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QUANTIFICATION OF LONG-CHAIN MONO- AND POLYUNSATURATED FATTY ACIDS

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ABSTRACT

In this work are presented various chromatographic systems, TLC, HPLC, GC, designed to identify and accurately quantify the long-chain mono- and polyunsaturated fatty acids of different samples with a focus on literature that was published in the last decade.

KEYWORDS: Omega fatty acids, TLC, HPLC, GC.

INTRODUCTION

Long chain fatty acids (LC-FA) are organic compounds in which the length of the hydrocarbon chain may vary from 10 to 30 carbon atoms. The hydrocarbon chain may be saturated or unsaturated (containing one or more double bonds). Based on the number of double bonds, unsaturated fatty acids are classified into the following groups[1, 2]

- monounsaturated fatty acids (MUFA), containing one double bond, for example, oleic acid,
- polyunsaturated fatty acids (PUFA), which has two or more double bonds, for example, γ-linolenic acid,
- eicosanoids, which are derived from polyunsaturated fatty acids, prostaglandins,

Omega-3 fatty acids and omega-6 are known as essential (essential) fatty acids (EFAs) because they are important for good health. The human body can not produce these fatty acids, therefore omega-3 fatty acids must be obtained through food. Their content is found in foods such as cold-water fish, including tuna, salmon and mackerel. Other important omega-3 fatty acids are found in dark green leafy vegetables, flaxseed oil and certain vegetable oils. Seaweed and phytoplankton are also sources of omega-3 fatty acid. As common sources of

vegetable oils containing omega-3 ALA fatty acids include nuts, edible, sage - seed oil, whose - seed oil, linseed oil, algal oil, oil from hemp, and sources animal omega-3 EPA and DHA fatty acids include - fish oil, squid oil and krill oil.[3, 4] Therefore, it can be noted that the PUFA are nutrient sources are used and in pharmacy.[4]

Thin layer chromatography (TLC) is the classical method of separation, identification and quantification of fatty acids.[5] The study of literature of the last decade dedicated to lipid analysis shows that among the various analytical methods, thin layer chromatography (TLC) and its modern version of high performance thin layer chromatography (HPTLC) are still a very important tool in lipid analysis as well as analysis of fatty acids.

As reported by Fuchs et al[5], there are many advantages that make TLC very competitive with HPLC (high performance liquid chromatography) in the field of fatty acids, such as ease of use, less expensive costs, less consumption of solvent as compared with HPLC, the presence of an analysis of several samples in parallel, and the possibility of easy visualization of unsaturated fatty acids after TLC fractionation using suitable colorants.[5] In the report on TLC analysis in food and agricultural samples Sherma confirmed that quantification by HPTLC equipped with a densitometer may lead to results comparable to those obtained by using gas chromatography (GC) or high performance liquid chromatography (HPLC).[6]

MATERIAL AND METHODS

Reagents: Chloroform, Cyclohexane, Ether, Supplement containing mixture from cod liver oil (Atlantis Gradus morrhuna) 300 mg and DHA 400 mg;

TLC conditions for densitometry determination

- TLC glass sheets with Kieselgel G60, F254, 20/20, 0.25 mm;
- UV – lamp at 254 nm;
- mobile phase : cyclohexane - ether in volume ratio 80 : 20 (v/v);
- run - 12 cm;
- volumes - 5 to 20l.

Test solutions – (a) 1.0 ml from tested supplement mixture containing mixture from cod liver oil 300 mg and DHA 400 mg was diluted with solvent chloroform up to 10.0 ml;
(b) 0.1 ml from test solution (a) was diluted with the same solvent to appropriate concentrations.

Reference solutions – solutions from reference compound DHA were prepared by diluting in solvent chloroform up to adequate to (b) test solution concentrations.

Procedure - after the development of TLC plates and removing of the mobile phase on the air they were scanned with System - TLC-Scanner E-BOX Vilber Lourmat using PC Software - E-capt, version 12.7 for Windows and detection at 254 nm. 3D volumes, Areas and Heights were found and used in the validation procedure and distance calculations.

RESULTS AND DISCUSSION

For the development of the validation procedure of TLC-densitometry method the following analytical parameters were studied.

1. Selectivity - There no spots corresponding by RF values in the "placebo" chromatograms obtained from solutions containing all compounds without DHA.

