Automated Preparation and Analysis of Fatty Acid Methyl Esters

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INTRODUCTION

The preparation of fatty acid methyl esters (FAMEs) for the lipid analysis by capillary gas chromatography (GC) is the commonest chemical reaction performed by lipid analysts. The reaction is very laborious and time consuming to perform, although it is a relative simple operation.

In this presentation, a fully automated procedure based on the ATAS Focus XYZ Sample Preparation Robot is described and compared with the classical manual method.
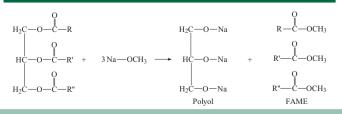
CLASSICAL PROCEDURE

- Introduce lipids into a reaction flask
- Add 5 - 12 mL borontrifluoride(BF_3)-methanol reagent
- Attach condenser and boil for 2 minutes
- Add hexane through the condenser and boil for 1 minute longer
- Add about 15 mL of saturated sodium chloride (NaCl) solution
- Shake the tepid solution vigorously for 15 seconds
- Add sufficient saturated NaCl solution to float the hexane layer in the neck of the reaction flask
- Transfer about 1 mL of the hexane into another test tube and add anhydrous sodium sulfate $NaSO_4$
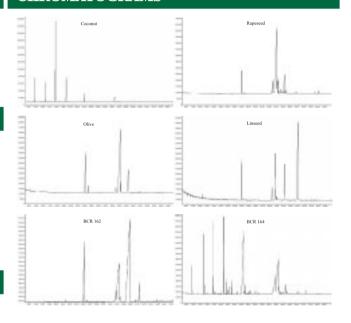- The dry solution is ready for injection into the GC

AUTOMATED PROCEDURE

- Introduce lipids into a 2 mL autosampler vial and add hexane
- Shake to disolve the lipids
- Add an excess of satureted sodium methylate ($NaOCH_3$)-methanol reagent
- Allow some time for the polyols to settle
- Inject the clear top layer into the GC

CHEMICAL REACTION

Polyol   FAME

CHROMATOGRAMS

Coconut — Rapeseed — Olive — Linseed — BCR 162 — BCR 164

FOCUS SYSTEM

- Optic 2 programmable injector
- Focus sample preparation robot
- HP6890 gas chromatograph
- Column: HP-23 Cis/Trans 30 m x 0.20 mm x 0.25 µm

THEORETICAL DATA

	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1
Coconut		<1.2	3.4-15	3.2-15	41-56	13-23	4.2-12		1.0-4.7	4.4-12	0.9-3.7 4			
Rapeseed					<1.0	1.5-6.4	<3.0	0.5-3.1	8.0-45	11.0-29	5.0-16	<3.0	3.0-15	
Olive					<0.05	7.5-20	0.3-3.5	0.5-3.5	56-83	3.5-20	<1.5			
Linseed							±6		±5	±20	±16	±53		
BCR 162							10.5-10.8		2.8-2.9	23.9-24.4	56.1-57.2	4.5-4.9		
BCR 164	±4.3	2.2-2.6	1.3-1.5	2.8-3.0	3.9-4.1	10.4-11.1	26.1-27.8	±1.5	9.7-10.9	24.2-25.4	2.3-3.1	0.47-0.55	±0.1	±0.2

PRACTICAL DATA

	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1
Coconut		0.0	8.1	6.4	49	18	8.7		8.8	0.6	0.4			
Rapeseed					0.1		5.8	0.2	8.0	74	8.8	0.9	0.7	1.3
Olive							14	1.5	2.5	65	12	0.6		
Linseed							6.2		5.2	19	15	54		
BCR 162							11.5		2.7	23.9	56.3	4.5		
BCR 164	2.5	1.7	1.2	2.8	4.0	11.0	27.3	1.4	10.7	24.4	2.8	0.60	0.17	0.12

RSDs for coconut	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1
100% method		1.8	1.2	0.6	0.4	1.7			2.6	3.4			
quantitative		2.1	2.2	2.3	2.6	3.4			2.4	3.4			

CONCLUSION

The use of the ATAS Focus XYZ sample preparation robot gives the possibillity to replace the laborious and time consuming classical method for the preparation of the FAMEs for a fully automated one. The possibility of doing over 200 analyses, the use of a simple procedure and the automation makes the system both robust and user friendly.

ACKNOWLEDGEMENTS

A Total Analytical Solution