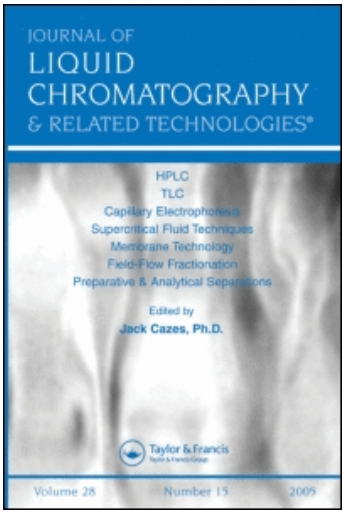

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Analysis of Triglyceride Methanolysis Mixtures Using Isocratic HPLC With Density Detection

Bernd Trathnigg[a]; Martin Mittelbach[a]
[a] Institute of Organic Chemistry Karl-Franzens-University, 28 Graz, Austria

To cite this Article Trathnigg, Bernd and Mittelbach, Martin(1990) 'Analysis of Triglyceride Methanolysis Mixtures Using Isocratic HPLC With Density Detection', Journal of Liquid Chromatography & Related Technologies, 13: 1, 95 — 105
To link to this Article: DOI: 10.1080/01483919008051790
URL: http://dx.doi.org/10.1080/01483919008051790

JOURNAL OF LIQUID CHROMATOGRAPHY, 13(1), 95-105 (1990)

ANALYSIS OF TRIGLYCERIDE METHANOLYSIS MIXTURES USING ISOCRATIC HPLC WITH DENSITY DETECTION

BERND TRATHNIGG AND MARTIN MITTELBACH
Institute of Organic Chemistry
Karl-Franzens-University
A-8010 Graz, Heinrichstrasse 28
Graz, Austria

ABSTRACT:

A simple and reliable method is described, which allows the determination of the overall content of tri-, di-, and monoglycerides in fatty acid methyl esters (FAME) by isocratic liquid chromatography using a density detector. The separation is achieved by coupling a cyano-modified silica column with two GPC- columns; chloroform with an ethanol content of 0.6 % is used as an eluent. The response factors were determined experimentally for the pure triglycerides and methyl esters of various fatty acids as well as for the di- and monoglyceride of palmitic acid and calculated for the mixed products obtained in the methanolysis of vegetable oils. The performance of the method is demonstrated in the analysis of methanolysis mixtures of varying conversion.

INTRODUCTION:

The methanolysis of vegetable oils yields mixtures of fatty acid methyl esters (FAME), which may be used as intermediates in oleo chemistry or as diesel fuel substitute [1,2]. Espe-

cially in the latter case it is very important to reduce the content of mono-, di-, and triglycerides to a very low level in order to avoid formation of deposits in the engine during combustion.

This would require a simple and reliable method of analysis. The numerous methods for analysis of FAME reported in literature are, however, not very well suited for this purpose, because they separate rather according to the fatty acids[3]. There are only a few papers dealing with the determination of the overall content of mono-, di-, and triglycerides in FAME: In general, the methyl esters and lower triglycerides can be analyzed directly by CGC, while mono- and diglycerides require a derivatization prior to CGC - analysis. In TLC and HPLC, both methyl esters and glycerides can be analyzed directly.

Freedman, Pryde and Kwolek[4] used TLC coupled to an FID and, in a further communication[5], CGC (after silylation), a technique, which was also applied by Goh and Timms[6]. Aizetmüller[7] analyzed partial glycerides and other lipid mixtures by gradient elution HPLC with a moving wire detector (Pye Unicam LCM-2), and Bruns[8] used a similar separation system in combination with a (evaporative) light scattering detector (Zinsser - ACS 750/14).

