PAL SYSTEM
Ingenious sample handling

GC Application Note

FOOD SAFETY

Fast Fat Analysis: Determination of Fatty Acid Methyl Esters (FAME) using an Automated Workflow.

www.palsystem.com

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
BGB Analytik AG, Adliswil, Switzerland

Reto Bolliger, Günter Böhm
CTC Analytics AG, Zwingen, Switzerland

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). Most often the samples are processed manually, which is labour 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 PAL workstation 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 10µl syringe to inject the sample into the GC [4].

The software of the workstation allows overlapped sample processing, which increases sample throughput.

The method enables the determination of 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 sausage.

Concept of the method using three different internal standards

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 C14:1, non reactive, to check for complete turnover.

2. Triglyceride of C11 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 C11-FAME / alkane peak ratio is smaller than 0.75, transesterification was not complete e.g. through lack of the reactant, or the FAMEs were saponified already.
If the FAME-9 / alkane peak ratio is smaller than 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: Robotic Tool Change (RTC), parkstation.

Workflow

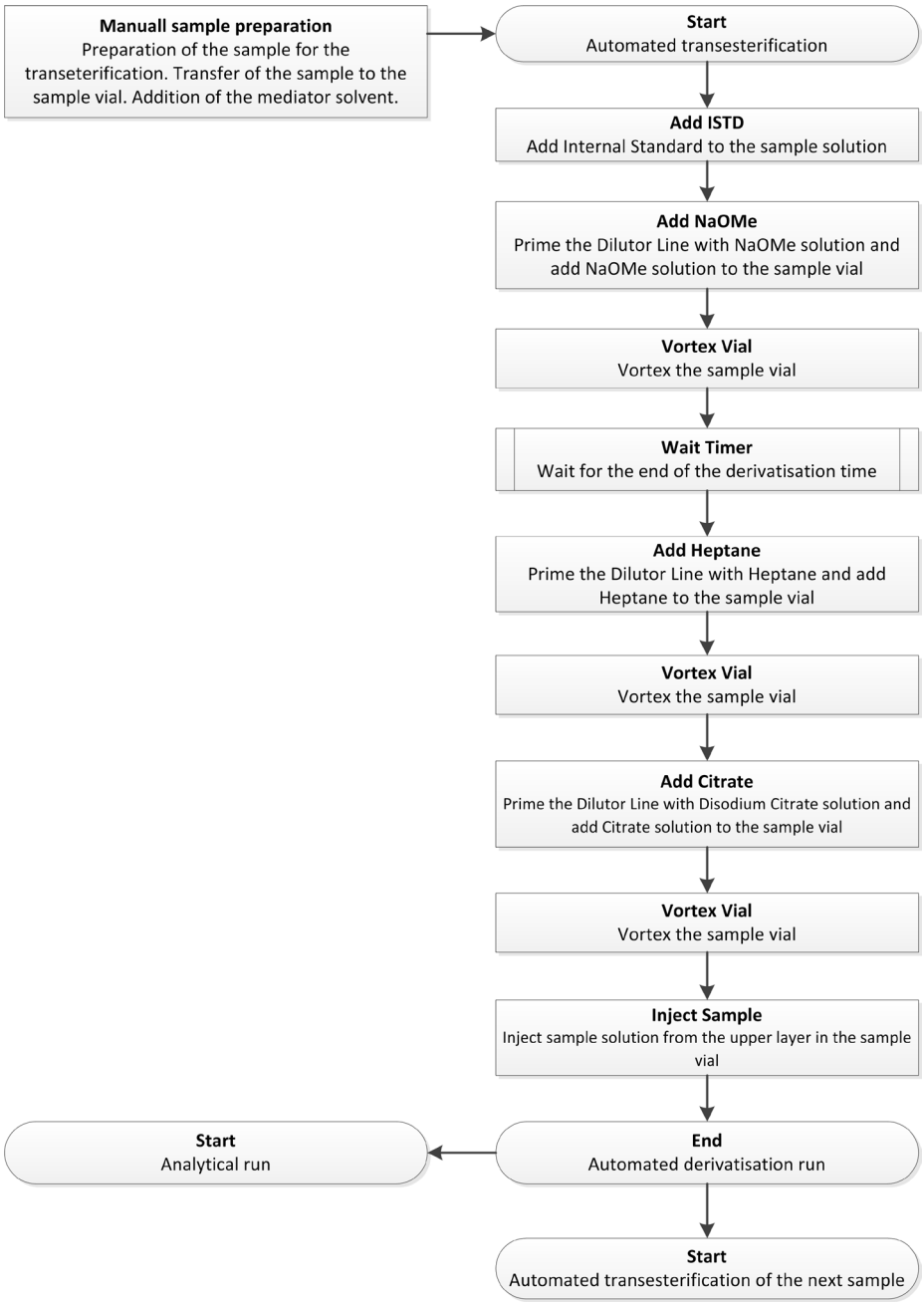

Figure 2: Workflow for the automated generation and analysis of FAMEs.

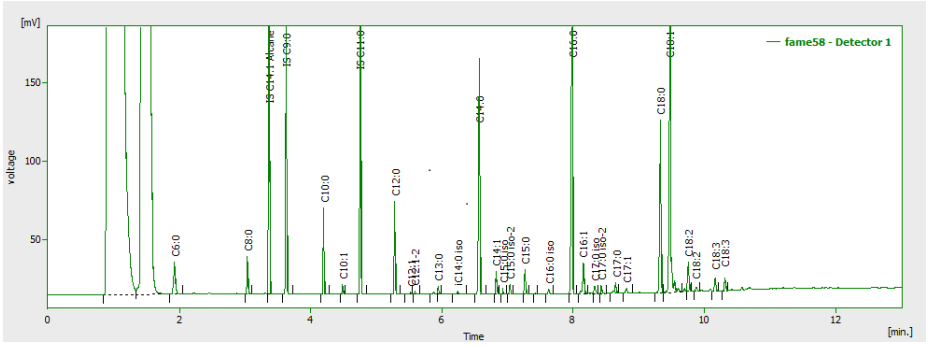

Figure 3: Good chromatographic separation of FAMEs is achieved.

Results

