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Simultaneous determination of glycerol, and mono-, di- and triglycerides in vegetable oil methyl esters by capillary gas chromatography

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Abstract

A gas chromatographic procedure for the simultaneous determination of glycerol, mono-, di- and triglycerides in vegetable oil methyl esters has been developed. Quantitative information about this group of organic contaminants is very important for the quality of these oleochemical products when used as automotive diesel fuel substitutes.

Trimethylsilylation of glycerol, mono- and diglycerides, followed by GC using a 10-m capillary column coated with a 0.1-μm film of DB-5 allows the determination of all analytes in a single GC run. Calibration is performed by analysis of standard solutions containing glycerol, mono-, di- and triolein as well as two internal standards, 1,2,4-butanetriol and tricaprin. The recovery of the procedure at different concentration levels and the repeatability of the quantitative results are evaluated.

1. Introduction

Vegetable oil methyl esters (VOMEs), obtained by alkali-catalyzed transesterification of vegetable oils with methanol, can be significantly contaminated with glycerol, mono-, di- and triglycerides due to incomplete transesterification and insufficient purification. For the use as automotive diesel fuel substitutes, now the most important field of application, the presence of these minor components can lead to serious engine problems and hazardous emissions [1]. Limits for the permissible levels of glycerol, mono-, di- and triglycerides have therefore been introduced by existing standard specifications [2] and will be also included in future standard specifications. As a consequence, continuous quality control of these oleochemical products is essential.

During the last few years, several analytical procedures have been developed for both the determination of glycerol as well as for mono-, di- and triglycerides in VOMEs. Methods, based on the extraction of glycerol from VOMEs into an aqueous phase and its subsequent gas chromatographic (GC) analysis, were described by Bondioli et al. [3] and Hödl and Schindlbauer [4]. An enzymatic determination of glycerol in an aqueous extract of fatty acid methyl esters was described by Bailer and De Hueber [5]. Mittelbach analyzed glycerol after derivatization with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) directly in the VOME sample [6].

Capillary GC is normally used for the analysis of mono-, di- and triglycerides in VOMEs.

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Freedman et al. [7] applied capillary GC for studying transesterification of soybean oil to fatty esters. Mariani et al. [8] described a GC method for the determination of fatty acid methyl esters together with mono-, di- and triglycerides in vegetable oil derivatives. Recently, we reported on a GC method for the determination of mono-, di- and triglycerides in VOMEs, which was developed especially for quantitative analysis of these compounds in VOMEs used as diesel fuel substitutes [9]. Isocratic HPLC–gel permeation chromatography (GPC) with density detection was applied by Trathnigg and Mittelbach for the analysis of triglyceride methanolysis mixtures [10]. For kinetic studies, Freedman et al. analyzed reaction mixtures of transesterified soybean oil by thin-layer chromatography with flame ionization detection (TLC–FID) [11].

All methods, reported so far, were designed to determine the content of either free glycerol or of mono-, di- and triglycerides in VOMEs. In this work, we have developed a rapid and reliable GC procedure for the simultaneous determination of glycerol, mono-, di- and triglycerides in VOMEs, predominantly consisting of C_{18}-fatty acid methyl esters, such as rapeseed oil methyl ester, sunflower oil methyl ester, soybean oil methyl ester and used frying oil methyl ester. This method can be used for both the routine quality control of VOMEs in the course of production and the inspection of their compliance with the required specifications.

Trimethylsilylation of the free hydroxyl groups of glycerol, mono- and diglycerides, followed by GC using a short thin film capillary column allows the determination of all analytes, varying considerably in polarity and volatility, in a single GC run. By the use of two internal standards, 1,2,4-butanetriol and tricaprin, reliable quantitative analysis of glycerol, mono-, di- and triglycerides can be carried out within a run time of 30 min. Calibration is performed by analysis of standard solutions containing glycerol, mono-, di- and triolein and both internal standards. The recovery of the procedure at different concentration levels and the repeatability of the quantitative results are carefully evaluated.