All of these methods are either laborious or suffer from detection problems (such as poor linearity). This lead us to the development of a new method, which was based on the following considerations :

1. As in GC a derivatization is necessary for the analysis of mono- and diglycerides, HPLC should be preferred.
2. In order to avoid detection problems, an isocratic separation seemed to be desirable. In this case a differential refractometer or a density detector could be applied.
3. A typical methanolysis mixture from an average vegetable oil contains (after sample cleanup, which removes the glycerol and free fatty acids), the following components, which differ in their molecular mass:

   - triglycerides ..... 800 - 900
   - diglycerides ..... 550 - 650
   - monoglycerides ..... 330 - 350
   - methyl esters ..... 270 - 300

4. A separation according to molecular mass (using a GPC - column set) would resolve the tri- and diglycerides, but not the monoglycerides and the methyl esters.
5. A separation according to hydroxyl content (using a polar column) would resolve the di- and monoglycerides, but not the triglycerides and the methyl esters.
6. Hence an adequate combination of both separation mechanisms should resolve all of the components in an isocratic mode.

In this way, a convenient method was developed, which allows a determination of the overall content of mono-, di-, and triglycerides and FAME in a methanolysis mixture from vegetable oils. The analysis time is less than 20 minutes, no re-equilibration is required.

EXPERIMENTAL:

Materials:

Reference standards used in this study were purchased from Sigma GmbH, Deisenhofen, FRG, and were chromatographically pure (99%), vegetable oils were edible grade. The corresponding methyl esters were prepared according to the procedure of Freedman [9] using sodium methoxide as a catalyst.

CHROMATOGRAPHY:

The composition of the vegetable oils was determined by CGC of the fatty acid methyl esters according to standard procedures. For the HPLC analysis the following instrumentation and conditions were used:

Pump: Gynkotek 300 C; Columns: Waters Bondapak CN (30 cm), Waters -Styragel 100 Å + 500 Å (30 cm each); Eluent: chloroform LiChroSolv (Merck 2444, stabilized with 2-

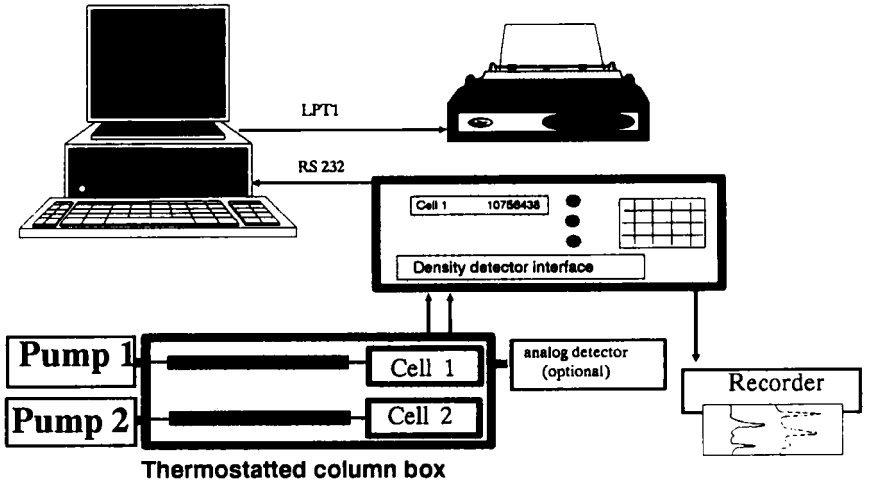

Fig. 1: Chromatographic system using the density detector (schematically)

methyl-butene) + 0.6 % ethanol; flow rate: 1.00 ml/min. Detector: density detection system DDS 70 (developed in our lab[10], commercially available by A.PAAR KG, Graz, Austria), consisting of a density microcell placed in a thermostatted column box, an intelligent interface DDI, coupled to an IBM - PC. Data acquisition and processing was performed using the software package CHROMA. The system is shown schematically in Fig.1.

PRINCIPLE OF DENSITY DETECTION:

Density measurement according to the mechanical oscillator method [11] may be used with advantage for the detection of non UV- absorbing samples in GPC or isocratic HPLC, as we have shown previously [10,12].

The density difference Δd between a solution and the pure solvent is given by

$$\Delta d = c_i \cdot (1 - d_0 \cdot v_i^*) = c_i \cdot a_i \qquad (1)$$

wherein d_0 is the density of the pure solvent, c_i the concentration of the solute and v_i^* its partial (or apparent) specific volume, a_i is the response factor.