Transesterification of fatty acid esters with Na-methoxide is a fast, efficient and very robust method for fat analysis in in food samples. With the use of three ISs the completeness of the transesterification as well as the extent of undesired saponification can be checked.

The described setup can prepare and analyze 50 samples fully automatically in 18h30min. This is possible because the PAL Sample Control software allows to process one sample while another sample is being analyzed ("prep ahead").

The good chromatographic separation achieved for all FAMEs (Fig. 3) enables robust quantitation. GC peak shapes remained perfect even after 75 injections (Fig. 4). Contamination of the injector liner or the column inlet was not observed.

Typical results for the analysis of the fatty acid composition of different vegetable oils are listed below (Tab.1).

Conclusions

The PAL RTC workstation allows to fully automate the FAME preparation, incuding injection into the GC. A dilutor module was used to dispense Na-methoxide, heptane and Na-citrate. It was also used for intermediate washing steps with methanol and water. The vortex mixer ensured rapid mixing. The fast wash module is required for efficient cleaning of the dilutor tool and the syringe including washing of the outside of the needle. No carryover was detected (Fig. 6).

The PAL System offers a wide range of tools and modules for the preparation and injection of samples. Click here for more information about tools and modules for sample preparation.

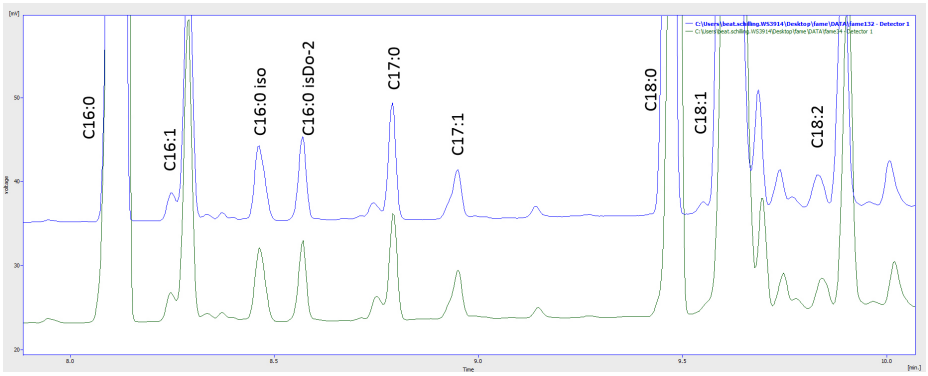

Figure 4: Good chromatographic stability: detail of the analysis of butter FAMESs (inj. #1 blue, inj. #75 green).