2. Experimental

2.1. Chemicals

The reference substances used in this study, glycerol, (*S*)-(−)-1,2,4-butanetriol, 1-mono[*cis*-9-octadecenoyl]-*rac*-glycerol (monoolein), 1,3-di-[*cis*-9-octadecenoyl]glycerol (diolein), 1,2,3-tri-[*cis*-9-octadecenoyl]glycerol (triolein) and 1,2,3-tridecanoylglycerol (tricaprin), were purchased from Sigma (Deisenhofen, Germany) and were chromatographically pure (>99%). N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) was obtained from Fluka (Buchs, Switzerland). Analytical grade *n*-heptane and pyridine were supplied by Loba Feinchemie (Fischamend, Austria).

2.2. Preparation of samples and standard solutions

Stock solutions of glycerol (0.5 mg/ml), monoolein (4.0 mg/ml), diolein (4.0 mg/ml), triolein (5.0 mg/ml), 1,2,4-butanetriol (3.0 mg/ml) and tricaprin (8.0 mg/ml) in pyridine were used to prepare standard solutions at 5 different concentration levels. The concentration of glycerol in the standard solutions varied from 0.001 to 0.006 mg/ml, of mono- and diolein from 0.005 to 0.063 mg/ml, and of triolein from 0.007 to 0.110 mg/ml; the concentration of the internal standards was 0.022 mg/ml for 1,2,4-butanetriol and 0.090 mg/ml for tricaprin in all standard solutions. Appropriate amounts of the stock solutions of glycerol, mono-, di- and triolein, 70 μl of butanetriol stock solution and 100 μl of tricaprin stock solution were transferred into 10 ml screw-cap vials.

For preparation of samples, butanetriol stock solution (70 μl) and tricaprin stock solution (100 μl) were added as internal standards to 100.0–110.0 mg of VOME in a 10 ml screw-cap vial. MSTFA (100 μl) was added to the standard and to the sample material. After 15 min at room temperature, the silylated mixtures were dissolved in *n*-heptane and diluted to 9 ml.

2.3. Instrumentation

GC analyses were performed with a Fisons Instruments GC 8000 gas chromatograph (Milan, Italy). The instrument was equipped with an on-column injector and a flame ionization detector, and was fitted with a 2 m × 0.53 mm I.D. uncoated, deactivated fused-silica pre-column (Carlo Erba) connected in series with a 10 m × 0.32 mm I.D. fused-silica capillary column coated with a 0.1-μm film of DB-5 (J&W Scientific, Folsom, CA, USA) by means of a butt connector. Acquisition and processing of data was performed with a Chromcard (Fisons Instruments) in combination with an IBM compatible personal computer.

Samples (1 μl) were injected on-column by an AS 800 automatic sampler (Fisons Instruments) at an oven temperature of 50°C. After an isothermal period of 1 min, the GC oven was heated at 15°/min to 180°C, at 7°/min to 230°C and ballistically to 370°C (held for 10 min). Hydrogen was used as carrier gas at a flow-rate of 3 ml/min measured at 50°C. Detector temperature was 370°C; nitrogen served as detector make up gas at an inlet pressure of 0.5 bar. Run time was 30 min.

3. Results and discussion

This GC method for the simultaneous determination of glycerol, mono-, di- and triglycerides was developed especially for rapeseed oil methyl ester (RME). The reference substances were selected with respect to the fatty acid composition of rapeseed oil. Temperature programming was adjusted to obtain group-type separation of glycerol, mono-, di- and triglycerides, typically occuring in RME. VOMEs with fatty acid compositions or chain lengths similar to that of RME, such as sunflower oil methyl ester, soybean oil methyl ester and used frying oil methyl ester, can be analyzed by the outlined method without any adjustment of conditions. However, this GC method can not be applied to methyl esters obtained by transesterification of lauric oils without modifications, because superimpositions of peaks of long-chain fatty acid methyl esters and short-chain monoglycerides make a reliable quantitative analysis impossible.