In a density detector, this density difference will cause a change ΔN_B in the detector signal N_B, which is the number of periods of the time base (in the density detection interface an oven controlled 10 MHz quartz oscillator, $T_B = 1.10^{-7}$ sec) within one measuring interval $t = N_B.T_B$:

$$\Delta d = 2.(d_0 + B).\Delta N_B / N_B \qquad (2)$$

wherein B is a constant for each individual cell.

At a given flow rate F, within each measuring interval t, a volume $V = F . N_B.T_B$ passes the cell, which contains a mass m_i of the sample :

$$m_i = c_i . V = c_i . F . N_B.T_B \qquad (3)$$

Hence, the mass eluted within one measuring interval is given by

$$m_i = 2 . T_B . (d_0 + B) . F / a_i . \Delta N_B \qquad (4)$$

as follows from combination of the equations above. This means, that the digital output of the detector is inherently integrated over each measuring interval. The total mass eluted within a peak is thus easily obained by a simple summation of the digital values of the detector response ($x_i = \Delta N_B$) :

$$m_p = 2 T_B . (d_0 + B) . F / a_i . \Sigma x_i \qquad (5)$$

As long as d_0 and F are constant, the "peak area" $A_p = \Sigma x_i$ represents the eluted mass of a component of the response factor a_i with high accuracy. a_i can be determined by density measurement for each component or better in the conventional way from the peak area and the sample size m_p obtained from chromatograms of standards, which eliminates errors in flow rate and the injected volume.

RESULTS AND DISCUSSION :

Separation of the components :

Using the chromatographic conditions described above, the tri- and diglycerides are sufficiently, the methyl esters and the mono glycerides are completely separated, the free acids, which do not occur in the methanolysis mixtures after sample cleanup, would also be separated, as can be seen from Fig.2 and Tab.1 .

RESPONSE FACTORS OF FATTY ACID METHYL ESTERS AND GLYCERIDES:

Using equation 5, the response factors of FAME and glycerides can easily be determined from the ratio A_p/m_p, or better by linear regression. To show the linearity of the detector response,a typical plot of peak area vs. sample size is shown in Fig.3.

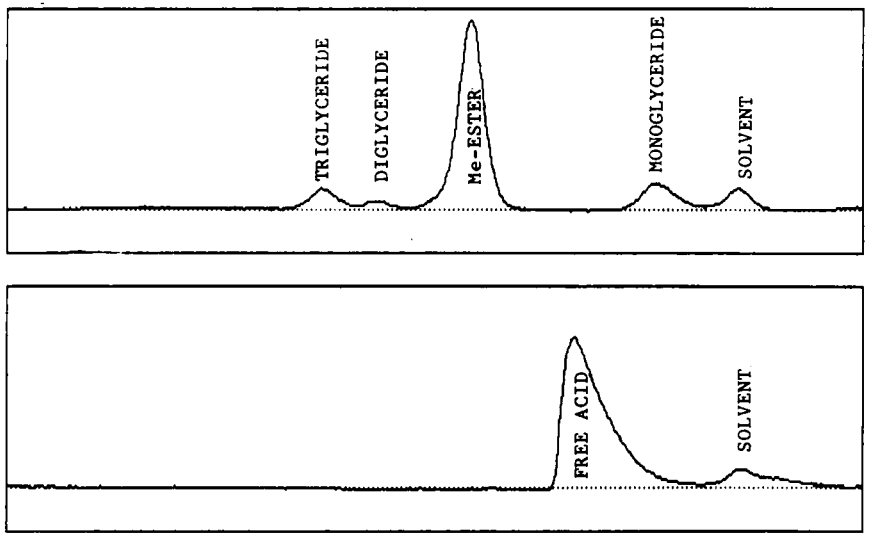

Fig. 2:Comparison of chromatograms of a typical methanolysis mixture from rapeseed oil (top) and of linoleic acid (bottom).

