acids: with 22 or more carbon atoms.

Depending on their length, they are distributed in the blood differently.

Fatty acids can be classified according to the presence or the absence of double bonds in the carbon chain:

- Saturated fatty acids: there are no double bonds in the carbon chain (for example, caprylic acid, C 8: 0, palmitic acid C 16: 0, stearic acid C 18: 0);
- Unsaturated fatty acids: there are double bonds in the carbon chain; in turn are divided into:
 - monounsaturated fatty acids or monoenoics, if there is only one double bond C = C (for example, oleic acid C 18: 1);
 - polyunsaturated fatty acids or polyenoics, if there are two or more double C = C bonds (for example linoleic acid C 18: 2, linolenic acid C 18: 3, arachidonic acid C 20: 4).

The metabolism of saturated and unsaturated fatty acids activates the same enzymes, and the body is not able to adjust to this competitive inhibition. Therefore, it is important to maintain the proper balance in the consumption of these two types of fats. An overload of fatty acids in general reduces the amount of enzymes and aggravates the problem of competition.

Some unsaturated fatty acids are considered particularly important for the human metabolism, and are classified as follows:

- omega-3: when the last double bond is present on the third carbon from the end (for example α -linolenic acid C 18: 3);
- omega-6: when the last double bond is present on the sixth carbon from the end (for example linoleic acid C 18: 2);
- omega-9: when the last double bond is present on the ninth carbon from the end (for example oleic acid C 18: 1). These are not considered essential fatty acids (EFA).

Fatty acids are biosynthesized in the human body from dietary fat, fat storage or endogenous lipids. Some polyunsaturated fatty acids cannot be biosynthesized through metabolic processes and need to be engaged under the diet. These fatty acids are called "essential" (EFA English essential fatty acid). The main EFAs are linoleic acid (C 18: 2 ω 6) and α -linolenic acid (C18: 3 ω 3). The degradation of fatty acids through β -oxidation pathway and results in the production of the acetyl-coenzyme A, which is used for the biosynthesis of new fatty acids, or is degraded in the Krebs cycle into water and carbon dioxide, releasing energy.

Free fatty acids represent the circulating fraction and the energy reserve of the organism lipids, which can be easily picked up and metabolized by the liver and muscles. Due to their insolubility, serum lipoprotein (albumin) is required to circulate in the blood.

In order to prevent obesity and related metabolic disorders, as well as atherosclerotic and thromboembolic diseases it is necessary to analyze, quantify and monitor the concentration of long chain fatty acids in the plasma matrix.

There are several studies that relate high levels of polyunsaturated fatty acids omega-3 longchain (EPA and DHA) to a reduced risk of developing cardiovascular disease, the main cause of death in the world.

Given the chemical structure of the molecules and their consequent poor solubility in water, over 98% of the fatty acids do not circulate in free form in the blood, but require carriers such as albumin.

Since this protein is one of the main transporter in plasma, we have chosen to analyze fatty acids in plasma. Only a small fraction, <2% is able to circulate in free form in whole blood.

This fraction is only increased in cases of a prolonged fast, intense physical activity, pheochromocytoma (tumor chromaffin tissue) or hyperthyroidism.

This increase is always accompanied by a high levels of fatty acids present in the plasma, thus confirming the importance of monitoring fatty acids in the plasma matrix. A further confirmation of the importance of plasma matrix is suggested by the latest studies in the field. It is currently possible to find many scientific work aimed at the determination of the concentration of long chain fatty acids in the plasma matrix rather than in whole blood.

The kit allows you to determine the main fatty acids in plasma (C14-C22) using GC-MS procedure.



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This product fulfills all the requirements of Directive 98/79/EC on in vitro diagnostic medical devices (IVD). The declaration of conformity is available upon request.

N° 003	Differentiated Fatty Acids in plasma by detector GC/MS	11/2025
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TECHNICAL FEATURES

Principle of the Method :

This method allows to determine the fatty acids by GC / MS. The sample after extraction and washing, is treated with a derivatizing for 15 minutes at 100 ° C; The solution, after various dilutions, is directly injected into the GC.

