Application Note No. 016

Fully Automated Preparation and Analysis of Fatty Acid Methyl Esters Using the FOCUS Sample Processing Robot

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Introduction

The preparation of the methyl esters of fatty acids for analysis by gas chromatography is the commonest chemical reaction performed by lipid analysts. Although it is a relatively simple operation, it is still a time consuming process. Automated preparation, extraction and injection of the methyl esters of fatty acids for gas chromatographic analysis is now possible using the FOCUS Sample Processing Robot in conjunction with GC-FID.

Classical Method

The commonly used BF_3 method, besides being laborious, also uses large quantities of solvents and reagents. To the lipid sample a specified amount of BF_3-methanol reagent is added and the solution boiled. After the addition of hexane, the solution is boiled again. Saturated sodium chloride solution is then added and the mixture is shaken vigorously. The hexane layer is transferred into another test tube and anhydrous sodium sulfate is added. The dry hexane solution is then ready for injection into the gas chromatograph.

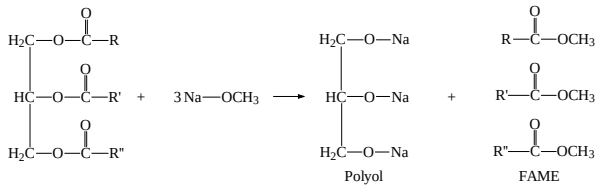
Automated Method

FOCUS is controlled by a macro running under Windows. This tells the robot what to do and when to do it. For the sodium methoxide method used by FOCUS, the FAME macro provides for derivatisation, liquid-liquid extraction and automated injection of the sample. In the procedure described, a known amount of the lipid sample is weighed into an autosampler vial and dissolved in hexane. The vial is then placed into the sample tray. Prior to each analysis, FOCUS adds sodium methoxide to each vial and agitates the mixture. After allowing some time for the polyols formed, to settle to the bottom of the vial, the top layer is injected into the GC.

Procedure:

1. 10 mg lipids in 1 ml n-hexane
2. Shake the vial
3. Add an excess of sodium methoxide ($NaOCH_3$)
4. Shake the vial
5. Allow some time for the phases to stabilize
6. Inject 1 µl from the upper layer

Reaction



Instrumentation

- ATAS FOCUS Sample Processing Robot
- ATAS OPTIC Programmable Injector
- Hewlett-Packard HP6890 GC with FID
- Hewlett-Packard ChemStation
- HP-23 Cis/Trans column (30m x 0.20mm x 0.25µm)

Samples Analysed

- Olive Oil
- Margerine
- Soybean Oil
- Sunflower Oil
- Maize Oil
- Massage Oil
- Palm Oil
- Walnut Oil
- Sesame Oil
- Linseed Oil
- Coconut Oil
- Rapeseed Oil
- BCR 162 Reference
- BCR 164 Reference

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Results

Table 1. RSD values in % (n=5) from the 100% method and the quantitative method for coconut oil.

	RSD% (Coconut oil)		Coconut oil
Fatty Acid	100% method	Quantitative method	100 % method (%)
8:0	1.8	2.1	8.1
10:0	1.2	2.2	6.4
12:0	0.6	2.3	49
14:0	0.4	2.6	18
16:0	1.7	3.4	8.7
18:0	2.6	2.4	8.8
18:1	3.4	3.4	0.6
18:2	-	-	0.4

Table 2. Comparison 100% method for the BF_3 and the FOCUS procedures using an EU standard sample.

	BCR 164	
Fatty Acid	Certified and Indicative Value	$NaOCH_3$ Method
4	(4.3)	2.50
6	2.17-2.55	1.72
8	1.26-1.46	1.16
10:0	2.77-3.01	2.73
10:1 w9	(0.3)	0.32
12	3.93-4.13	3.99
12:1 w9	(0.1)	0.10
14:0 iso	(0.1)	0.11
14:0	10.44-11.14	10.95
14:1 w9	(1.1)	1.05
15:0 iso	(0.3)	0.27
15:0 ante-iso	(0.5)	0.54
15:0	(1.0)	1.10
16:0 iso	(0.2)	0.25
16:0 total	26.07-27.75	27.3
16:1 w7	(1.5)	0.33
16:1 total	(1.5)	1.35
17:0 iso	(0.5)	0.41
17:0 ante-iso	(0.4)	0.46
17:0	(0.5)	0.49
17:1	(0.3)	0.53
18:0 iso	(0.1)	0.00
18:0 total	9.70-10.91	10.67
18:1 total	24.21-25.43	24.39
18:2 w6 cis	(1.8)	2.38
18:2 conj.	(0.9)	0.43
18:2 total	2.28-3.08	2.81
18:3	0.47-0.55	0.60
20:0	(0.1)	0.17
20:1	(0.2)	0.12

Chromatograms

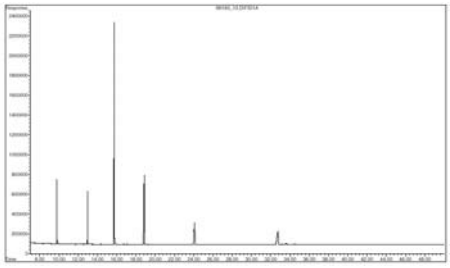

Figure 1: *Coconut Oil*

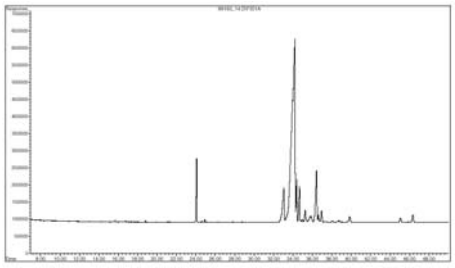

Figure 2: *Rapeseed Oil*

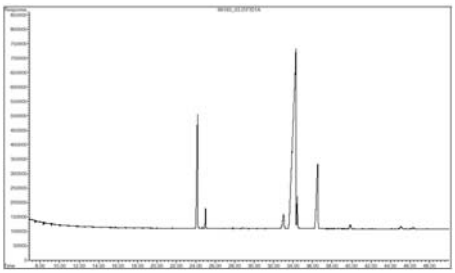

Figure 3: *Olive Oil*

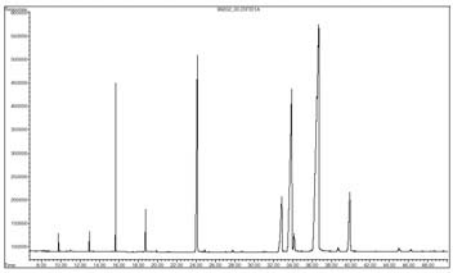

Figure 4: *Margarine*

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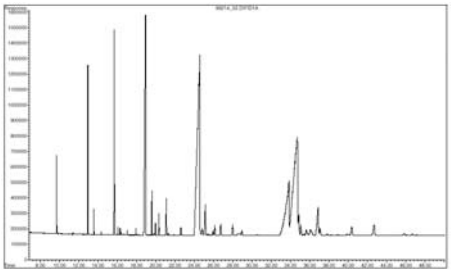

Figure 5: *BCR 164 Reference*

ATAS FOCUS Sample Processing Robot on HP6890

Conclusion

The results show good agreement between the new automated method and the classical approach. As such there would seem to be no disadvantage to adopting this new automated procedure. The high degree of automation and limited degree of manual work leads to high sample throughput due to the ability of the system to run 24 hours per day.
Small amounts of reagents are used and so running costs and disposal costs are also lower.

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