AUTOMATED WORKFLOW FOR THE DETERMINATION OF FATTY ACID METHYL ESTERS (FAME) IN FAT AND FAT CONTAINING FOOD SAMPLES USING A 90 SEC. TRANSESTERIFICATION

Beat Schilling[1], Reto Bolliger[2], Guenter Boehm[2]

Agilent Technologies

BGB Analytik AG[1], Lettenstrasse 97, 8134 Adliswil, Switzerland
CTC Analytics AG[2], Industriestrasse 20, 4222 Zwingen, Switzerland

email: gboehm@ctc.ch

PAL SYSTEM Ingenious sample handling

Introduction

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 labor intensive and exposes the lab personnel to potentially hazardous chemicals [1,2].

This work presents a fully automated workflow using a workstation with robotic tool change (RTC, Fig. 1) based on a method using sodium methoxide in methanol as reactant [3]. The workflow improves process safety, optimizes throughput and minimizes handling errors. The PALworkstation was equipped with a Dilutor to dispense the liquids for the reactions, the extraction and the cleaning steps, a Vortex module to provide fast mixing and extraction and a tool for a 10 µl syringe to inject the sample into the GC.
The software of the workstation allows overlapped sample processing, which increases sample throughput.
The method allows the determination of the total fat content, quantitative analysis of saturated and unsaturated cis- and trans-fatty acids. Three internal standards are used to control extraction, transesterification and undesired saponification. The method was applied to a number of different vegetable oils and water containing animal fats such as butter, cheese and salami.

Concept of the Method using three Internal Standards (IS)

Sodium methoxide transesterifies triglycerides within a very short time at ambient temperature. In the presence of water, methoxide also forms hydroxide, which may saponify the triglycerides directly or via the methylesters of the fatty acids. This reaction is about thousands times slower. Saponification is undesired but can be detected and quantified via the Internal Standard FAME-9.

Three IS are used:

1. Alkane C_{14}:1, non reactive, to check for complete reaction.
2. Triglyceride of C_{11} fatty acid, to check for complete transesterification.
3. FAME-9, to check whether saponification occurred.

Peak areas of the three ISs are checked for every analysis. If the C_{11}-FAME/alkane peak ratio is < 0.75, transesterification was not complete e.g. through lack of the reactant (Fig. 4), or the FAMEs were saponified already. If the FAME-9/alkane peak ratio is < 0.67 saponification occurred already. In the work of Grob et al. [3] the use of a fourth IS was proposed when injecting into a SSL injector to check for thermal peak discrimination. Nowadays, thermal discrimination due to solvent evaporation in the syringe needle can be avoided by performing fast injections.