Recovery:

>98%

Sensitivity (LLOQ):

Fatty Acid	mg/l
Myristic ac.	0,5
Palmitic ac.	0,25
Palmitoleic ac. (ω7)	0,25
Stearic ac.	2,0
Elaidic ac. (trans)	0,25
Oleic ac.	0,5
Linolelaidic ac. (trans,trans)	0,25
Linoleic ac. (ω6)	0,1
γ-Linolenic ac. (ω6)	0,25
α-Linolenic ac. (ω3)	0,25
Dihomo-γ-linolenic ac. (ω6)	1,0
Arachidonic ac. (ω6)	1,0
All cis 5,8,11,14,17 Eicosapentaenoic ac. (EPA ω3)	0,5
All cis 4,7,10,13,16,19 Docosahexenoic ac. (DHA ω3)	0,5

Reference Values:

Fatty Acids	mg/l
Myristic ac.	3,43-9,57
Palmitic ac.	298,00-460,00
Palmitoleic ac. (ω7)	4,00-14,73
Stearic ac.	144,00-253,00
Elaidic ac. (trans)	0,90-2,40
Oleic ac.	98,00-195,00
Linolelaidic ac. (trans,trans)	0,00-2,00
Linoleic ac. (ω6)	133,00-271,00
γ-Linolenic ac. (ω6)	0,67-2,40
α-Linolenic ac. (ω3)	2,03-5,68
Dihomo-γ-linolenic ac. (ω6)	28,90-69,50
Arachidonic ac. (ω6)	98,00-166,50
All cis 5,8,11,14,17 Eicosapentaenoic ac. (EPA ω3)	10,19-40,20
All cis 4,7,10,13,16,19 Docosahexenoic ac. (DHA ω3)	61,00-125,00

<u>Components of the kit :</u>	All the reagents are ready to use and stable 3 years at 2-8 °C
Reagent A – Extraction Solution 1, 1 x 320 ml	
Reagent C – Internal Standard, 1 x 1 ml	
Reagent D – Wash Solution, 1 x 50 ml	
Reagent E – Derivatization Solution, 1 x 25 ml	
Reagent F – Extraction Solution 2, 1 x 25 ml	
Reagent G – Blocking Solution, 1 x 25 ml	
Reagent H – Stabilizing Solution, 1 x 5 ml	
Calibrator liophyl. in plasma, 1 x 1 ml	<u>Code GC75016 (pace separately – see data sheets)</u>

Minimum Instrumental equipment required:

GC/MS
Operational Computer
Drying System / evaporating solvents

Collection Procedure :

Test tube with EDTA anticoagulant or heparin. If you analyze not immediately, freeze the sample a- 20 ° C and protected from light. Stable for 6 months.

ANALYTICAL PROCEDURE

STEP 1 : Sample Preparation

Dispense in pyrex of 10 ml:

	Calibrator	Control	Sample
Calibrator	200 µl		
Control		200 µl	
Sample			200 µl
Reagent A – Extraction Solution	3200 µl	3200 µl	3200 µl
HCl 37%(concentrated hydrochloric acid)	20 µl	20 µl	20 µl
Reagent C – Internal Standard	10 µl	10 µl	10 µl

Cap the tube and place on Vortex for 30 sec.

STEP 2 : Centrifuge at 4.000 rpm for 7 minutes for the complete separation of the two phases

STEP 3 : Take 2 ml of inferior phase and transfer it in a clean pyrex of 10 ml.

STEP 4 : Add 0,5 ml of **Reagent D – Wash Solution**

Cap the tube and place on Vortex for 30 sec.

STEP 5 : Centrifuge at 4.000 rpm for 5 minutes and remove and waste with a pasteur pipette the upper phase (including any interface)

Dry with evaporator or nitrogen flow

STEP 6 : Into the tube containing the dried fat add in sequence 250 ul of **Reagent E – Derivatization Solution** and 250 ul of **Reagent F – Extraction Solution 2**

Vortex for 20 sec.