Tab.1 : Elution times of the glycerides, FAME and free acids

Component	elution time (sec)
Triglycerides	845
Diglycerides	881
FAME	973
Free acids	1030
Monoglycerides	1060

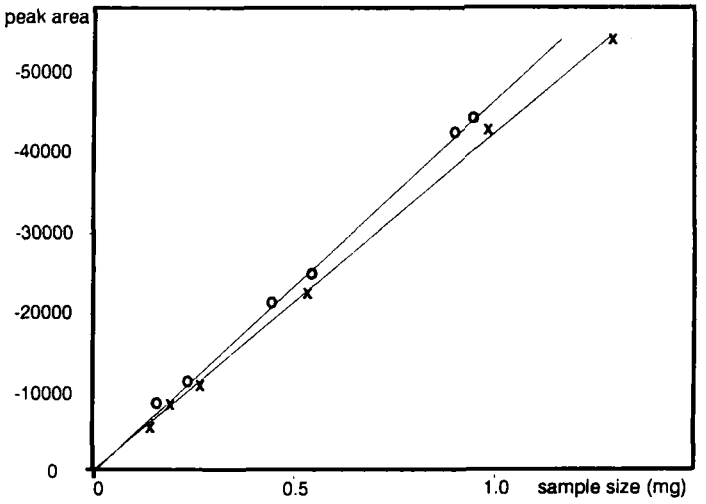

Fig. 3: Plot of peak area vs. sample size from chromatograms obtained with varying concentrations of triolein (+) and methyl oleate (o)

From the slope of the regression line, a_i was calculated (with $T_B = 1.10^{-7}$ sec, $d_0 = 1.47$ g/cm^3, and $B = 2.90$ g/cm^3, $F = 1.00$ ml/min).

The response factors of various pure triglycerides and methyl esters as well as the corresponding correlation coefficients of the regression line are given in Tab.2 .

Tab.2 : Response factors a_i of triglycerides and methyl esters of various fatty acids (determined by linear regression) and correlation coefficients r

Fatty acid	Triglycerides		Methyl esters	
	a_i	r	a_i	r
Stearic acid	-0.716	-0.99996	-0.795	-0.99997
Palmitic acid	-0.706	-0.99999	-0.796	-0.99999
Oleic acid	-0.671	-0.99994	-0.761	-1.00000
Linoleic acid	-0.633	-0.99995	-0.716	-0.99992
Linolenic acid	-0.587	-0.99970	-0.689	-0.99992

Tab. 3 : Response factors of derivatives of palmitic acid	
Tripalmitin	-0.706
Dipalmitin	-0.677
Methyl palmitate	-0.796
Monopalmitin	-0.612

The response factors of pure mono- and diglycerides may be determined in the same way, provided that there are any standards of sufficiently high purity available, which was the case only with palmitic acid. The response factors of the glycerides and the methyl ester of palmitic acid are given in Tab.3.

ANALYSIS OF METHANOLYSIS MIXTURES:

Since vegetable oils are mixtures of the triglycerides of various fatty acids, also mixtures of the di- and monoglycerides and the methyl esters will occur during the methanolysis.

Tab. 4 : Composition of various vegetable oils (with respect to fatty acids) obtained by CGC of methyl esters.
(a : at complete conversion, b : at low conversion)

Oil: / Acid :	Linseed	Peanut	Corn	Olive	Rapeseed		Sunflower	
					a	b	a	b
Palmitic	5.7	12.7	11.0	12.6	5.3	5.5	6.7	7.1
Stearic	3.0	4.1	2.6	2.9	1.6	1.7	4.6	5.0
Oleic	18.1	33.9	28.9	72.5	61.5	61.5	21.7	21.8
Linoleic	14.9	34.9	56.1	9.3	21.5	21.4	66.3	65.6
Linolenic	58.3	0.4	1.0	0.7	8.7	8.1	0.4	0.3
Palmitoleic	0	0	0	1.3	0	0	0	0
Eicosanic	0	1.3	0.4	0.4	0.4	0.5	0.3	0.2
Eicosenic	0	0.7	0	0.3	1.0	1.3	0	0
Behenic	0	2.0	0	0	0	0	0	0

The composition of some typical vegetable oils (determined in the usual way by CGC of the methyl esters) is shown in Tab. 4.