3.1. Derivatization

In principle, glycerol, mono-, di- and triglycerides can be analyzed on highly inert columns coated with apolar stationary phases without derivatization. The inertness of the column, required to obtain good peak shapes and satisfactory recoveries, cannot be easily maintained in routine analysis. Trimethylsilylation of the free hydroxyl groups of glycerol, mono- and diglycerides, however, ensures excellent peak shapes, good recoveries and low detection limits, and enormously improves the ruggedness of the procedure.

For complete silylation of glycerol and partial glycerides, the conditions of the derivatization reaction have to be controlled carefully. Extensive studies on the silylation of partial glycerides, previously carried out in our laboratory, showed that complete silylation can be obtained under the following conditions [12]: (i) BSTFA as silylating agent, addition of pyridine or dimethylformamide and heating to 70°C for 15 min; (ii) BSTFA + 1% trimethylchlorosilane as silylating agent, addition of pyridine and a reaction time of 15 min at room temperature; (iii) MSTFA as silylating agent, addition of pyridine and a reaction time of 15 min at room temperature; (iiii) MSTFA as silylating agent and heating to 70°C for 15 min. With the mentioned conditions, the degrees of conversion were determined by GC analysis of the corresponding reaction mixtures to be higher than 98% for monoglycerides and 100% for glycerol and diglycerides. This method employs derivatization conditions according to (iii), as the consumption of silylating agent is reduced when using MSTFA instead of BSTFA, and as heating is not necessary when pyridine is added as a catalyst.

The internal standard 1,2,4-butanetriol serves as a very sensitive indicator of incomplete derivatization. In case of insufficient silylation (not

all of the three hydroxyl groups are silylated), the peak of 1,2,4-butanetriol appears splitted and drastically reduced in height.

3.2. Qualitative analysis

A gas chromatogram of silylated rapeseed oil methyl ester with 1,2,4-butanetriol and tricaprin as internal standards (as trimethylsilyl derivatives) is shown in Fig. 1. Separation of compound classes only is required, not of compounds within a class. Peak identification was achieved by analysis of samples spiked with reference substances or by comparison with reference chromatograms.

Glycerol and 1,2,4-butanetriol elute in advance of the fatty acid methyl esters at column temperatures lower than 100°C. The enlargements in Fig. 1b show the regions of the gas chromatogram, where the peaks of mono-, di- and triglycerides appear. On 5% phenyl polydimethylsiloxane, mono-, di- and triglycerides are mainly separated according to carbon numbers (CN).

For monoglycerides, the peak with the relative retention time (RRT) of 0.78 with respect to the internal standard tricaprin (t_R = 19.7 min) clearly dominates and corresponds to the signals of monoolein, monolinolein and monolinolenin. The asymmetric peak shape is due to the superimposition of these monoglyceride peaks with equal carbon number (CN = 18) but different number of double bonds. In addition, the signals of monopalmitin (CN = 16; RRT = 0.70) and monostearin (CN = 18; RRT = 0.79) could be identified.

The diglycerides are also primarily separated according to carbon number and appear in groups of peaks corresponding to 1,2- and 1,3-isomers or to diglycerides with equal carbon number but different number of double bonds. The individual diglyceride peaks could not be reliably identified. Nevertheless, the signals with relative retention times from 1.09 (t_R = 21.4 min) to 1.17 (t_R = 23.0 min) could clearly be assigned to diglycerides with carbon numbers of 34, 36 and 38 by comparison of chromatograms of samples with high and low degrees of transesterification and reference chromatograms.

At the end of the chromatogram, the triglycerides form a group of peaks separated only according to carbon number. Signals with relative retention times from 1.35 (t_R = 26.6 min) to 1.47 (t_R = 29.2 min) corresponding to triglycerides with carbon numbers of 52, 54, 56 and 58 were included in quantitation.

3.3. Calibration

For the quantitative determination of free glycerol, mono-, di- and triglycerides in VOMEs a calibration with the reference substances glycerol, mono-, di- and triolein was carried out. Due to structural differences between the individual analyte classes and to partial thermal degradation observed for the high boiling di- and triglycerides, calibration is of great importance for a reliable quantification.

