Online Hydrolysis and Derativization of Fatty Acids

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Introduction

Fats are extracted from matrices with non-polar solvents such as ether or naphtha and subsequently saponified to produce free fatty acids. Afterwards, fatty acids are derivatised to their corresponding methyl esters to increase analyte volatility, improve peak symmetry and decrease activity, hence providing more accurate analytical results. AOAC method 969.331 and AOCS method Ce 2-662 describe derivatization procedures for the esterification of fatty acids. Glycerides are saponified by refluxing with a methanolic sodium hydroxide solution. The free fatty acids are esterified in the presence of boron trifluoride (BF3) in methanol and extracted with heptane for analysis by GC. The autosampler executes the entire derivatisation procedure with great precision, freeing costly analyst time. The setup is available in either an offline configuration or an online configuration.

Equipment

Sample preparation carried out by means of a Thermo TriPlus RSH robotic sampler, placed on top of a Trace1300 GC instrument with i-connect COC and FID modules.

Software control is embedded in the Chromeleon instrument method. Method overlapping is enabled which means that the next sample is prepared during the analysis of the previous one. Instrumental procedure:

- Vial is transported to the agitator (65°)
- Heat and shake for 15 seconds to melt the sample
- Add 400 µL methanolic NaOH solution
- Heat and shake for 300 seconds to hydrolyze
- Add 200 µL methanolic BF3 solution
- Heat and shake for 240 seconds
- Add 900 µL of i-octane
- Shake the vial for 2 minutes
- Add 600 µL saturated NaCl solution
- Shake the vial for 1 minute
- Transport vial to the sample tray

When using a split/splitless inlet the sample is ready for split injection. During this test a Cold on Column inlet was used, therefore the sample needs to be diluted. The procedure for this is described below:

- Transport 25 µL of the upper layer to an empty 2 mL vial
- Add 1420 µL i-octane
- Transport vial to the agitator
- Shake the vial for 1 minute
- Transport vial to the sample tray
- Inject 1 µL in the Cold on Column inlet

Please note that prior to the automated steps described above, samples need to be prepared accordingly in order to allow proper preparation. These steps involve:

- Add 2,5-4,0 mg of sample to an empty 2,5 mL vial
- Place the vial in the robotic sampler tray

Chromatography

A typical chromatogram of a Restek Marine Oil FAME Mix (20 components) is depicted in Figure 1.

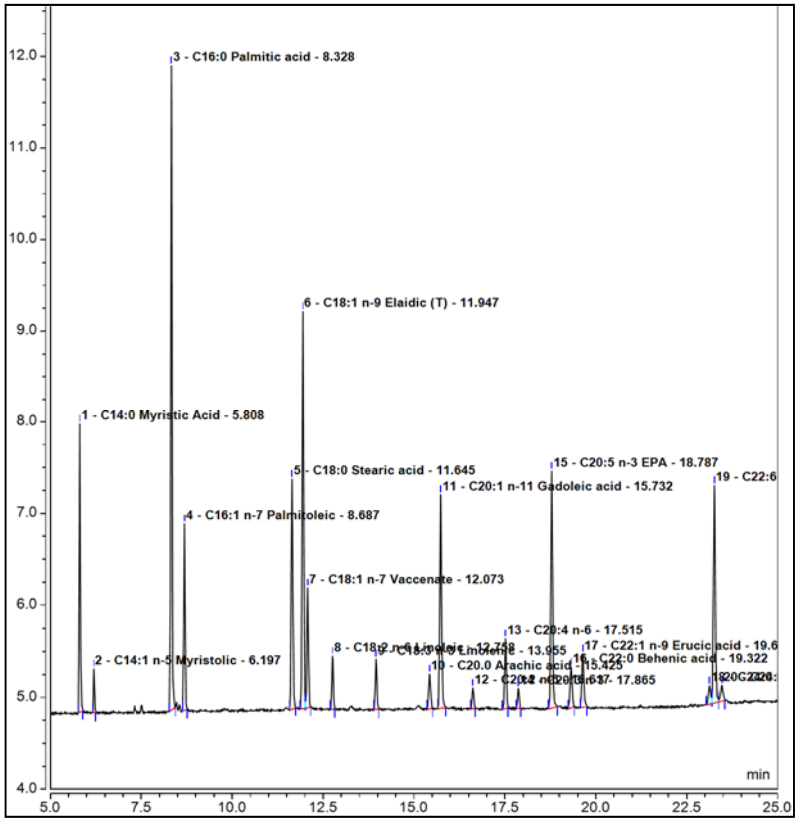
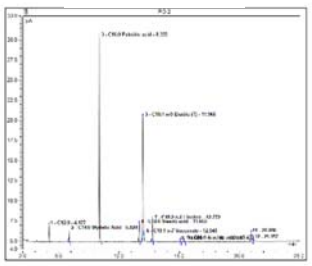


Figure 1. Chromatogram of Restek Marine Oil Mix.

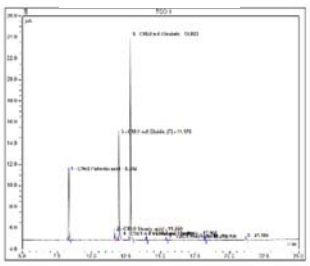
The results of three different samples are given in the following chromatograms.

Sample 1



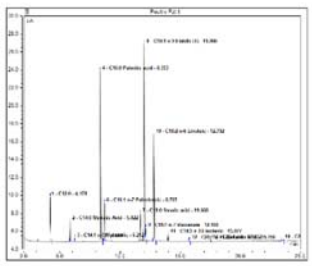
Name	RT (min)	%Area
C12:0	4,177	2,03
C14:0	5,820	1,94
C16:0	8,355	43,92
C18:0	11,653	5,15
C18:1 n-9 (T)	11,968	32,70
C18:1 n-7	12,048	2,57
C18:2 n-6	12,772	5,46
Unknown	15,090	0,33
C20.0	15,433	0,42
Unknown	18,180	0,39
Unknown	20,958	4,21
	21,157	0,86
Total		100,00

Sample 2



Name	RT (min)	%Area
C16:0	8,342	13,57
C18:0	11,660	2,59
C18:1 n-9 (T)	11,970	26,86
C18:1 n-7	12,092	0,92
C18:2 n-6	12,803	53,83
C18:3 n-3	13,968	1,07
C20.0	15,458	0,36
Unknown	18,212	0,36
unknown	21,185	0,44
Total		100,00

Sample 3



Name	RT (min)	%Area
C12:0	4,178	3,50
C14:0	5,822	2,26
C14:1 n-5	6,212	0,36
C16:0	8,353	22,91
Unknown	8,600	0,46
C16:1 n-7	8,705	5,23
C18:0	11,668	5,54
C18:1 n-9 (T)	11,988	35,88
C18:1 n-7	12,100	2,20
C18:2 n-6	12,792	19,45
C18:3 n-3	13,977	1,32
C20:1 n-11	15,750	0,20
C20:4 n-6	17,532	0,34
C24:1	23,492	0,34
Total		100,00

In Summary

Unattended hydrolysis/derivatization and extraction of fatty acids (as FAMEs) provides serious reductions in cost of analysis. The procedure has been validated and performs equally well as the manual procedure. With respect to precision, the automated procedure is superior.

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