STEP 7 : Put the capped tubes in oven at 100 °C for 15 minutes.

STEP 8 : Cool quickly and add 250 ul of **Reagent G – Blocking Solution**

Cap the tube and place on Vortex for 20 sec.

STEP 9 : Centrifuge at 4.000 rpm for 3 minutes and wait 1 minute so that the two phases to separate completely

STEP 10 : Collect 150 ul of the upper phase in which they are dissolved FAME (careful not to draw the bottom!) and transfer to a glass insert in an amber vial

Dry with evaporator or nitrogen flow

STEP 11 : Put in insert 50 ul of **Reagent H - Stabilizing Solution**

Vortex for 10 sec.

STEP 12 : Centrifuge at 4.000 rpm for 3 minutes

STEP 13 : Inject 1 µl in GC

N.B.: at this step, the sample is stable 24 hours at 2-8 C°

DIFFERENTIATED FATTY ACIDS IN PLASMA BY DETECTOR GC/MS - Warnings

SET OF GAS-CROMATOGRAPH:

- Durabond HP-88 100 m x 0,25 mm 0,2 um
- Injector Temperature 250 °C
- Splitting Ratio: ON split 10
- Temperature 100 °C x 0 minutes + 10 °C/min till up 220 °C x 4 minutes + 10 °C/min till up 240 °C x 12 minutes (run 30 minutes)
- Helium Gas 1 ml/min

SET OF MASSA DETECTOR (4000) :

- Massa Range 40 – 400
- Massa Temperature: 190 °C;
- Transfer Line Temperature: 270 °C;
- Manifold Temperature: 80 °C
- Filament on: 10 minutes

CONDITIONING OF Durabond HP-88 100 m x 0,25 mm 0,2 um COLUMN

Follow the manufacturer prescription.
Do not condition column/s if connected to the Massa Detector.

CLEANING OF THE COLUMN AND THE SYRINGE

Wash the syringe before the injection with hexane and after injection with iso-octane. Disconnect the detector. Keep the maximum temperature for the recommended time. (See the manufacturer's instructions) **MAKE SURE THE FUNCTIONALITY OF THE SYRINGE BEFORE EACH SESSION.**