Freshly prepared standard solutions (5 concentration levels), containing known amounts of the reference substances glycerol, mono-, di- and triolein and both internal standards were analyzed three times each by capillary GC. A corresponding gas chromatogram is shown in Fig. 2. Table 1 summarizes the regression data of the linearly fitted calibration functions, which show excellent linearity for glycerol, mono- and diolein and acceptable linearity for triolein in the concentration range of interest.

3.4. Quantitative results

For the quantitative determination of monoglycerides, the corresponding peaks were integrated separately, and the concentrations were calculated according to the calibration function obtained for monoolein based on the internal standard tricaprin. The total concentration was then calculated by summing up the concentrations of the individual monoglycerides. Di- and triglycerides were quantified analogously. The concentration of glycerol was calculated according to its calibration function based on the internal standard 1,2,4-butanetriol.

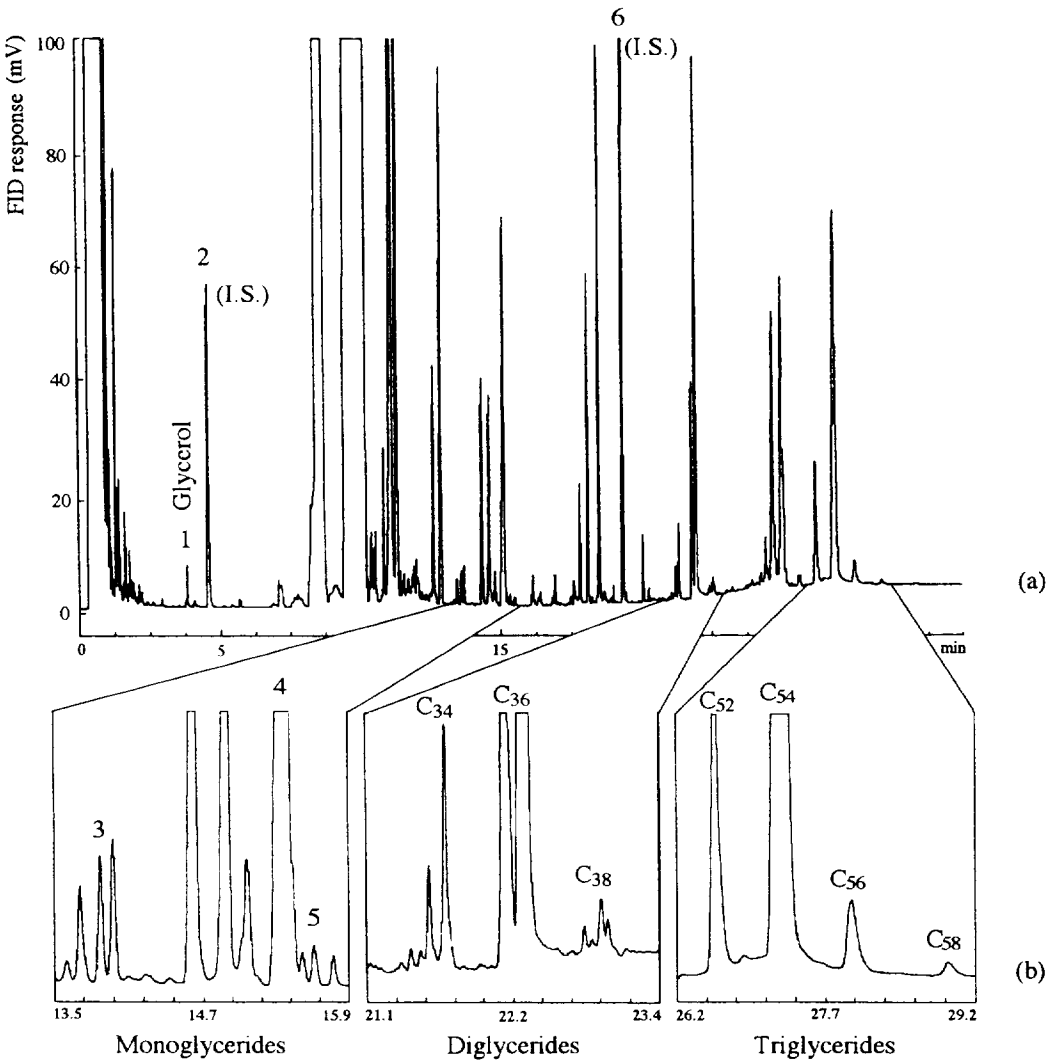

Fig. 1. (a) Gas chromatogram of silylated rapeseed oil methyl ester with 1,2,4-butanetriol and tricaprin as internal standards. (b) Enlargements of the regions, where the signals of mono-, di- and triglycerides appear in the upper gas chromatogram. GC column: 10 m × 0.32 mm I.D. fused-silica capillary column coated with DB-5 (0.1 μm film thickness), equipped with a 2 m × 0.53 mm I.D. uncoated, deactivated pre-column. GC temperature