SEG-A-081

Quality Control According to DIN EN 14103, 14105, 14110

Application Book Volume 4

Biodiesel Quality Control

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Application Book Volume 4

Biodiesel Quality Control

Contents

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Biodiesel Quality Control

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1. Determination of methanol in biodiesel

Determination of methanol in biodiesel

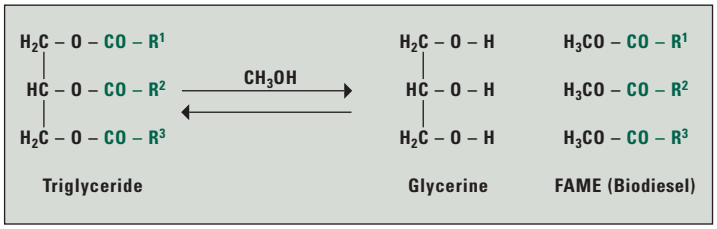


Figure 1: Equilibrium reaction of the transesterification of plant oils (triglycerides) to fatty acid methyl esters (biodiesel)

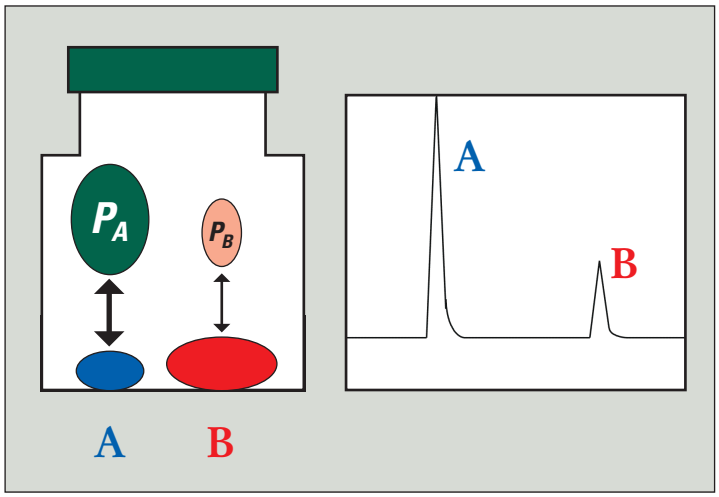


Figure 2: Equilibrium between the liquid- and gas-phase during headspace sample preparation. Although component A is present at a lower concentration in the liquid phase, the concentration ratio in the gas-phase may shift, depending on the vapor pressure of the individual components. Correspondingly, the chromatograms resulting from headspace injection usually produce different signal ratios when compared with those resulting from liquid sample injection.

- **System with headspace autosampler for high precision results**
- **Requirements of DIN EN 14110 exceeded**

Biodiesel production has increased considerably in Europe in recent years. In 1996, production was nearly 0.5 million tonnes. Today, the production capacity is much higher. This development has also been supported by the automobile industry. In fact, several million passenger cars and commercial vehicles have been approved by their manufacturers to run on biodiesel fuel or higher biodiesel blends with mineral diesel. Compliance with the European Biodiesel standard DIN EN 14214 ensures a continuously high quality level.

The production of biodiesel is based on transesterification of plant oils or animal fats to fatty acid methyl esters (FAME, Figure 1). At a temperature of approximately 60 °C, the plant oil, consisting mainly of glycerin esters, is esterified using methanol and a catalyst (alkali hydroxide or alkali alcoholate). In order to quantitatively shift the equilibrium reaction towards the biodiesel or FAME side, methanol is added in excess.

After the reaction is finished, the excess methanol together with several by-products must be removed.

The DIN EN 14214 standard specifies all legal limits for possible by-products. For safety-related reasons and in order to comply with requirements of the automobile industry, the legal limit for methanol residue has been defined as 0.2 mass percentages (% m/m). DIN EN 14110 describes the required method for methanol determination.

Figure 4: GC-2014 and HT200H

GC with headspace injection

Biodiesel can consist of up to 100 different fatty acid methyl esters, depending on the plant oils used during production. For the determination of methanol in such a complex matrix, gas chromatography in combination with headspace injection is the recommended method. This enables separation of low-boiling point methanol from the high-boiling point fatty acid methyl esters already during the sample preparation step.

The biodiesel samples are heated in a gas-tight 20 mL vial. Methanol is a low-boiling point compound and is enriched in the gas-phase while the high-boiling point compounds remain in the liquid phase (Figure 2). After a specific time, equilibrium is

reached and a constant volume can be sampled from the gas-phase (headspace) of the vial and injected into the GC system.

Calibration with minimum complexity

Since the biodiesel matrix can influence the equilibrium between the methanol concentrations in the liquid- and gas-phase, calibration standards must be prepared in methanol-free biodiesel. The DIN EN 14110 biodiesel standard recommends preparation of a calibration curve containing 0.01, 0.1 and 0.5 % (m/m) methanol in FAME. An internal standard should always be used when applying manual headspace injection. When using a headspace autosampler, an internal standard will not be necessary but is, however, recommended to increase the reliability of the results. The additional work is kept within reasonable limits: to each standard and each biodiesel sample, 5 µL of 2-propanol is added (Figure 3).

All measurements are carried out on a Shimadzu GC-2014AFsc system in combination with the HT200H headspace autosampler (Figure 4). Both instruments are easy to operate and designed for daily routine analysis.

For the calibration, 5 g of the respective standard solution as well as 5 µL of 2-propanol are added to a 20 mL headspace crimp vial. Corresponding to 5 g of biodiesel sample, 5 µL of 2-propanol is added as internal standard. The HT200H can handle up to 40 sample vials per tray.

Automated sample preparation

Sample preparation is fully automated, whereby each sample is shaken and heated to 60 °C for

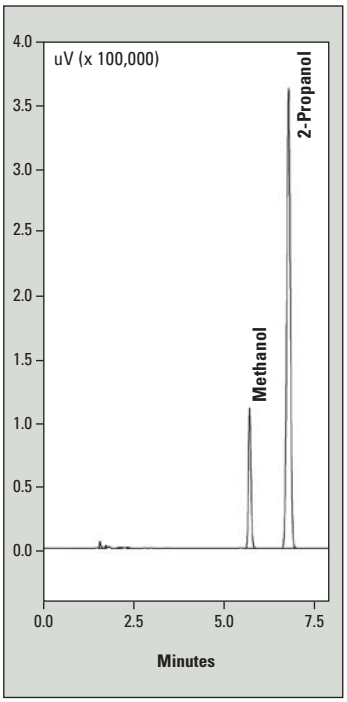


Figure 3: Chromatogram of 0.01 % (m/m) methanol in biodiesel. 2-propanol was added as internal standard.

a given time. The autosampler prepares several samples simultaneously to ensure the highest possible sample throughput and to prevent hold-up at the gas chromatograph. A gas volume of 500 µL is sampled from the headspace of each vial and injected into the GC-2014AFsc system with a split ratio of 1:10.

Additional parameters are recommended according to DIN EN 14110:
Temperature split injector: 150 °C
Column oven (isothermal): 50 °C
Flame ionization detector: 150 °C
Carrier gas (helium) linear velocity: 35 cm/s
Separation column used for the measurements: Restek, Stabilwax-DA 30 m, 0.32 mm, df 1.0 µm

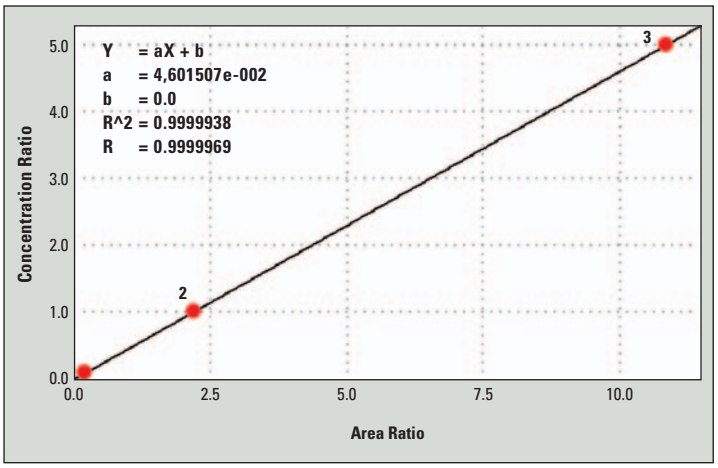


Figure 5: Calibration curve of methanol in biodiesel in accordance with DIN EN 14110 using internal standard calibration

Sample	1	2	3
Average concentration % (m/m)	0.009	0.100	0.500
Reproducibility standard deviation % (m/m)	0.00005	0.00100	0.00485
Reproducibility % RSD	0.54	0.99	0.97

Table 1: Biodiesel sample spiked with three different methanol concentrations. Table 1 shows the reproducibility over 6 measurements of the same sample using the same method on the same instrument.