Figure 1: PAL RTC 120 (G7370A) on Agilent GC

Experimental

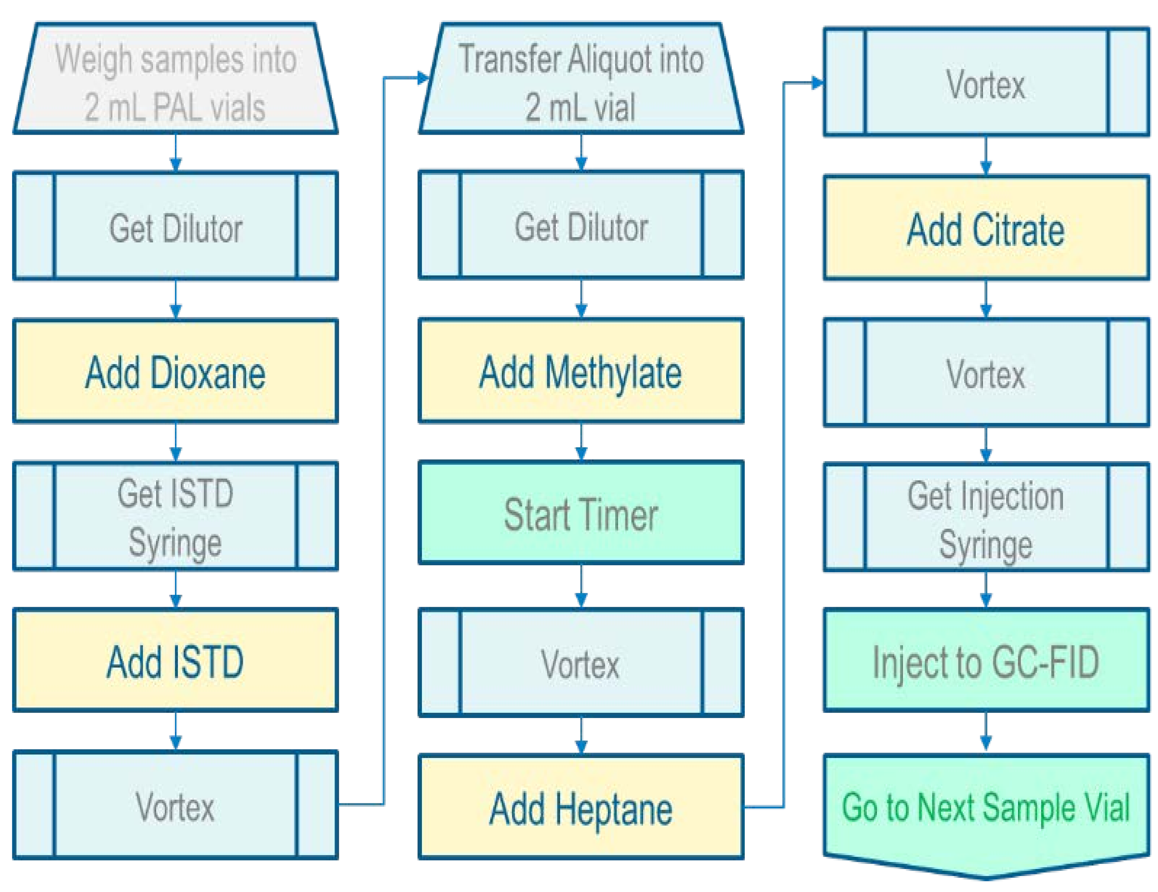
The following solutions were used:
Reactant: 5 % Na-methoxide in methanol
IS solutions: C_{14}:1 Alkane, FAME-9, Triglyceride C_{11}, @ 1 mg/mL in dioxane
Solution to stop the reaction: 15 % Na-citrate in Water

Instrumentation and Chromatography: PAL RTC 120 (G7370A) with additional Park Station, PAL 3 Dilutor Multistream (G7387-64100), PAL 3 Vortex Mixer (G7379-60001), PAL 3 Fast Wash (on request)

GC: Agilent 6890
Injector: SSL @ 250 °C, split flow 5 mL/min.
Column: 25 m x 0.25 mm ID, 0.25 µm BGB-WAX
Oven: 40 °C @ 25 °/min → 180 °C @ 15 °/min → 250 °C → 3 min. hold
Detection: FID @ 300 °C

A weighed amount of fat or fat containing food sample (e.g. 15.3 mg oil) was dissolved in the corresponding amount of dioxane containing the three Internal Standards (1.53 mL). 100µl of this solution was transferred to a 2 mL vial. The sequence of preparation steps is shown below. The phase separation occurs usually in less than 30 s. For some food samples, such as chocolate cream containing emulsifiers, more time is needed, in some cases even centrifugation. A prototype centrifuge for the PALsystem was used in theses cases. For some samples e.g. salami a pre-treatment with DMF is necessary to make the fat extractable from the cells. In this case about 100 mg of sample was heated up to 120 °C with 100 µL DMF for 10 min. before processing the samples. Dioxane has been chosen as a good solvent mediator between water and the fat containing sample and the reactant solution containing methanol.

Workflow



Conclusions

--Transesterification of fatty acids with Na-methoxide is a fast, efficient and very robust method for fat analysis in food samples.

-- With the use of three ISs the completeness of the trans-esterification as well as the extent of undesired sapo-nification can be checked.

-- Automation ensures process safety and high productivity (50 samples, unattended in 18.5 h).

