Application Note

Food & Agriculture

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Analysis of Oil and Fat containing foods by fully automated sample preparation using a PAL3 coupled with a 7890 GC and a 5977 MSD system according to AOAC 996.01

Abstract

The analysis of oils, fat and fat containing food via fatty acid methyl esters (FAME) is a common task in governmental, quality control (QC) or contract research laboratories (CRO). In most cases the samples are processed manually, which is labour intensive and exposes the lab personnel to potentially hazardous chemicals. This application note describes the analysis of Oil and Fat containing food applying automated sample preparation for GCMS, according to AOAC 996.01

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Introduction

The food industry routinely performs fatty acid (FA) analysis since lipids are a major component in oils, meats, seeds, and other products. Furthermore, with the increased importance on fat as part of dietary health and its role in maintaining a healthy disposition, the determination of FA composition has become increasingly common [1]. The general public has an interest in the relationship between diet and health and in the choice of an appropriate diet to suit individual needs.

Nutrition labelling is one important method of informing consumers about the composition of foods and of helping them to make an informed choice. Empowering consumers to make informed nutritional choices is essential to both consumer welfare and effective competition. Knowledge of the basic principles of nutrition and appropriate nutrition information on foods would contribute significantly towards enabling the consumer to make such an informed choice.[2]

Biomedical applications use FA profiles as a diagnostic tool since FA composition effects biological membranes [3-4,5-6].

Fatty acids are also found in many household products and are used industrially in cosmetics and surfactants, among other things [7,8].

Biodiesel is made by transesterification of vegetable oil or animal fats to produce a mixture of fatty acid methyl esters (FAMEs). Pure biodiesel is called B100 and must meet industry standard specifications before it can be used as a fuel or blending stock. The distribution of FAMEs in a B100 mixture depends on the feedstock source. The relative amounts of FAMEs in biodiesel can vary widely and have different effects on both the fuel and handling properties. [7]

This application note describes a complete and fully automated sample preparation and analysis of FA using a PAL3 with robotic tool change (RTC) a 7890 GC and a 5977 MSD (add picture) according to AOAC 996.01 which uses boron trifluoride in methanol as catalyst. The workflow improves process safety, optimizes throughput and minimizes handling errors. The method allows the determination of the total fat content and quantitative analysis of saturated and unsaturated cis- and trans-fatty acids. The method was applied to a number of different vegetable oils and water containing animal fats such as butter, cheese and salami.

Experimental

Materials

1-Tetradecene (C14 Alkene)	Aldrich; ≥ 97%	PN: 87189-25ML CAS: 1120-36-1
Methyl nonanoate (FAME-9)	Aldrich; 98%	PN 24895-10G CAS 1731-84-6
Glyceryl triundecano-ate (FAME-11)	Aldrich; ≥ 98%	PN: T5534-1G CAS: 13552-80-2
FAME Mix C14-C22	Supelco; neat	PN: CRN18917 Lot: LC04905V
Palm oil analytical standard	Aldrich	PN: 70905-100G CAS: 8002-75-3
Sodium hydroxide	Aldrich; reagent grade; ≥ 98%;	PN: S5581-500G Lot: SZBF3240V CAS: 1310-73-2
Boron trifluoride	Acros; 12% in MeOH	PN: 402765000 Lot: A037-6838 CAS: 373-579
Sodium chloride	Aldrich; puriss p.a. ≥ 99.8%	PN: 31434-1KG-R Lot: SZBE2110V CAS: 7647-14-5
Methanol	JT Baker; HPLC Grade	PN: 8402 CAS: 67-56-1
Water	JT Baker; HPLC Grade	PN: 4218 CAS: 7332-18-5
Heptane	Biosolve; 99%	PN: 08070501 CAS: 142-82-5
Acetone	Aldrich; ReagentPlus. ≥ 99%	PN: 17,997-3 CAS: 67-64-1