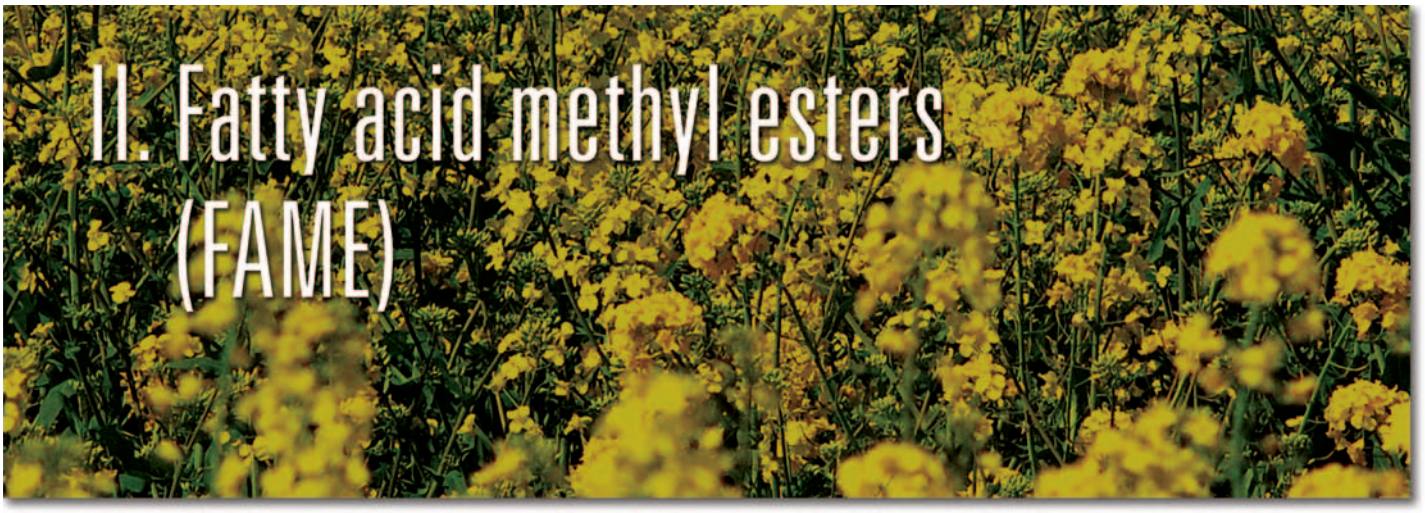
At a methanol concentration of 0.01 % (m/m) a signal-to-noise ratio of higher than 2000:1 was obtained. Thus, the technique offers many options for improvements via application of larger split ratios or faster chromatographic separation.

Results exceeding the standard requirements

The calibration curve in Figure 5 demonstrates that the combination of the HT200H autosampler and the GC-2014 results in high linearity over the concentration range recommended in DIN EN 14110. Reproducibility of the measurement values for biodiesel samples is higher than the requirements according to DIN EN 14110 (Table 1). Biodiesel samples with very low methanol content were spiked with variable amounts of methanol.

Samples with the lowest concentrations showed the highest difference of two out of six measurement values 0.00012 % (m/m). DIN EN 14110 allows for 0.00151 % (m/m), a ten-fold higher deviation.

The combination of the HT200H and GC-2014AFsc offers an efficient as well as accurate automation solution for the routine analysis for methanol determination in biodiesel. Smaller scale biodiesel producers will profit from this robust analytical system with its excellent price/performance ratio.


II. Fatty acid methyl esters
(FAME)

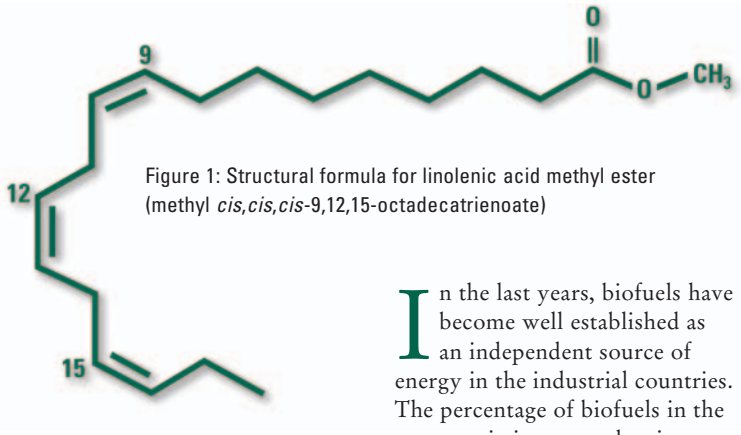


Figure 1: Structural formula for linolenic acid methyl ester (methyl *cis,cis,cis*-9,12,15-octadecatrienoate)

Fatty acid methyl esters (FAME)

Determination of total ester content and linolenic

- **Determination of iodine value**
- **Requirements of DIN EN 14103 exceeded**
- **GC system prepared for future challenges**

In the last years, biofuels have become well established as an independent source of energy in the industrial countries. The percentage of biofuels in the energy mix is expected to increase considerably within the next 10 years in order to reduce dependency on fossil fuels and to maintain energy prices at long-term affordable levels.

Based on the amount of freight transportation, the need for diesel fuels and consequently biodiesel, is significant compared to other biofuels in the European Union. Should the European Parliament agree with the proposal of the European Commission, EU member states will add at least 10 % biofuel to fossil fuels before the year 2020. In Europe, the demand for biodiesel will further increase, leading to a continuous search for suitable plant oils and animal fats that can be used as raw materials. Experience shows that the quality of the raw material has an enormous impact on the quality of biodiesel. Continuous quality control therefore remains necessary in order to meet the demands of modern diesel generators.

According to DIN EN 14103 biodiesel must consist of 90 % (m/m) or more of fatty acid methyl esters within the range of C14:0 up to C24:1. The designation Cx:y refers to the carbon number or C-number (x) of the corresponding fatty acid as well as the number of double bonds (y). When y is larger than 0, it is an unsaturated fatty acid.

Accordingly, the polyunsaturated linolenic acid (Figure 1) is designated as C18:3. The content of linolenic acid methyl ester is also determined. While this compound is a welcome essential acid in salad oils, it is an interfering reactive compound in biodiesel that might influence long-term stability of biodiesel.

Instrumentation and measurement

A Shimadzu GC-2010AF with AOC-20i autosampler (Figure 2) was used for FAME analysis. The GC is equipped with a Split/Splitless injector (SPL) as well as a Flame Ionisation Detector (FID). Control of the GC-2010 and AOC-20i autosampler as well as data acquisition was performed by GCsolution software. Alternatively the AOC-5000 sampler can be used if the system will also be used for quality control according to DIN EN 14410 (headspace injection technique).

Using the special Restek FAME-WAX column it is possible to completely separate almost all fatty acid methyl esters from C6:0 to C24:1. For identification of the individual fatty acid methyl esters the Supelco standard "37 Component FAME Mix" (Cat. No. 47885-U) was used. The standard contains 37 fatty acid methyl esters in the range from C4:0 up to C24:1.

The chromatogram in Figure 3 shows the complete separation except for C4:0 and the oleic acid methyl ester C18:1 *cis/trans* isomers. The chromatogram time can be reduced drastically since a complete separation of all fatty acids methyl esters is not required for the evaluation according to DIN EN 14103. Good separation is required for linolenic acid methyl ester and the internal standard C17:0 which is added to each biodiesel sample. All other fatty acid methyl esters from C14:0 to C24:1 are evaluated as sum area. Therefore, separation of single peaks is not needed.

Problems could occur if animal fats are used as feedstock for biodiesel production. In contrast to plant oils which consist of fatty acids with even chain length numbers, animal fats include also uneven numbers including C17:0. Thus co-elution with the added internal standard amount must be corrected for if necessary.