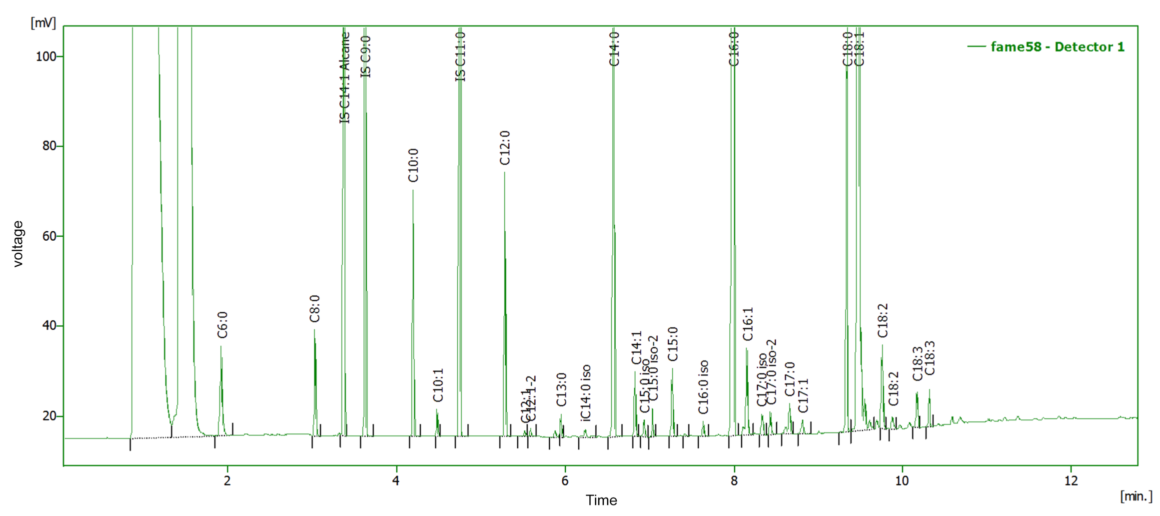


Figure 2: Typical result of butter FAMEs. Complete separation within 11 minutes.

Coconut Oil	%	Peanut Oil	%	Safflower Oil	%	Olive Oil	%	Sunflower Oil	%
C8:0	7.5	C16:0	8.9	C16:0	6	C16:0	12.3	C16:0	4.7
C10:0	5.8	C18:0	3.2	C16:1	0.1	C16:1	0.7	C16:1	0.1
C12:0	45.8	C18:1	68.8	C18:0	2.5	C17:0	0.1	C18:0	1.9
C14:0	18.5	C18:2	16.3	C18:1	17.1	C17:1	0.2	C18:1	13.3
C16:0	9.3	C18:3	0.1	C18:2	73.2	C18:0	2.4	C18:2	57.1
C18:0	2.9	C20:0	1.3	C18:3	0.3	C18:1	74.5	C18:3	0.2
C18:1	8.2	C20:1	1.4	C20:0	0.4	C18:2	8.2	C20:0	0.3
C18:2	21			C20:1	0.2	C18:3	0.8	C20:1	0.2
						C20:0	0.5		
						C20:1	0.4		

Table 1: Typical result of the FAME composition of vegetable oils.

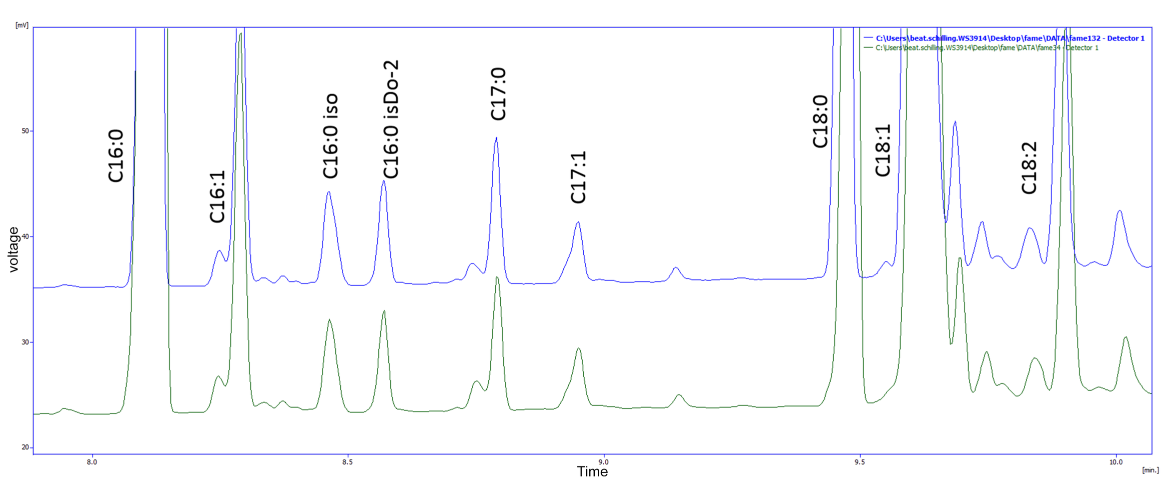


Figure 3: Detail of butter FAMEs before (blue) and after (green) 75 injections.