Instrumentation

GC System	Agilent 7890B	Injector SSL
MS System	Agilent 5977A MSD	
Data System	MassHunter GC/MS	B.07.03.2129
Liner	Agilent, split, single taper, glass wool, deactivated, low pressure drop	P/N 5183-4647
Column	Standard FAME column	Cat 12423 SN 1369648
PAL:	PAL3 RTC85	
Tools	D7 and D8	
Syringes:		
10 µL; G26S; Teflon plunger		PAL3-SYH-207837
1000 µL; G23		PAL3-SYH-207849
1000 µL; G22		PAL3-SYH-207836
Additional Modules:		
MHE Module		
Agitator		
Vortex Mixer		
Fast Wash Station		
Tray Holder		
Large Wash Module		
Dilutor Module		

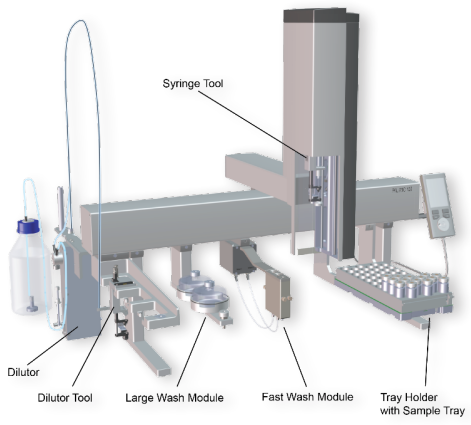
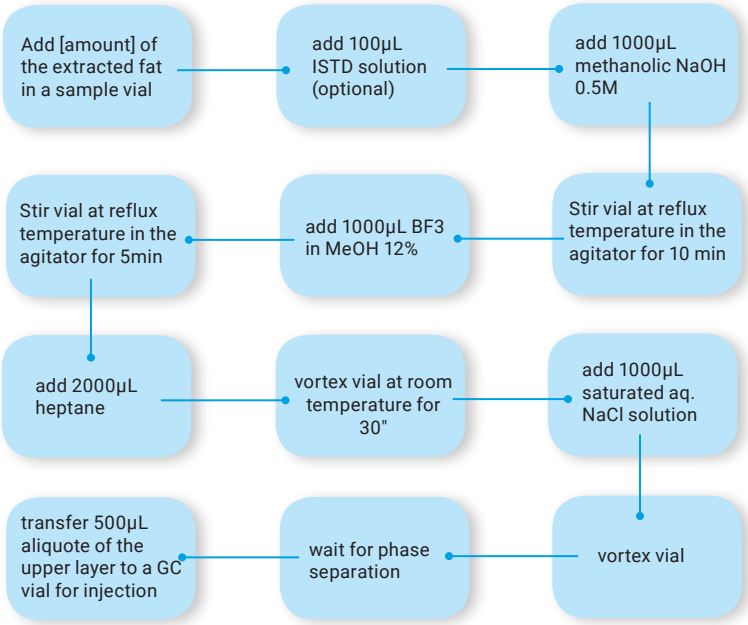


Figure 1. The RTC configuration used for the sample preparation

Automated Sample Preparation

Here is a detailed description of the sample preparation done by the PAL3 RTC



Results & Discussion

Automating the sample preparation procedure proves to be advantageous in many ways. By adapting this method to an automated one, the scale of the reaction was reduced. In doing so, the level of chemical exposure is reduced as well as the amount of solvent and reagent used. This increases the safety of the method and reduces the cost of the analysis. With this approach, it takes only 1 hour and 50 minutes to have the results for 6 samples while a single sample preparation alone requires 44 minutes. The usage of the RTC for this method resulted also in better recoveries and reproducibility compared to a manual method.

Reproducibility

In the following paragraph, we evaluate the reproducibility of the automated method for different matrices: palm oil, fish oil and milk chocolate.

Palm Oil

Palm oil contains relatively high amounts of saturated fatty acids such as palmitic acid. Crude palm oil is mainly composed of a mixture of mono-, di- and triglycerides of C14, C16 and C18 fatty acids. It is extensively used as an important raw material in the manufacture of soaps, washing powder and other hygiene and personal care products. In recent years, palm oil has gained importance in the production of biodiesel, however, its significance is still limited in comparison to rapeseed as a major source.