programme: 50°C (1 min), at 15°/min to 180°C, at 7°/min to 230°C, ballistically to 370°C (10 min). Peak assignment: 1 = glycerol; 2 = 1,2,4-butanetriol, I.S.; 3 = monopalmitin; 4 = monoolein, monolinolein, monolinolenin; 5 = monostearin; 6 = tricaprin, I.S.; C_{34}–C_{38} = diglycerides with carbon numbers of 34, 36 and 38; C_{52}–C_{58} = triglycerides with carbon numbers of 52, 54, 56 and 58.

For the evaluation of the recovery, reference samples and spiked RME samples containing known amounts of standard substances at different concentration levels were analyzed. The reference samples contained glycerol, mono-, di- and triolein at concentrations, typical of authentic VOME samples. Distilled RME (containing small amounts of glycerol and monoglycerides but no detectable amounts of di- and triglycerides) was spiked with the standard substances mentioned. By comparing the quantitative results obtained by GC analysis and the

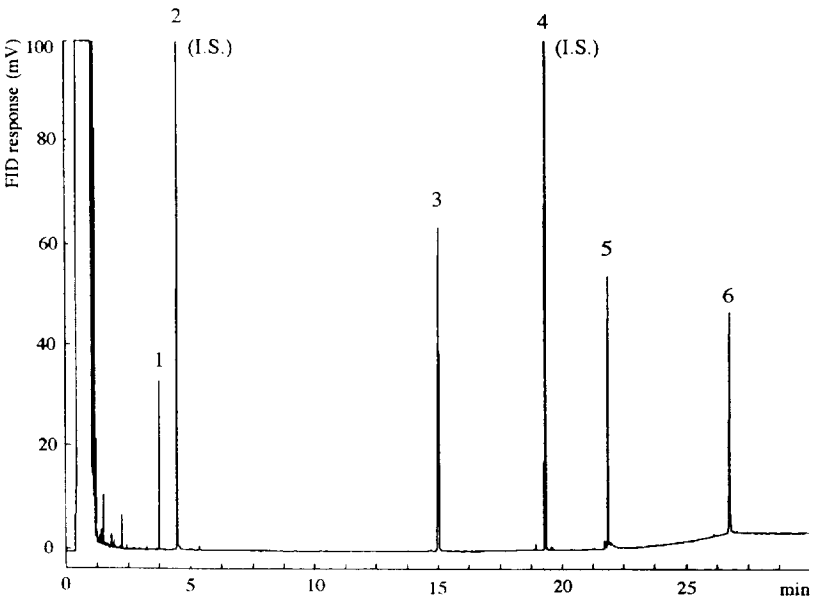

Fig. 2. Gas chromatogram of a standard solution containing the trimethylsilyl derivatives of glycerol, 1,2,4-butanetriol, monoolein and diolein, as well as tricaprin and triolein. Conditions as in Fig. 1. Peak assignment: 1 = glycerol; 2 = 1,2,4-butanetriol, I.S.; 3 = monoolein; 4 = tricaprin, I.S.; 5 = diolein; 6 = triolein.

actual concentrations of the standard substances, the percentage recoveries were calculated and are given in Table 2. Except for triolein in the lowest concentration level (5 μg/ml), the recovery of all solutes of interest is excellent.