2. Precision - six (6) equal homogenous samples from reference mixtures containing DHA were analyzed by TLC-densitometry method. Standard deviation (SD) and relative standard deviation (RSD) were found based on the obtained 3D volume in mobile phase : cyclohexane - ether in volume ratio 80 : 20 (v/v). The results are shown on table 1.

Table 1. Study of linearity.

Samples (n)	Obtained 3D volumes of DHA	X_{mean}	SD	RSD%
1.	3624718	3538334,66	50181,74	1,41
2.	3559293			
3.	3538301			
4.	3530515			
5.	3494749			
6.	3486302			

In order to be study the analytical parameter linearity densitometric method is applied at chromatographic conditions: stationary phase: Silicagel $G_{60}F_{254}$, mobile phase: cyclohexan-ether 80 : 20 v/v, UV-detection at λ = 254 nm, start-front distance: 120 mm.

Measured are the areas of the spots of solutions with increasing concentrations: 5.10-4 g / ml ÷ 5.10-3 g / ml for Docosahexaenoic acid summarized in Table, and data are presented for: C [g / ml] - concentration, V [µl] - applied volume, [µg / patch] - applied quantity, d [cm] - the diameter of the spot, r [cm] - radius of the spot, S [cm2] - area of the stain. Table 2

Table 2. Densitometric parameters for standard solutions with DHA

Docosahexaenoic acid						
C [g/ml]	**V [µl]**	**[µg/петно]**	**Rf**	**d [cm]**	**r [cm]**	**S [cm^2]**
5.10^{-4}	10	5	0.65	0.2	0.1	0.0304
1.10^{-3}	10	10	0.64	0.3	0.15	0.0763
$1.5.10^{-3}$	10	15	0.64	0.4	0.2	0.1246
2.10^{-3}	10	20	0.63	0.5	0.25	0.1953
$2.5.10^{-3}$	10	25	0.64	0.6	0.3	0.2816
3.10^{-3}	10	30	0.64	0.7	0.35	0.3837
$3.5.10^{-3}$	10	35	0.65	0.8	0.4	0.5014
4.10^{-3}	10	40	0.64	0.9	0.45	0.6349
$4.5.10^{-3}$	10	45	0.63	0.96	0.48	0.7225
5.10^{-3}	10	50	0.64	1.1	0.55	0.9489

In order to be studied the analytical parameter linearity of the solutions with Docosahexaenoic acid values of the areas of the spots for each concentration were measured. (Table 3)

Table 3. Study of linearity for DHA

	Docosahexaenoic acid	
N:	**C [g/ml]**	**A**
1.	5.10^{-4}	13700
2.	1.10^{-3}	50400
3.	$1.5.10^{-3}$	76900
4.	2.10^{-3}	101900
5.	$2.5.10^{-3}$	126900
6.	3.10^{-3}	152300
7.	$3.5.10^{-3}$	176500
8.	4.10^{-3}	200200
9.	$4.5.10^{-3}$	224100
10.	5.10^{-3}	270600

Linearity - the parameter linearity was studied in the concentration interval 50 – 150 % from theoretical quantity. The accordance between the 3D volumes, measured in relative units and concentration in mg/ml is proportional in the detected intervals. The correlation coefficient was found to be about 1 for DHA in model mixtures.

Table 4. The correlation coefficient

S.No	**Parameter**	**Docosahexaenoic acid**
1.	Linear interval [g/ml]	5.10-4 ÷ 5.10-3
2.	Regressive equation	y = 53256970.x – 7007
3.	Slope (a)	53256970
4.	SE_{slope}	1389175
5.	Segment (b)	– 7007
6.	$SE_{segment}$	4310
7.	Correlation coefficient (R2)	0.994

System suitability test - For system suitability test determination of some chromatographic parameters such as Retardation factors (RF), LOD and LOQ were appointed for optimization of conditions in according with ICH and European Pharmacopoeia criteria.

For Distance calculations were analyzed 11 test solution containing DHA with concentration in interval 75 – 125 % of theoretical calculated quantity. RSDs (in %) of the marked areas and heights on the TLC plates are 9.89 % and 4.77 %. The obtained results for Retardation factors (RF) of DHA show excellent reproducibility (RSD = 0.66 %). The results are shown on table 3.

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