Another problem might occur with plant oils containing fatty acids with chain lengths higher than C24. The DIN EN 14103-

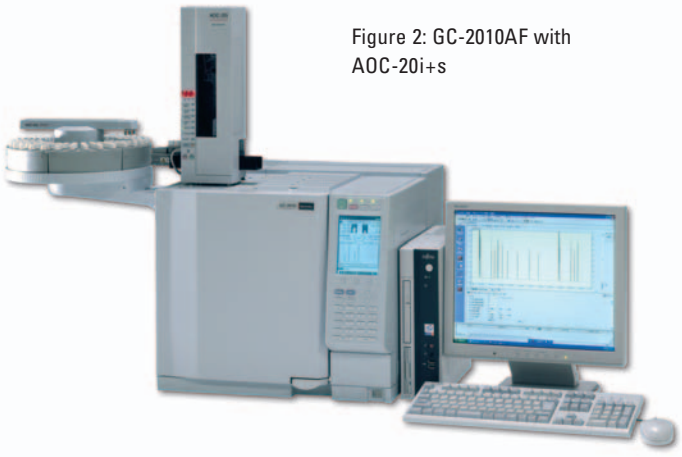


Figure 2: GC-2010AF with AOC-20i+s

acid methyl ester contents

2003 determines that all peaks within the chromatogram range from C14:0 to C24:1 are evaluated for fatty acid methyl ester content C but peaks outside this range must not be included in the evaluation (for calculation of C see equation on the right). Thus biodiesel produced from plant oils containing a considerable amount of fatty acids C25:0 or higher might fail in quality control according to DIN EN 14103.

Gas chromatographic method

GC:	GC-2010AF
Autosampler:	AOC-20i
Injection vol.:	1 µL
Column:	Restek FAMEWAX 30 m, ID 0.25 mm, df 0.1 µm (Cat. No. 12497)
Carrier gas:	Helium
Control mode:	Constant linear velocity
Injection mode:	Split 1:50
Linear velocity:	35 cm/s (constant)
Septum purge:	3 mL/min
Injector temp.:	250 °C

Oven temperature program FID settings:

Rate (°C/min)	Temp. (°C)	Hold time (min)
	150.0	1.00
5.00	240.0	6.00

Equilibrium time: 1.0 min

Temperature:	250 °C
H_2 flow:	40 mL/min
Air flow:	400 mL/min
Makeup flow:	30 mL/min (He)
Sampling rate:	40 ms
Filter time const.:	200 ms

Figure 4 on Page 12 shows the measurement of a biodiesel sample using the method described. For evaluation, the sum of all signal areas between C14:0 and C24:1 is calculated, taking also into account the areas of unidentified signals. Using the following equation, the fatty acid methyl ester content C in % (m/m) can be calculated according to DIN EN 14103 as:

$$C = \frac{(\sum A) - A_{ISTD}}{A_{ISTD}} x \frac{C_{ISTD} x V_{ISTD}}{m} x\ 100\ \%$$

∑A	total signal area of all methyl esters from C14:0 up to C24:1 (15734000 µV*s)
A_{ISTD}	signal area of the internal standard heptadecanoic acid methyl ester C17:0 (2644200 µV*s)
C_{ISTD}	concentration of the heptadecanoic acid methyl ester in the internal standard solution used (10 mg/mL)
V_{ISTD}	volume of the internal standard solution added (5 mL)
m	mass of the weighed biodiesel sample (250 mg)

For the biodiesel sample analyzed (Figure 4), a fatty acid methyl ester content of 99.0 % was determined. A fatty acid methyl ester content of 90 % or higher is required. The linolenic acid methyl ester content L is calculated using the following equation as:

$$L = \frac{A_L}{(\sum A) - A_{ISTD}} x\ 100\ \%$$

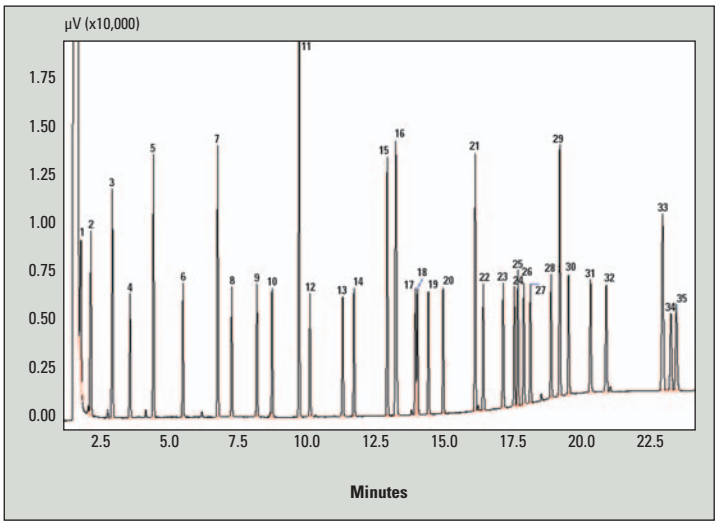


Figure 3: Supelco standard "37 Component FAME Mix" separated on a Restek FAMEWAX column. Not separated are the *cis*/*trans* isomers C18:1 (16). C4:0 not separated from the solvent and the signals (1)-(6) representing the fatty acid methyl esters C6:0 up to C13:0 are not required for the quality control according to DIN EN 14103. It was measured with the described gas chromatographic method except for the split ratio was 1:20. The naming of the individual components is given in Table 3 on Page 13.

A_L	signal area of linoleic acid methyl ester C18:3 (991200 µV*s)
∑A	total signal area of all methyl esters from C14:0 to C24:1 (15734000 µV*s)
A_{ISTD}	signal area of the internal standard heptadecanoic acid methyl ester C17:0 (2644200 µV*s)

The result is 7.6 % linolenic acid which is well within the acceptable range of 1 % up to 15 % (m/m).

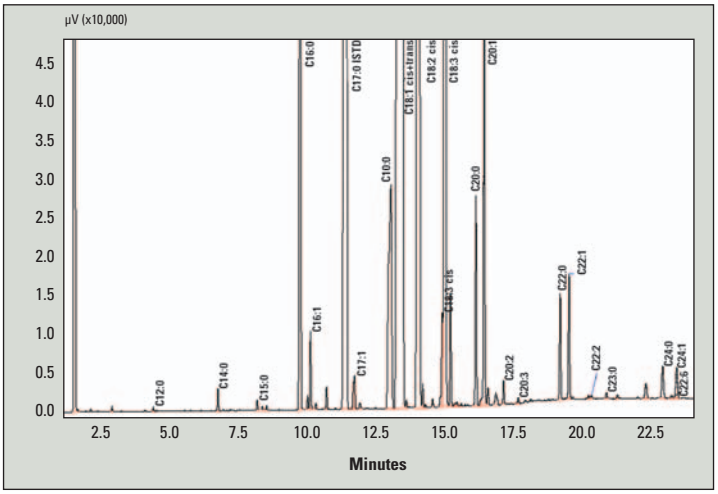


Figure 4: Biodiesel sample measured with the gas chromatographic method described. For detailed information on the compounds see Table 3.

According to DIN EN 14214 Annex B the iodine value can also be calculated from the methyl ester pattern obtained. However, in case of a doubt DIN EN 14111 is the preferred method. For calculation according to DIN EN 14214 Annex B a good separation of the compounds in Table 3 marked with asterisk is required. For these compounds a factor to calculate the iodine value is given.

The percentage by mass obtained from the chromatogram is then used to calculate the iodine value of the sample, being the sum of the individual contributions of each methyl ester, obtained by multiplying the methyl ester percentage by its respective factor.

For the biodiesel sample in Figure 4 an iodine value of 109 g iodine/100 g was determined (Table 1, the limiting value is 120 g iodine/100 g).

Compound	% (mm)	Factor iodine value	g iod / 100 g
C16:1	0.20	0.95	0.2
C18:1	64.1	0.86	55.1
C18:2	18.7	1.732	32.4
C18:3	7.55	2.616	19.8
C20:1	1.32	0.785	1.0
C22:1	0.40	0.723	0.3
Calculated iodine value:			**108.8**

Table 1: Calculation of the iodine value for the sample analyzed (Figure 4). The factors for the respective unsaturated fatty acid methyl esters are taken from DIN EN 14214 Annex B. The mass percentages are calculated from the chromatogram in Figure 4.

When the same biodiesel sample is measured repeatedly with the same instrument, DIN EN 14103 allows a maximum difference in methyl ester content of 1.6 % (m/m) for two arbitrary results. Using the GC-2010 in combination with the AOC-20i autosampler, 24 measurements resulted in a range over all results of 0.2 % (m/m) – the difference between the largest and the smallest measured value was therefore 8 times less than the required value in the norm.

For the determination of linolenic acid, a similar result is obtained. Over 24 measurements the range over all results was only 0.01 % (m/m).