In the following chromatogram we are going to show the FA composition of raw palm oil as well as the reproducibility data for the samples run with the automated system.

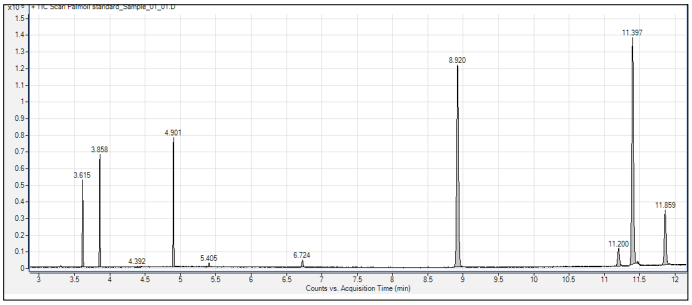


Figure 1. Example chromatogram of the analysis of Palm Oil run with the automated sample preparation

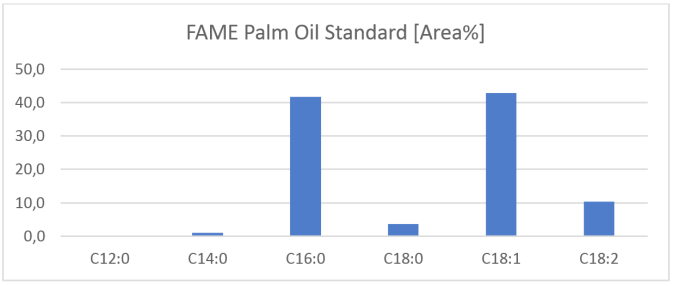


Figure 2. FA composition of palm oil

	AVG	STDEV	%RSD	%Area
C14 Alkene	34924	1395	4.0	26.8
FAME-9	44341	2038	4.6	34.0
FAME-11	50987	2242	4.4	39.1
C12:0	1809	193	10.7	0.3
C14:0	5657	524	9.3	1.0
C16:0	226390	23987	10.6	41.7
C18:0	19794	1975	10.0	3.6
C18:1	232569	17371	7.5	42.9
C18:2	56314	5312	9.4	10.4

Tab 1. Palm Oil Reproducibility results

Fish Oil

Fish oil is oil derived from the tissues of oily fish. Fish oils contains mainly the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), precursors of certain eicosanoids that are known to reduce inflammation in the body, and have other health benefits. Referring to the EU legislation 1196/2011, which aims for a better clarity in food product and because Fish Oil capsules are frequently used as Omega 3 supplement in daily diet we analysed their FA composition.

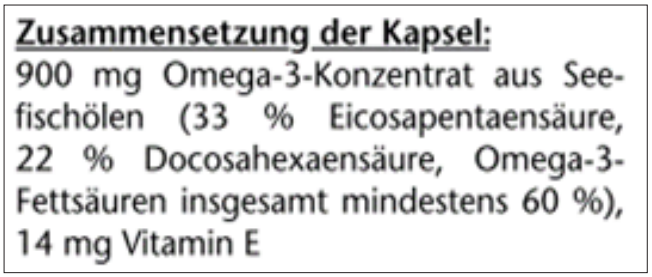


Figure 3. Omega 3 tablets label

The results of the testing are as follows:

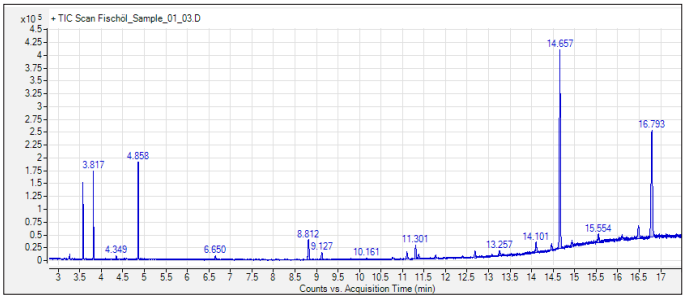


Figure 4. FA Total Ion Chromatogram

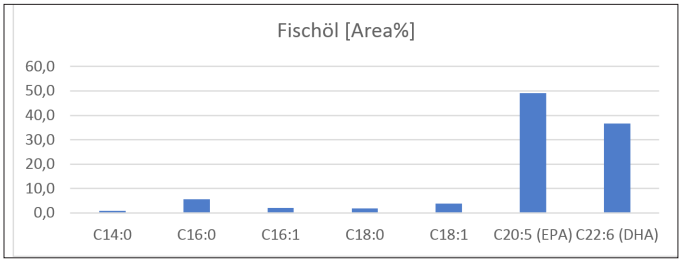


Figure 4. FA fish oil composition

	AVG	STDEV	%RSD	%Area
C14 Alkene	10472	537	5.1	29.1
FAME-9	11577	599	5.2	32.2
FAME-11	13927	745	5.3	38.7
C14:0	1145	62	5.5	0.8
C16:0	7837	434	5.5	5.7
C16:1	2790	151	5.4	2.0
C18:0	2614	115	4.4	1.9
C18:1	5206	371	7.1	3.8
C20:5 (EPA)	67966	3011	4.4	49.1
C22:6 (DHA)	50772	2464	4.9	36.7

Table 2. Fish oil FA analysis reproducibility

Milk Chocolate

Being one of the most popular and delicious treat consumed worldwide, quality control for this product is crucial, and is a duty of the producer to provide clear and understandable information about the products they sell. The main source by law of FA in milk chocolate must be Cocoa Butter.

Fatty acid	Percentage
Arachidic acid (C20:0)	1.0%
Linoleic acid (C18:2)	3.2%
Oleic acid (C18:1)	34.5%
Palmitic acid (C16:0)	26.0%
Palmitoleic acid (C16:1)	0.3%
Stearic acid (C18:0)	34.5%
Other Fatty Acids	0.5%

Table 3. Typical cocoa butter FA composition (ref. Wikipedia)

	AVG	STDEV	%RSD	%Area
C14 Alkene	57206	2959	5.2	27.4
FAME-9	69177	3687	5.3	33.2
FAME-11	82142	4152	5.1	39.4
unknown	1424	74	5.2	0.4
C10:0	1477	83	5.6	0.5
C12:0	1657	42	2.5	0.5
C14:0	4969	250	5.0	1.5
C16:0	91303	5131	5.6	28.4
C18:0	112465	6576	5.8	34.9
C18:1	101476	7499	7.4	31.5
C18:2	8580	439	5.1	2.7

Table 4. Composition and reproducibility of FA in milk. There's a clear match between the typical composition and the results obtained by the automated method.

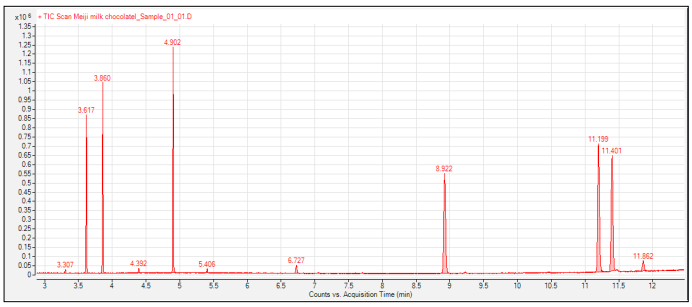


Figure 5. Total Ion Chromatogram of FA in Milk Chocolate

Conclusion

- The PAL3 RTC coupled with a 7890 GC and 5977MSD allows a fully automated sample preparation and analysis of FAME in different matrices.
- Six samples can be run in 1 hour and 50 minutes without human intervention.
- The amount of solvents and toxic chemicals is reduced to at least a factor of 10 compared to a manual sample preparation.
- Good reproducibility and accuracy were achieved as highlighted by the analysis of three different food matrices.
- Agilent provides a complete solution from the sample preparation to the analysis of the results

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