For the accuracy of the analysis, it is very important, that the fatty acid composition of all of the components occuring in a methanolysis mixture, should be the same over the entire range of the methanolysis. Under the assumption of similar rate constants for the solvolytic cleavage of all of the esters bonds, the FAME as well as the glycerides should have the same average fatty acid composition. This was proven by CGC - analysis of methanolysis mixtures at complete and at rather low conversions (approximately 50 %) : in both cases, the fatty acid composition of the FAME was found to be the same (within experimental error), as can be seen from Tab.4.

Hence, the best approach for practical use seemed to be the following : The response factors of the vegetable oils and the corresponding methyl esters can be determined experimentally; for each vegetable oil a correction factor (based on tripalmitin) can be used to

Tab.5 : Response factors a_i of various vegetable oils, mixed di- and monoglycerides, and methyl esters obtained therefrom.
(c : calculated, based on glycerides of palmitic acid, Tab.2)

Oil :	Triglycerides	Diglycerides[c]	Monoglycerides[c]	Methyl esters
Olive	-0.644	-0.618	-0.558	-0.749
Rapeseed	-0.639	-0.613	-0.554	-0.719
Peanut	-0.637	-0.611	-0.552	-0.724
Corn	-0.618	-0.593	-0.536	-0.720
Sunflower	-0.618	-0.593	-0.536	-0.707
Linseed	-0.590	-0.566	-0.511	-0.699

Tab. 6 : Integration results from two chromatograms of methanolysis mixtures from typical vegetable oils at low conversion

Component :	Rapeseed oil wt. %	Sunflower oil wt. %
Triglycerides	55.25	49.19
Diglycerides	11.59	18.56
Monoglycerides	6.85	6.65
Methyl esters	26.19	26.47
Total	99.88	100.87

calculate the response factors for the di- and mono glycerides. The values obtained in this way (using the data from Tab.3) are given in Tab.5.

The performance of the method was evaluated by analyzing methanolysis mixtures of varying conversion, which contain all of the glycerides and the methyl esters, but no free fatty acids, as can be seen from Fig.2.

The accuracy of the integration data can be estimated as follows: If the response factors are correct, the sum of the components found should equal the sample size, or, in other words, the fractions of peak mass should sum up to yield 100 %, which is proven by the integration data shown in Tab. 6. Hence, the described method yields the overall content of glycerides in fatty acid methyl esters with good accuracy.

Acknoledgement:

Financial support by the Austrian "Forschungsförderungsfonds für die gewerbliche Wirtschaft" is gratefully acknowledged.

REFERENCES:

1. M.Mittelbach, M.Woergetter, J.Pernkopf, H.Junek, Energy in Agriculture 2 (1983) 369
2. M.Mittelbach, Öst.Chem.Ztg. 1989, in press
3. CHROMPACK Application notes Nr. 80, 90-2, 94,129-30,195, 201-205, 234-240
4. B.Freedman, E.H.Pryde, W.F.Kwolek, J.Am.Oil Chem.Soc. 61 (1984) 1215
5. B.Freedman, W.F.Kwolek, E.H.Pryde, J.Am.Oil Chem.Soc. 63 (1986) 1370
6. E.M.Goh, R.E.Timms, J.Am.Oil Chem.Soc. 62 (1985) 730
7. K.Aizetmüller, J.Chromatogr. 139 (1977) 61
8. A.Bruns, Fat Sci.Technol. 90 (1988) 289
9. B.Freedman, E.H.Pryde, T.L.Mounts, J.Am.Oil Chem.Soc. 61 (1984) 1638
10. B.Trathnigg, Ch.Jorde, J.Chromatogr. 385 (1987) 17
11. O.Kratky, H.Leopold, H.Stabinger,Z.Angew.Phys.27(1969)273
12. B.Trathnigg, Ch.Jorde, J.Liq.Chromatogr. 7 (1984) 1185