The norm allows for a value ten times higher i.e. 0.1 % (m/m). All statistical results of the performed measurement sequence are summarized in Table 2.

Measuring value	Methyl ester content	Linoleic acid content
Mean value % (m/m)	98.9	7.55
Standard deviation % (m/m)	0.06	0.06
Maximum value % (m/m)	99.0	7.56
Minimum value % (m/m)	98.8	7.55
Difference maximum/minimum value % (m/m)	0.2	0.01

Table 2: Reproducibility over 24 measurements of one biodiesel sample. The table shows the statistical data including the relative standard deviation and the range of all results (max.-min. value).

Summary

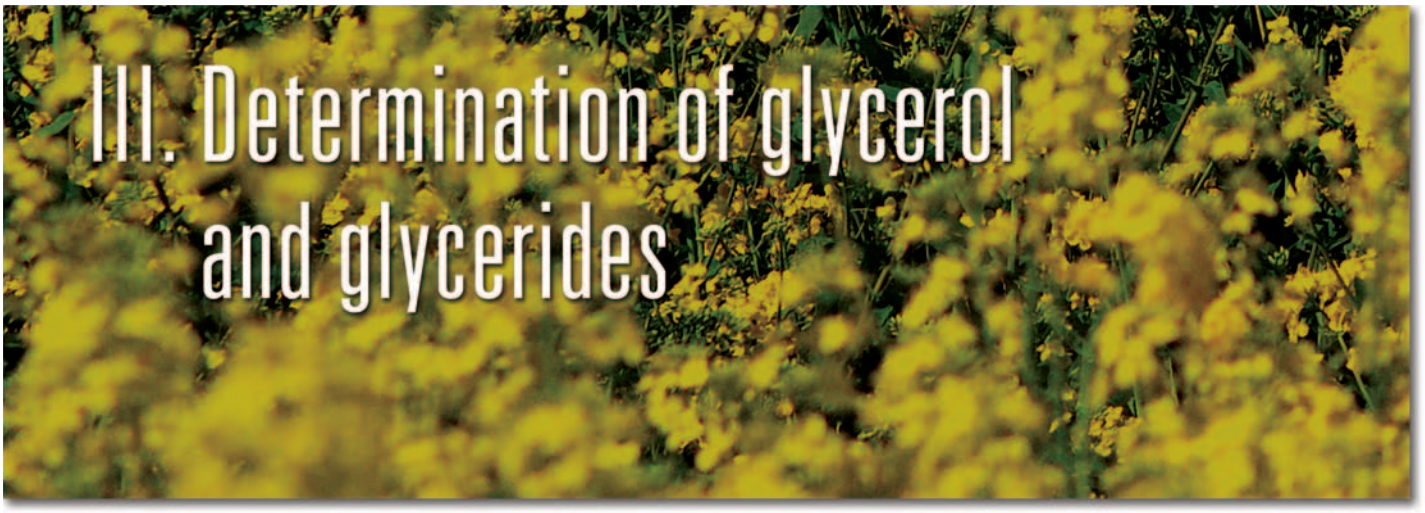
The price of biodiesel depends very much on the availability of cheap feedstock for the production process. This forces the producers to also use used oils or animal fats instead of fresh plant oil. Keeping up with this process will be the future challenge of quality control for biodiesel. Especially the importance of the quality control according to DIN EN 14103 will grow with this development.

The performance of GC-2010 in combination with AOC-20i – or alternatively with AOC-5000 – not only meets today's requirements but is also prepared for future challenges. The system is able to separate almost the whole composition of fatty acids, including those with odd carbon numbers from animal fat, in a reasonable chromatogram time. This is the requirement for the correct determination of the iodine value.

The precision of the system was shown to exceed the requirements in the DIN EN 14103 by a factor of ten.

Peak No.	Compound	Alternative naming	Saturation	Molecular formula	CAS No.
01	Methyl hexanoate	Methyl caproate, Hexanoic acid methyl ester, Methyl capronate	C6:0	$C_7H_{14}O_2$	106-70-7
02	Methyl octanoate	Octanoic acid methyl ester, Caprylic acid methyl ester	C8:0	$C_9H_{18}O_2$	111-11-5
03	Methyl decanoate	Decanoic acid methyl ester, Capric acid methyl ester, Caprate	C10:0	$C_{11}H_{22}O_2$	110-42-9
04	Methyl undecanoate	Undecanoic acid methyl ester	C11:0	$C_{12}H_{24}O_2$	1731-86-8
05	Methyl dodecanoate	Dodecanoic acid methyl ester, Lauric acid methyl ester, Methyl laurate	C12:0	$C_{13}H_{26}O_2$	111-82-0
06	Metyl tridecanoate	Tridecanoic acid methyl ester	C13:0	$C_{14}H_{28}O_2$	1731-88-0
07	Methyl tetradecanoate	Methyl myristate, Tetradecanoic acid methyl ester, Myristic acid methyl ester	C14:0	$C_{15}H_{30}O_2$	124-10-7
08	Myristoleic acid methyl ester	9-tetradecenoic acid, methyl ester, Methyl *cis*-9-tetradecenoate, Methyl myristoleate	C14:1	$C_{15}H_{28}O_2$	56219-06-8
09	Methyl pentadecanoate	Pentadecanoic acid methyl ester	C15:0	$C_{16}H_{32}O_2$	7162-64-1
10	Methyl *cis*-10-pentadecenoate	*cis*-10-Pentadecenoic acid methyl ester	C15:1	$C_{16}H_{30}O_2$	90176-52-6
11	Methyl palmitate	Hexadecanoic acid, Methyl ester	C16:0	$C_{17}H_{34}O_2$	112-39-0
12	Methyl palmitoleate*	Methyl *cis*-9-Hexadecenoate, Palmitoleic acid methyl ester	C16:1	$C_{17}H_{34}O_2$	1120-25-8
13	methyl heptadecanoate	Heptadecanoic acid methyl ester	C17:0	$C_{18}H_{36}O_2$	1731-92-6
14	*cis*-10-heptadecenoic acid methyl ester	Methyl *cis*-10-heptadecenoate	C17:1	$C_{18}H_{34}O_2$	75190-82-8
15	Methyl stearate	Methyl octadecanoate	C18:0	$C_{19}H_{38}O_2$	112-61-8
16	Methyl oleate*	Methyl *cis*-9-oleic methyl ester, Methyl *cis*-9-octadecanoate (Z)-9-Octadecanoic acid methyl ester	C18:1 *cis*	$C_{19}H_{36}O_2$	112-62-9
17	*trans*-9-elaidic methyl ester*	(E)-Methyl 9-octadecenoate	C18:1 *trans*	$C_{19}H_{36}O_2$	2462-84-2
18	Methyl linoleate*	Linolenic acid methyl ester; Methyl linoleate, *cis,cis*-Octadeca-9,12-dienoic acid methyl ester	C18:2	$C_{19}H_{34}O_2$	112-63-0
19	Linolelaidic acid methyl ester*	*trans, trans*-octadeca-9,12-dienoic acid methyl ester	C18:2	$C_{19}H_{34}O_2$	2566-97-4
20	γ-linolenic acid methyl ester*	Methyl (Z,Z,Z)-6,9,12-octadecatrienoate, 6,9,12-Octadecatrienoic acid methyl ester	C18:3	$C_{19}H_{32}O_2$	16326-32-2
21	Methyl linolenate*	Linolenic acid methyl ester, Methyl *cis,cis,cis*-9,12,15-octadecatrienoate	C18:3	$C_{19}H_{32}O_2$	301-00-8
22	Methyl arachidate	Eicosanoic acid methyl ester	C20:0	$C_{21}H_{42}O_2$	1120-28-1
23	Methyl eicosenoate*	Methyl *cis*-11-eicosenoate, *cis*-11-Eicosenoic acid methyl ester	C20:1	$C_{21}H_{40}O_2$	2390-09-2
24	*cis*-11,14-Eicosadienoic acid methyl ester		C20:2	$C_{21}H_{38}O_2$	2463-07-7
25	*cis*-11,14,17-Ecosatrienoic acid methyl ester		C20:3	$C_{21}H_{36}O_2$	55682-88-7
26	*cis*-8,11,14-Eicosatrienoic acid methyl ester	Dihomo-γ-linolenic acid methyl ester, Methyl-DGLA	C20:3	$C_{21}H_{36}O_2$	21061-10-9
27	Methyl arachidonate	Arachidonic acid methyl ester, Methyl *cis*-5,8,11,14-eicosatetraenoate	C20:4	$C_{21}H_{34}O_2$	2566-89-4
28	Methyl 5,8,11,14,17-eicosapentaenoate	*cis*-5,8,11,14,17-Eicosapentaenoic acid methyl ester	C20:5	$C_{21}H_{32}O_2$	2734-47-6
29	Methyl heneicosanoate	Heneicosanoic acid methyl ester	C21:0	$C_{22}H_{44}O_2$	6064-90-0
30	Methyl behenate	Docosanoic acid methyl ester	C22:0	$C_{23}H_{46}O_2$	929-77-1
31	Methyl erucate*	*cis*-13-Docosen, Methyl *cis*-13-docosenoate; Erucic acid methyl ester	C22:1	$C_{23}H_{44}O_2$	1120-34-9
32	*cis*-13,16-Docosadienoic acid methyl ester		C22:2	$C_{23}H_{42}O_2$	61012-47-3
33	Methyl tricosanoate	Tricosanoic acid methyl ester	C23:0	$C_{24}H_{48}O_2$	2433-97-8
34	Methyl lignocerate	Methyl tetracosanoate, Lignoceric acid methyl ester	C24:0	$C_{25}H_{50}O_2$	2442-49-1
35	*cis*-4,7,10,13,16,19-Docosahexaenoic acid methyl	Methyl *cis*-4,7,10,13,16,19-docosahexenoate, DHA methyl ester	C22:6	$C_{23}H_{34}O_2$	301-01-9
36	Methyl nervonate	Nervonic acid methyl ester; Selacholeic acid methyl ester, Methyl *cis*-15-tetracosenoate	C24:1	$C_{25}H_{48}O_2$	2733-88-2