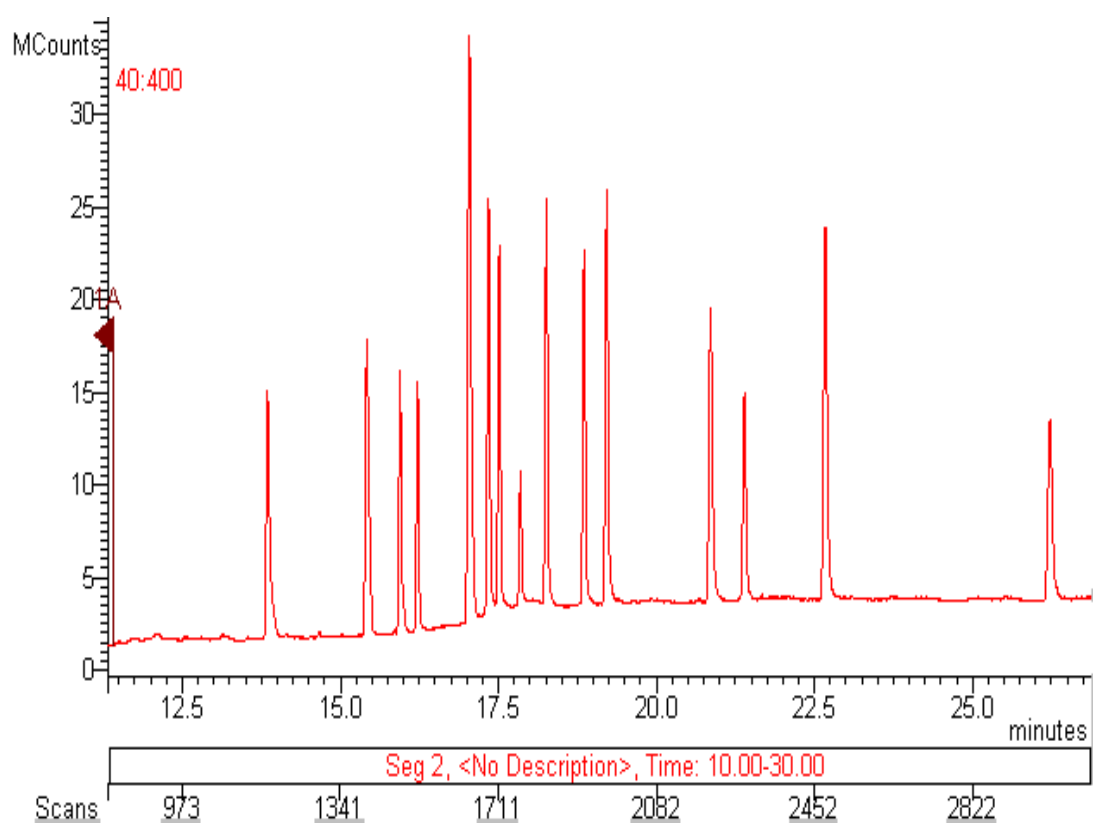
Fatty Acid	Fragments
Myristic ac.	74.1+87.0+143.0+243.0
Palmitic ac.	270.0+227.0
Palmitoleic ac. (ω7)	55.0+81.0+96.0+237.0
IS (Heptadecanoic ac.)	74.1+87.0+284.0+241.0
Stearic ac.	255.0+298.0
Elaidic ac. (trans)	81.1+96.0+264.0
Oleic ac.	81.1+96.0+264.0
Linolelaidic ac. (trans,trans)	81.1+95.0+67.0
Linoleic ac. (ω6)	81.1+67.0+95.0
γ-Linolenic ac. (ω6)	79.1+67.0+93.0+95.0
α-Linolenic ac. (ω3)	79.1+67.0+93.0+95.0
Dihomo-γ-linolenic ac. (ω6)	79.1+67.0+93.0
Arachidonic ac. (ω6)	79.1+67.0+91.0
All cis 5,8,11,14,17 Eicosapentaenoic ac. (EPA ω3)	79.1+91.0+105.0+67.0
All cis 4,7,10,13,16,19 Docosahexenoic ac. (DHA ω3)	91.1+79.0+67.0+117.0

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
GC75016	Calibrator for Differentiated Fatty Acids in plasma	4 x 1 ml
GC75019	Control for Differentiated Fatty Acids in plasma Levels 1 and 2	2 x 5 x 1 ml
S11288A7	Durabond HP-88 100 m, 0,25 mm - 0,2 um Column	1 Pc
S29004U	Amber Glass Vial of 2 ml with screw cap	1 x 100 Pcs
S51820717	Caps for glass Vial with reduced volume	1 x 100 Pcs
S24717	Insert 100 ul for vials of 2 ml	1 x 100 Pcs

FATTY ACIDS IN PLASMA

(Reference Chromatograms)



Fatty Acid	R. T. (min)
Myristic ac.	13,8
Palmitic ac.	15,4
Palmitoleic ac. (ω 7)	15,9
IS	16,2
Stearic ac.	17,1
Elaidic ac. (trans)	17,4
Oleic ac.	17,6
Linolelaidic ac. (trans,trans)	17,8
Linoleic ac. (ω 6)	18,3
γ -Linolenic ac. (ω 6)	18,9
α -Linolenic ac. (ω 3)	19,2
Dihomo- γ -linolenic ac. (ω 6)	20,9
Arachidonic ac. (ω 6)	21,5
All cis 5,8,11,14,17 Eicosapentaenoic ac. (EPA ω 3)	22,7
All cis 4,7,10,13,16,19 Docosahexenoic ac. (DHA ω 3)	26,3