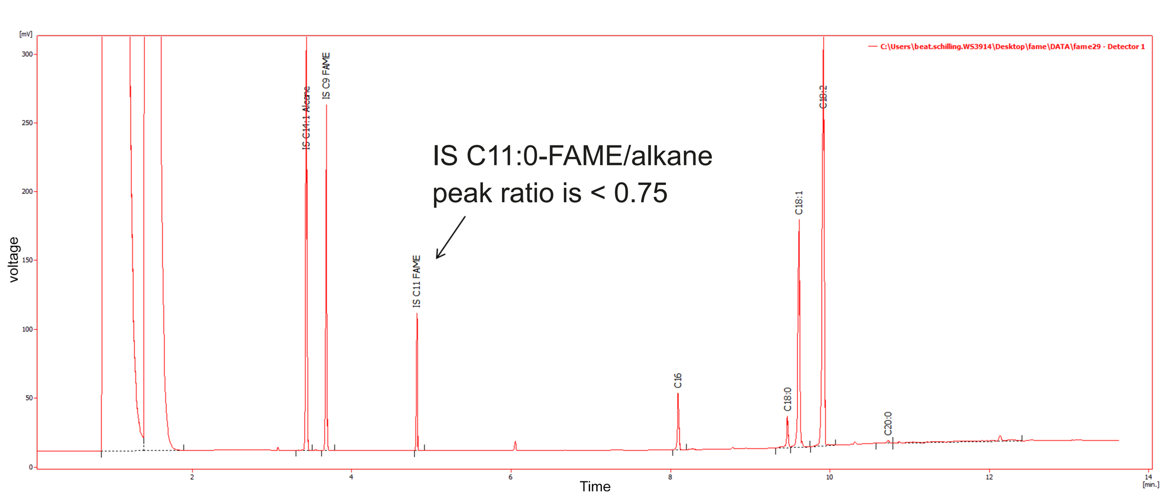


Figure 4: Example for incomplete esterification due to lack of reactant (sunflower oil)

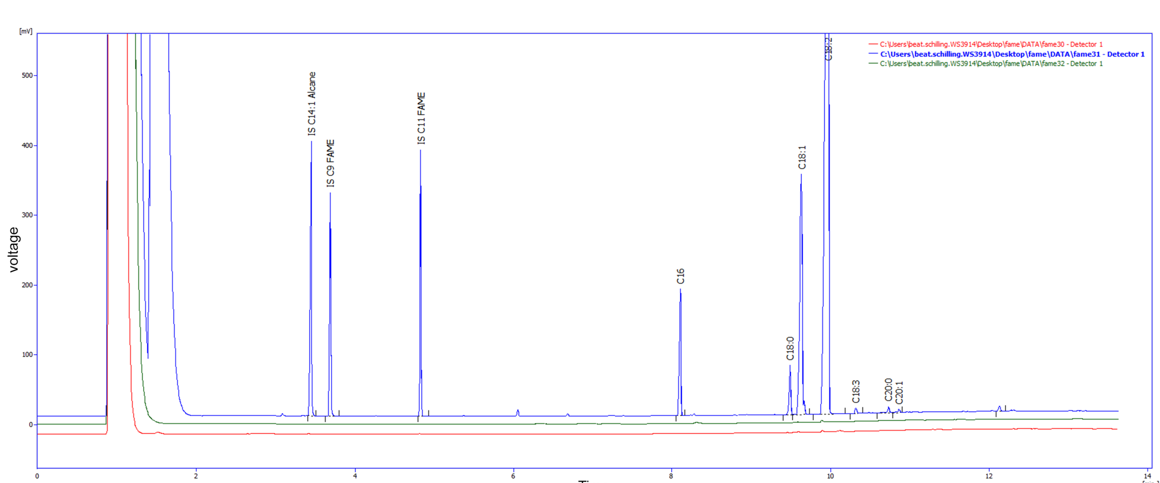


Figure 5: Blank before (red) and after (green) analysis of sunflower oil (blue)

Acknowledgments

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