Table 3: Components in the chromatograms in Figure 3 and 4. For the substances marked with an asterisk, the iodine value can be calculated with the given factor.


III. Determination of glycerol
and glycerides

Introduction and sample preparation

Property fatty acid in %	Rape seed	Sun flower	Palm tallow	Beef
Palmitic	5	6	42	28
Stearic	1	4	5	19
Oleic	60	28	41	45
Linoleic/Linolenic	30	61	10	5

Table 1: Approximate content of fatty acids in different oils and fats

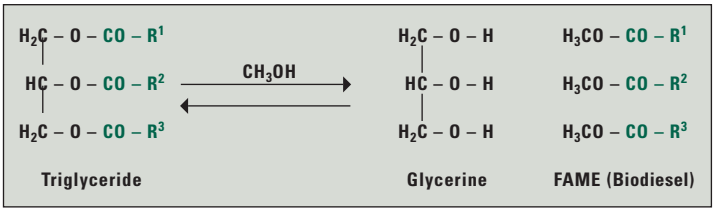


Figure 1: Transterification of triglycerols to FAME

Biodiesel is an interesting alternative to the decreasing resources of mineral fuels. It can be manufactured from all kinds of plant oils or even fats from fryers.

Because of the high viscosity direct use of those regenerative energy resources in diesel cars requires expensive modifications to engine and tank. Therefore, the transesterification (Figure 1) of the glycerol esters to fatty acid methyl esters (FAME) is preferred. FAMEs are less viscous and remain fluid even at low temperatures. They can be used in many diesel cars instead of mineral diesel without changes to the car. Presently the biggest market for biodiesel is the blending with mineral fuels (e.g. B5; up to 5 % biodiesel in mineral diesel).

Biodiesel mainly consists of methyl esters of the following fatty acids:

Palmitic acid	C_{16} (saturated)
Stearic acid	C_{18} (saturated)
Oleic acid	C_{18} (unsaturated)
Linoleic acid	C_{18} (poly unsaturated)

The distribution changes depending on the plant oil used (Table 1). In principle, all fatty acids with chain lengths from C_{14} to C_{24} may be found (in case of plant oils only the even numbers).

FAMEs are the products of the reactions in Figure 1.

Dependent on process parameters the transesterification is more or less quantitative. Generally, a certain amount of mono-, di-, and triglycerides remains in the biodiesel. Additionally, the surplus of methanol and the by-product glycerine must be removed during the biodiesel production. In quality control according to DIN EN 14105 or ASTM D 6584-00 the contents of the glycerine, mono-, di-, and triglycerides are determined. The methanol content is measured with headspace technique described in DIN EN 14110.

- **Control of transesterification process**
- **Simplified standard preparation**
- **Improved reproducibility**

Sample preparation

Gas chromatography is a relative technique. Every component must be calibrated before it can be quantified in unknown samples. In the case of biodiesel one mono-, di-, and triglyceride is calibrated, representative for ⏵

No.	Compound	Alternative naming	CAS No.	Comments
1	MSTFA	N-Methyl-N-(trimethylsilyl)-trifluoracetamide	24589-78-4	Derivatization of OH groups
2	Pyridine	Azine	110-86-1	Buffer
3	Heptane		142-82-5	Solvent
4	1,2,4-Butanetriol		3068-00-6	Internal Standard 1
5	1,2,3-Tridecanolyglycerol	Tricaprin	621-71-6	Internal Standard 2
6	Glycerin		56-81-5	
7	1-Monooleoglycerine (Monoolein)	1-Mono[*cis*-9-octadecenoyl]-rac-glycerol (monoolein)	111-03-5	Keep refrigerated at -20 °C
8	1,3-Dioleoylglycerine (Diglyceride)	1,3-Di[*cis*-9-octadecenoyl]glycerol (diolein)	2465-32-9	Keep refrigerated at -20 °C
9	1,2,3-Trioleoylglycerine (Triglyceride)	1,2,3-Tri[*cis*-9-octadecenoyl]glycerol (triolein)	122-32-7	Storage below 8 °C

Table 2: List of components required for biodiesel applications. Possible supplier: Sigma-Aldrich (Fluka).
Due to derivatization with MSTFA all components must be water-free.

Calibration level	1	2	3	4
Volume of stock solution A given into a 10 mL flask	100 µL	100 µL	100 µL	100 µL
Add the given volume of stock solution C into the same flask	10 µL	40 µL	70 µL	100 µL

Table 3: Preparation of the four standard solutions

Calibration level conc. in mass %	Glycerin	Monoolein	Diolein	Triolein
1	0.005	0.2	0.05	0.05
2	0.02	0.8	0.2	0.2
3	0.035	1.4	0.35	0.35
4	0.05	2.0	0.5	0.5

Table 4: Compound concentrations in the four calibration levels (given in mass %)

the other components of each group. Table 2 gives an overview of chemicals needed for preparation of standard and unknown samples. Required is a four level calibration of the components 6 to 9. Glycerin (6) is calibrated relative to the internal standard 1 (ISTD1). The glycerides (7-9) are calibrated relative to internal standard 2 (ISTD2).

Preparation of standard solutions

In deviation from DIN EN 14105, we recommend the following standard preparation. Three stock solutions A, B and C are prepared. Later, only the stock solutions A and C are needed for preparation of standard levels and unknown samples.

Stock solution A: Internal standard

50 mg 1,2,4-butanetriol and 400 mg tricaprin are given into a 50 mL flask. Pyridine is added up to the 50 mL marking.

Stock solution B: Glycerin dilution

50 mg glycerin are given into a 10 mL flask. Pyridine is added up to the 10 mL marking.

Stock solution C: Olein standard solution with glycerin

200 mg monoolein, 50 mg diolein and 50 mg triolein are given into a 10 mL flask. 1 mL of the stock solution B is added. Pyridine is added up to the 10 mL marking.

The three stock solutions can be kept in a refrigerator for weeks. For preparation of the standard levels add the given volumes of stock solution A and C (Table 3) into 10 mL flasks.

Derivatization of the calibration mixtures

Derivatization of the samples with MSTFA is crucial for improvement of peak shape and chromatographic separation.

100 µL MSTFA are added to each of the four flasks prepared according to Table 3. Close the flasks, tighten and shake them well. At room temperature the derivatization requires a minimum of 15 minutes. After that time add 8 mL of n-heptane to each flask and shake again. The sample is now ready for measurement.

Table 4 shows the concentrations in mass-% for the given components in the respective calibration levels.

Preparation and derivatization of unknown biodiesel samples

100 mg biodiesel is given into a 10 mL flask. 100 µL of stock solution A (internal standard) are added to the flask. Then add 100 µL of MSTFA, close the flask, tighten, and shake it well. The derivatization takes at least 15 minutes at room temperature. After that time add 8 mL of n-heptane to each flask and shake again.

Important

Derivatized standards or samples must be measured within 24 hours.