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 results of the determination of the fatty acid composition of different oils.

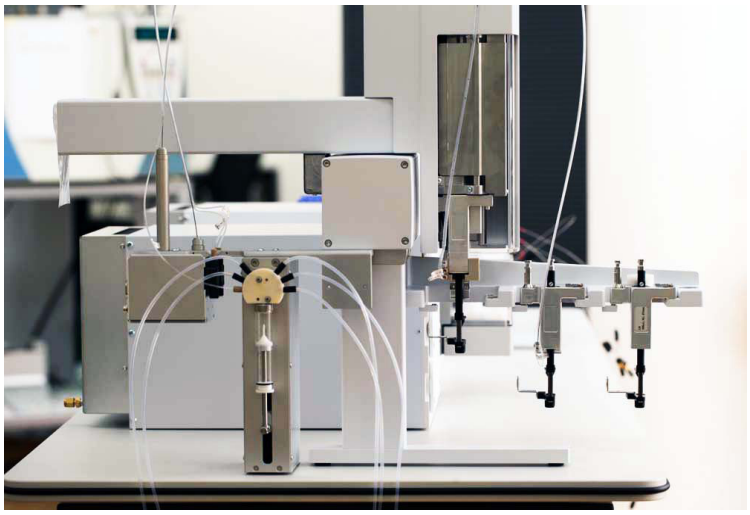

Figure 5: Dilutor module allowing the additon of 5 different solvents.

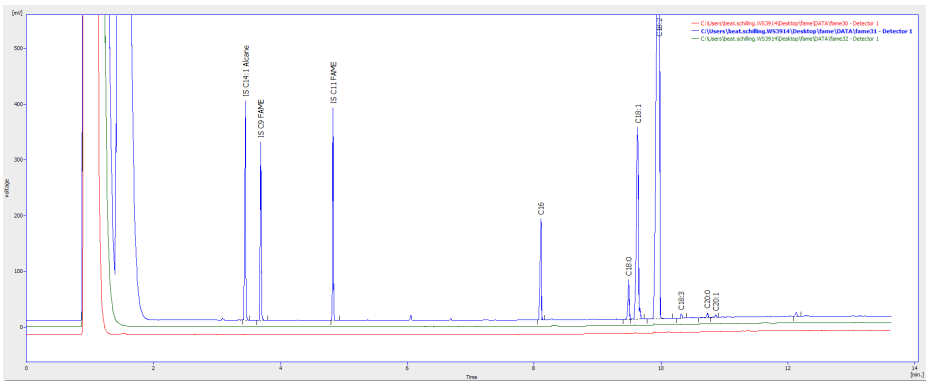

Figure 6: Blank before (red) and after (green) the analysis of sunflower oil (blue).

References

[1] Arens M, Schulte E, Weber K **(1994)**, Fat Sci Technol *96*; 67-68.

[2] House SD, Larson PA, Johnson RR, De Vries JW, Martin DL **(1994)**, J Assoc Off Anal Chem Intern *77*; 960-965.

[3] Suter B, Grob K, Pacciarelli **(1997)**, Z Lebensm Unters Forsch A *204*; 252-258.

[4] de Koning S, van de Meer B, Alkema G, Janssen HG, Brinkmann UT **(2001)**, J Chromatography A, *922*; 391–397.

Legal Statements

CTC Analytics AG reserves the right to make improvements and/or changes to the product(s) described in this document at any time without prior notice.

CTC Analytics AG makes no warranty of any kind pertaining to this product, including but not limited to implied warranties of merchantability and suitability for a particular purpose.

Under no circumstances shall CTC Analytics AG be held liable for any coincidental damage or damages arising as a consequence of or from the use of this document.

Imprint

Date of print: 08.2014

CTC Analytics AG
Industriestrasse 20
CH-4222 Zwingen
Switzerland
T +41 61 765 81 00
Contact: info@ctc.ch