In order to check the precision of the method, a sample of RME was prepared and consecutively analyzed seven times. The quantitative results for glycerol, mono-, di- and triglycerides are given in Table 3.

Additionally, seven samples of the same RME specimen were prepared and analyzed by GC. The results are summarized in Table 4. The data in Tables 3 and 4 indicate the excellent repeatability of the quantitative results obtained by the method outlined. Due to simple sample preparation (comprising sample weighing, the addition of the internal standards, derivatization and dilution), the reproducibility of the quantitative results obtained by repeated complete analy-

Table 1
Calibration functions for glycerol, monoolein, diolein and triolein: $W_c/W_{st} = b \cdot A_c/A_{st} + a$ ($n = 5$)

	b	a	Standard error	r^2
Glycerol	0.937 ± 0.005	-	0.002	0.999
Monoolein	0.748 ± 0.004	-	0.005	0.999
Diolein	0.819 ± 0.012	0.019 ± 0.005	0.007	0.999
Triolein	1.213 ± 0.052	0.085 ± 0.027	0.039	0.995

Abbreviations: W_c, weight of component; W_{st}, weight of internal standard; A_c, peak area of component; A_{st}, peak area of internal standard; r, correlation coefficient.

Table 2
Average recovery ($n = 3$) for glycerol, mono-, di- and triglycerides at three concentration levels

	Recovery (%)					
	Reference sample			Spiked RME		
Conc. (μg/ml)	1	2	4	1	2	4
Glycerol	104	103	100	106	103	101
Conc. (μg/ml)	5	50	100	5	50	100
Monoolein	95	101	101	103	101	101
Diolein	98	100	102	129[b]	101	102
Triolein	148[a]	95	98	201[a,b]	109	110

[a] The calibration function for triolein shows a higher error in this low concentration range.
[b] Distilled RME contains di- and triglycerides at concentrations below the detection limits which effect the recovery of di- and triolein in this low concentration range.

sis is as good as that obtained by consecutive injection of the same sample.

4. Conclusion

In a single GC run, the proposed method provides qualitative and quantitative information about glycerol, mono-, di- and triglycerides, the most important organic contaminants of vegetable oil methyl esters. High reliability of results, simple instrumentation and sample preparation, short analysis time and the possibility of complete automation make this method well suited for the quality control of these oleochemical products, gaining steadily in importance for technical applications, especcially as diesel fuel substitutes.

Table 3
Repeatability of the quantitative results for glycerol, mono-, di- and triglycerides, obtained by consecutive GC injections ($n = 7$)

Analysis	Concentration (weight%) in RME			
	Glycerol	Monoglycerides	Diglycerides	Triglycerides
1	0.009	0.355	0.505	1.152
2	0.009	0.357	0.510	1.168
3	0.009	0.365	0.509	1.162
4	0.009	0.363	0.509	1.169
5	0.009	0.358	0.510	1.173
6	0.009	0.350	0.510	1.174
7	0.009	0.364	0.513	1.171
Mean	0.009	0.359	0.509	1.167
S.D.	<0.001	0.005	0.002	0.007
R.S.D. (%)	1.3	1.4	0.4	0.6

Table 4
Repeatability of the quantitative results for glycerol, mono-, di- and triglycerides obtained by repeated complete analysis including sample preparation ($n = 7$)

Analysis	Concentration (weight%) in RME			
	Glycerol	Monoglycerides	Diglycerides	Triglycerides
1	0.009	0.362	0.512	1.176
2	0.009	0.366	0.516	1.179
3	0.009	0.365	0.514	1.170
4	0.009	0.358	0.514	1.183
5	0.009	0.367	0.511	1.170
6	0.009	0.360	0.508	1.168
7	0.009	0.359	0.513	1.173
Mean	0.009	0.362	0.513	1.174
S.D.	<0.001	0.003	0.002	0.005
R.S.D. (%)	1.3	0.9	0.5	0.5

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