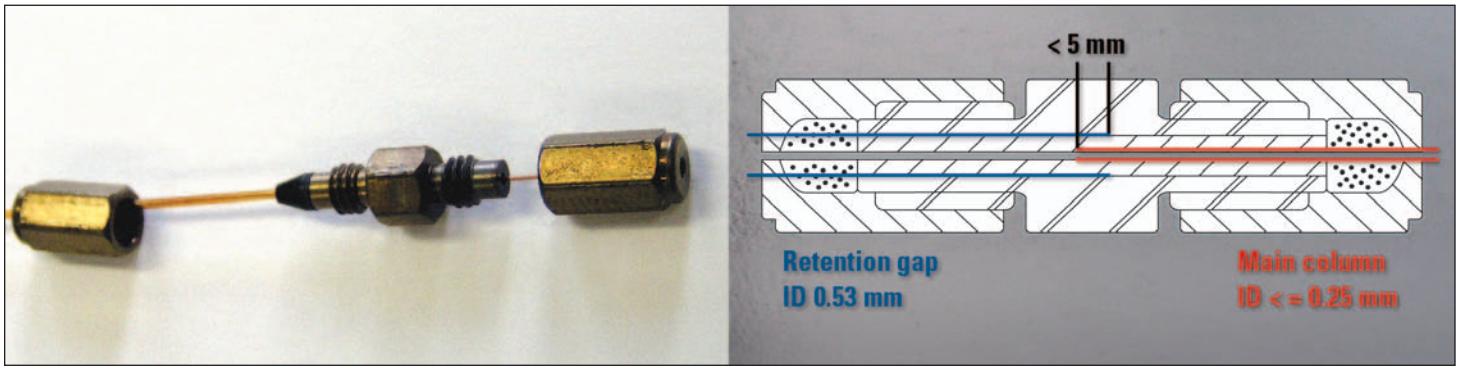


Figure 1: Mounting of retention gap kit with SGE metal connector

Instrument configuration

With this application the internal standard method is used for quantification so an auto sampler is not required, but recommended for better reproducibility of retention times.

The injection technique is "cool on-column" injection (OCI) to avoid discrimination effects. With OCI injection a thin needle of 26 gauge – outer diameter (OD) 0.47 mm – is inserted into the column and the sample is injected completely on the column. For accommodation of the syringe needle, the capillary column must have a minimum internal diameter (ID) of 0.53 mm. In principle, a 0.53 mm column can be used as main column for this application, but for better separation and faster chromatogram times a smaller ID is preferred.

Therefore, a retention gap – usually a 2 m uncoated fused silica capillary with an ID of 0.53 mm – is used as pre-column. Connection of the retention gap with the main column can be done with a metal connector (Figure 1). The preferred glass connectors cannot be used with this application due to the high oven temperatures – most glass connectors have a maximum temperature of 350 °C, an exception being the Restek Vu2 connector with a maximum operation temperature of 400 °C.

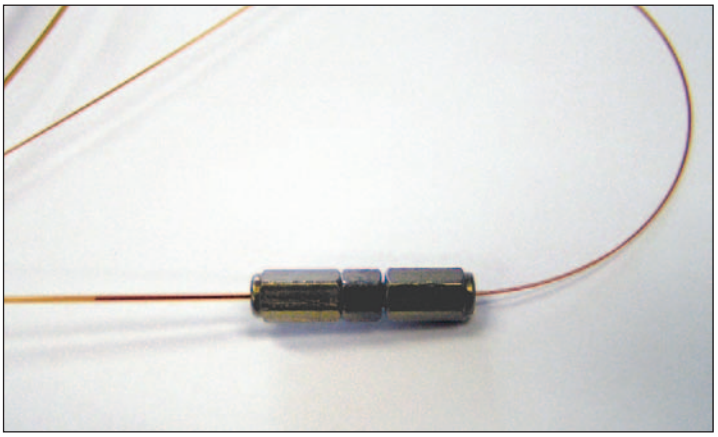
Another alternative is the "simple on-column" injection technique offered by Shimadzu. Instead of retention gap a special glass liner is used. Design of the liner is similar to a glass connector (see Figure 2). The injection is done into a small liner compartment directly above the column.

Column mounting with retention gap

If a retention gap is used the connection to the main column is often done with a metal connector (e.g. SGE retention gap kit art. no. 052296).

For best performance both columns should meet in the middle of the connector. Since columns with ID of 0.25 mm or smaller fit inside the ID 0.53 mm retention gap, the optimum position is not easy to find. A compromise could be pushing the main column up to 5 mm inside the retention gap (Figure 1). More than 5 mm would lead to peak tailing and poor results.

For accuracy and precision of the results it is essential that the connection of the retention gap to the main column is gas tight.



Column and retention gap connection with metal connector

- **"Simple on-column" injection technique**
- **Significantly simplified column mounting**
- **More precise results through glass liner**
- **Improved reliability**

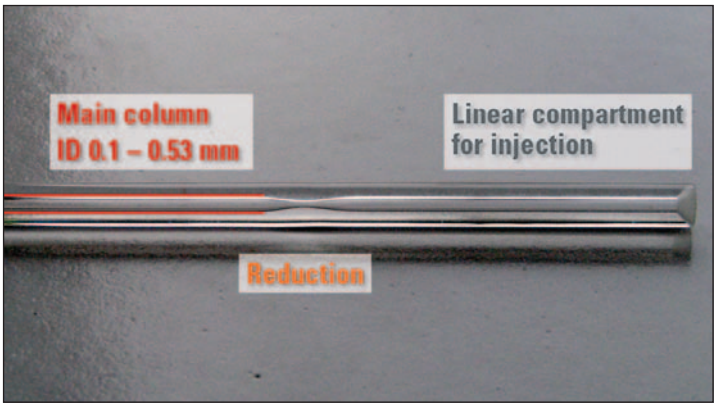


Figure 2: Mounting of column with simple on-column liner

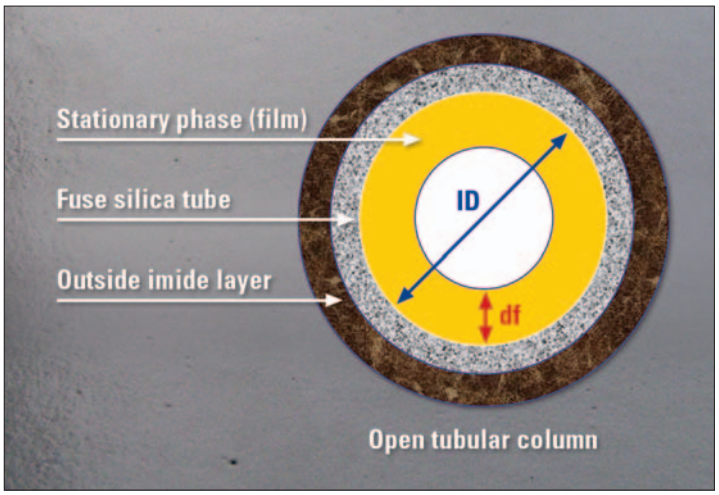


Figure 3: Cross-section through a capillary column

Due to the high oven temperature range the metal connector may eventually leak during operation. Therefore, it needs to be checked regularly. Because of this uncertainty it is not recommended to use metal connectors in combination with hydrogen as carrier gas.

Column mounting with liner

An alternative offers the "simple on-column" injection technique offered by Shimadzu. The task of the retention gap is taken by a glass liner inside the injector. Any column ID from 0.1 to 0.53 mm can be directly connected to the liner. The column sticks inside the liner reduction and is additionally tightened with a graphite ferrule on the injector capillary adapter.

This simplifies the column mounting significantly, and gas tightness even with hydrogen as carrier gas poses no problem anymore. Two different liners are available from Shimadzu depending on the column inner diameter.

- 221-49381-02 for column ID ≥ 0.32 mm
- 980-00371 for column ID ≤ 0.25 mm

The injection is performed in a small liner compartment above the column so a special on-column syringe is not required. A normal 23 gauge needle can be used.

Measuring the glycerol content in biodiesel, more precise results were found using the liner compared to retention gap solution.

Disadvantage of the liner is the lower capacity for contamination coming from biodiesel samples. Retention gaps are good traps for high-molecular components in biodiesel samples. If the first 20 - 30 cm of the retention gap are cut from time to time the main column is less affected by contaminants.

With the liner the main column is more affected by these contaminants and it might be necessary to cut 20 - 30 cm from the column regularly. However, the liner can be recycled. Best is to clean it in an ultrasonic bath using first heptane and then methanol as solvents. It can be dried using a nitrogen gas stream or alternatively, by heating the liner in an oven. It is important that it does not come into contact with contaminated surfaces. Deactivation of the glass liner is not necessary.

Capillary column

For good performance with this application the GC oven is operated at temperatures of 360 °C or higher. Unfortunately, most metal columns or aluminium coated columns show severe problems with recovery of di- and triglycerides. Generally with fused silica high-temperature columns, the recoveries are better.

Often used is the HT5 capillary column from SGE. It's a fused silica column with temperature limits 380/400 °C – meaning the column can be operated at 380 °C for longer times, at 400 °C only short term. Also possible is the HT8 phase from SGE but due to temperature limit 360/370 °C the chromatogram time is extended.

An alternative to the SGE columns could be the new Restek high-temperature columns. Restek offers an RTX-Biodiesel TG with a temperature limit of 380 °C (Cat. No. 10294). Upon request they also offer Shimadzu a Fast GC column of the same

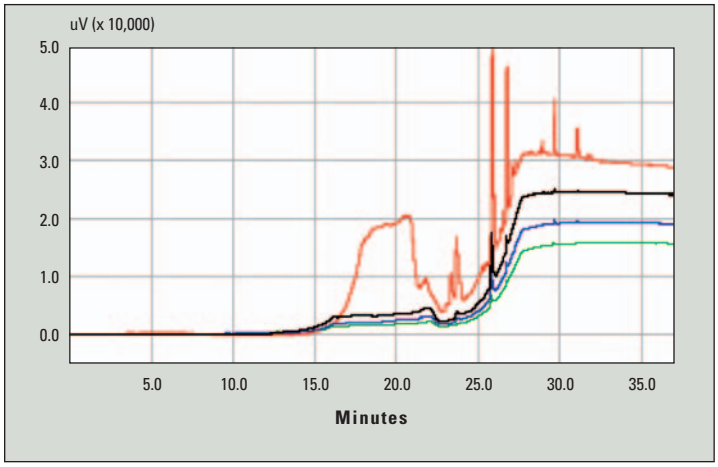


Figure 4: Column conditioning. Between red and green line the column was cycled about 20 times.

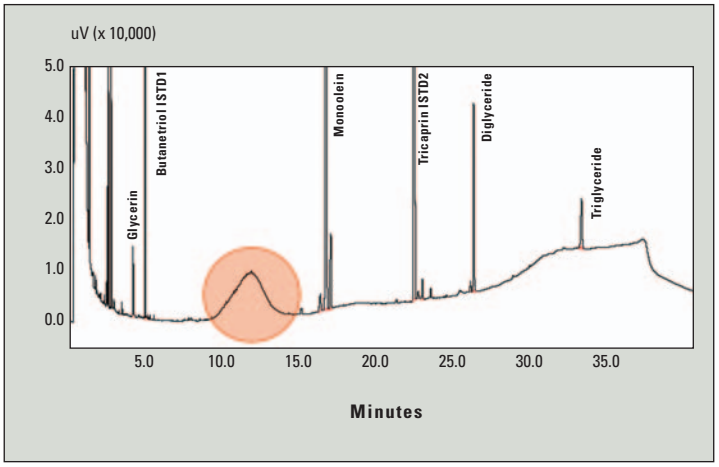


Figure 5: Baseline drift or "ghost peak" due to liner contamination

type, which reduces the analysis time to below 17 min. Restek also offers metal columns for biodiesel analysis e.g. MXT Biodiesel TG (Cat. No. 70289). Due to a special deactivation process the metal columns show a good recovery for all biodiesel components including the triglycerides. The MTX column is a mega-bore column with an Integra-Gap, trapping contaminants before they enter the separation column.

Conditioning of the system

Before starting with measurements the conditioning of the system is essential. A new column should be conditioned for 12 hours using the temperature program for injector and detector. A metal connector should be checked for leakage after the first two conditioning runs.

Figure 4 shows a typical trend of a baseline over more than 20 blank runs.

A temperature program was used heating with 10 °C/min from 65 °C up to 380 °C. At the beginning a baseline drift of about 30 mV or more was observed. After 12 hours it was reduced by 10 to 20 mV. Figure 4 shows some conditioning steps with a 0.1 µm film column. Of course, a 0.25 µm film column might show a more severe baseline increase. A lower baseline drift of about 5 - 15 mV was observed with the Restek Rtx-5 high-temperature column.

Ghost peaks

Even if the conditioning chromatogram looks good it is recommended to inject pure heptane once before measuring biodiesel samples. Sometimes a baseline effect might occur as shown in Figure 4.

Most probably the liner was contaminated by contact with surfaces covered by a fatty film (for example the human skin). If the effect occurs during operation of the system the reason may be the samples themselves. For example the biodiesel additives, if biodiesel from a gasoline station was measured, overaged MSTFA which was used for derivatization, or the chemicals and solvents used, which were not water-free.

Normally, it is not possible to eliminate these "ghost peaks" by several injections of heptane. A new or cleaned liner has to be installed and 10 cm should be cut from the capillary column.

Optimization of the gas chromatographic method

- **Optimization of the method according to columns used**
- **A good separation is crucial**

After leak check and conditioning of the instrument a first sample can be injected. DIN EN 14105 recommends a non-polar column with length of 10 m, ID (inner diameter) 0.32 mm, film thickness 0.1 µm.

This is a good column to start with, but most operators prefer a longer column (12 to 25 m). With smaller ID a better resolution can be achieved even with a shorter column (e.g. 12 m HT5, ID 0.22 mm, 0.1 µm). In general, a smaller ID allows a faster chromatography, but optimizing the gas chromatographic method might be more time-consuming.

Gas chromatographic method

GC: GC-2010AF with OCI
Autosampler: AOC-20i
Column: HT5, 25 m, ID 0.32 mm, df 0.1 µm with retention gap ID 0.53 mm
Carrier gas: Helium
Injection mode: Direct
Linear velocity: 50 cm/s (constant)
Septum purge: 3 mL/min

OCI temperature program

Rate (°C/min)	Temperature (°C)	Hold time (min)
	60.0	1.00
20.00	380.0	3.00

Table 1: OCI advanced settings fan: 100 °C

FID settings

Temperature: 400 °C
H_2 flow: 47 mL/min
Air flow: 400 mL/min
Make-up gas flow: 30 mL/min (He)
Sampling rate: 40 ms
Filter time constant: 200 ms

Oven temperature program

Rate (°C/min)	Temperature (°C)	Hold time (min)
	65.0	1.00
15.00	170.0	0.00
8.00	270.0	0.00
15.00	390.0	9.00

Table 2: Equilibrium time: 0.5 min

Autosampler

Heptane is used as solvent for biodiesel samples. The lifetime of micro-syringes with biodiesel can be extended if the syringe is rinsed regularly with a polar solvent (e.g. methanol) besides heptane. With AOC-20i+s this can be automated by using the three vial wash option. If no AOC-20s is used optional three wash stations can be inserted into the AOC-20i long rack. The wash cycle could be 1 x heptane, 1 x methanol and 1 x heptane again.

AOC-20i settings

Injection volume: 1 µL
 # Rinses solvent post-run: 2
or alternatively:
 # Rinses solvent post-run: 3
(if three vial wash option is used)
 # Rinses with sample: 2
Plunger speed (suction)**:** Low
Plunger speed (injection)**:** High
Syringe insertion speed: High
Injection mode: Normal
Pumping times: 5

The next step is the optimization of the method according to the column used. The first step is injection of the standard solution 4 (highest concentration).

The standard contains one representative component for each group of glycerides.

Butanetriol and tricaprin are added as internal standards. Even when refrigerated, the stock solutions for the standards alter slowly. This is the reason for the additional peaks in the chromatogram (Figure 1).

Since the glycerin concentration counts significantly in the final evaluation, a good separation from the solvent peak is crucial (Figure 1: more than 1 minute between solvent peak and glycerin).

Typically the baseline drift becomes steeper if the oven temperature rises above 340 °C (Figure 1: chromatogram time 23 - 26

Used naming	Alternative naming
Glycerin	
Butanetriol (ISTD1)	
Monoolein	1-Mono[*cis*-9-octadecenoyl]-rac-glycerol (monoolein)
Tricaprin (ISTD2)	1,2,3-Tridecanolyglycerol
Diglyceride	1,3-Di[*cis*-9-octadecenoyl]glycerol (diolein)
Triglyceride	1,2,3-Tri[*cis*-9-octadecenoyl]glycerol (triolein)

Table 3: Naming of biodiesel components

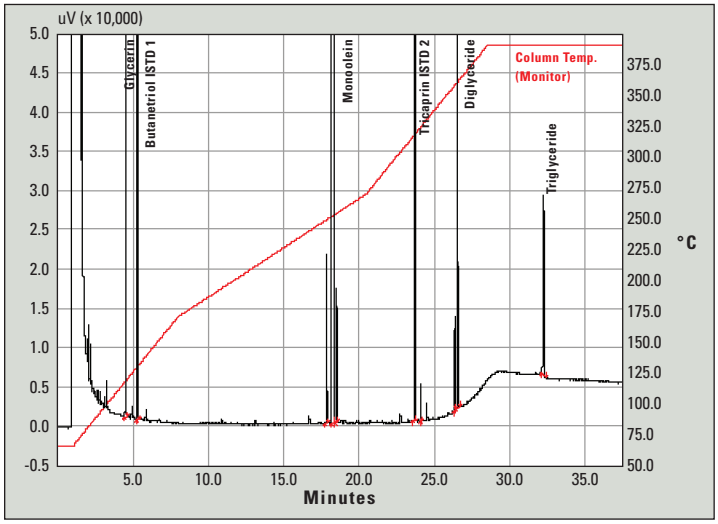


Figure 1: Chromatogram standard solution 4

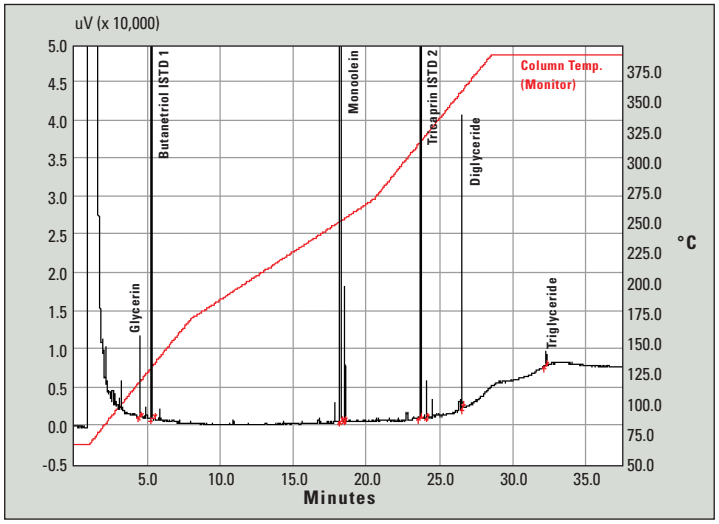


Figure 2: Chromatogram standard solution 1

min). Fortunately, the drift is not too large here. Therefore, it is acceptable that the diglycerides elute within this drift. A bigger drift could affect the integration accuracy of the whole group of diglycerides eluting from 23 - 24 minutes. In such cases an increase of the linear velocity (up to 70 cm/s) or changing of the oven temperature might help.

The oven temperature program can be slowed down by changing the end temperature (Table 4, blue marked temperature).

Alternatively, the temperature rate in line 4 can be decreased (red marked value in Table 4). A combination of both is also possible. In any case the hold time X in line 4 has to be adjusted so that the chromatogram time does not become too long but the triglyceride signals are still recorded.

Extending the temperature program has a disadvantage: the triglycerides remain too long on the column. The peak might disappear completely or become very small.

After optimization of the temperature program the standard solution 1 (lowest concentration) is injected to check the detection limits.

In the high-temperature range the FID noise is 40 µV. Peak height of the triglyceride peak is 2002 µV. Thus, the signal-to-noise ratio is 50. This is acceptable for a 0.32 mm column, but could be improved by accelerating the chromatography for example using a higher linear velocity.

Rate (°C/min)	Temperature (°C)	Hold time (min)
	65.0	1.00
15.00	170.0	0.00
8.00	**300.0**	0.00
10.00	390.0	**X**

Table 4: Variation possibilities of the oven temperature program for optimization of retention times

Signal identification

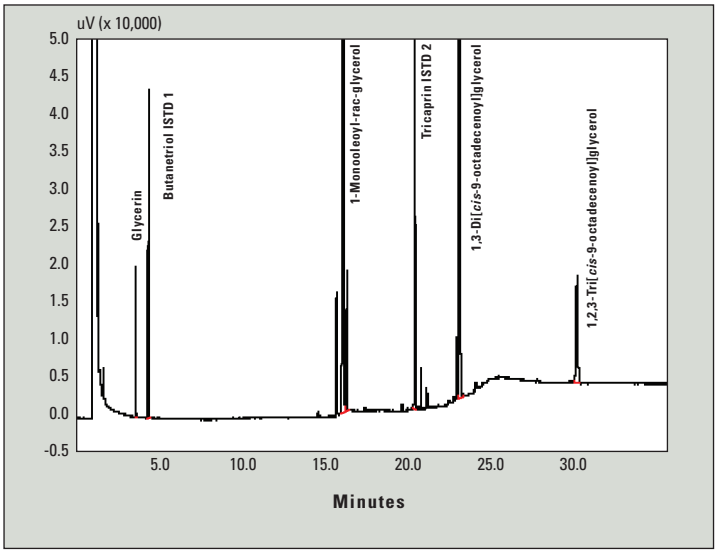


Figure 1: Standard chromatogram measured on a 25 m HT5, ID 0.32 mm, df = 0.1 µm with retention gap

Used naming	Alternative naming
Glycerin	"Free" Glycerin
Butanetriol (ISTD1)	
Monopalmitin	1-Monopalmitoyl-rac-glycerol
Monolinolenin	1-Monolinoleoyl-rac-glycerol
Monoolein	1-Monooleoyl-rac-glycerol
Monostearin	1-Monostearoyl-rac-glycerol
Tricaprin (ISTD2)	1,2,3-Tridecanolyglycerol
Diglyceride	1,3-Di[*cis*-9-octadecenoyl]glycerol
Diglyceride (rest)	*All other diglycerides*
Triglyceride	1,2,3-Tri[*cis*-9-octadecenoyl]glycerol
Triglyceride (rest)	*All other triglycerides*

Table 1: Naming of biodiesel components

- **Four groups to be evaluated in biodiesel quality control**
- **Correct identification of monoglycerides is challenging**

Since the standards contain only one component of each group (mono-, di- and triglycerides, Figure 1) the identification of all components must be carried out in a real biodiesel sample (Table 1).

Four groups are evaluated in the biodiesel quality control – "free" glycerin, the monoglycerides, diglycerides and triglycerides. Glycerin and monoglycerides are evaluated as single peaks and grouped by the software. Di- and triglycerides are integrated as one group respectively.

Identification

The biodiesel chromatogram (Figure 3) starts with glycerin and the first internal standard butanetriol. After the FAME peaks

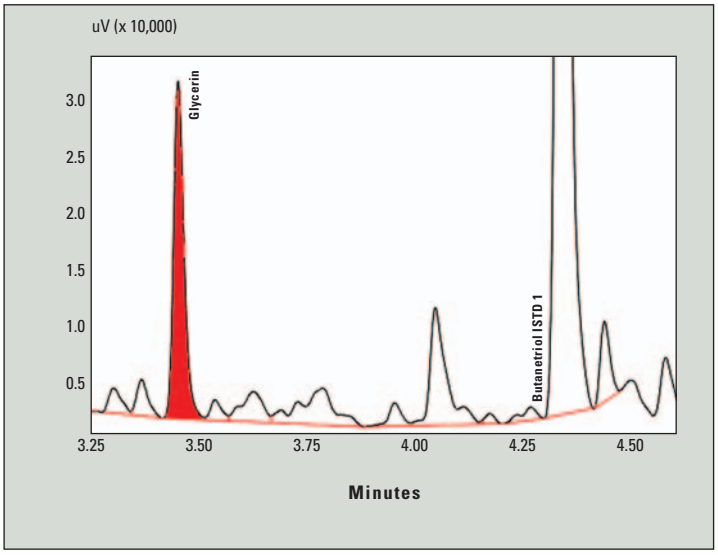


Figure 2: Glycerin and butanetriol peak zoomed from the biodiesel chromatogram in Figure 3

(from 10 to 14.5 minutes) the monoglycerides elute followed by the second internal standard tricaprin, and the di- and triglycerides.

From the standard chromatogram (Figure 1) the retention times of glycerin, butanetriol (Figure 2) as well as tricaprin are known.

Additionally one component of each group
- 1-monooleoyl-rac-glycerol (monoglycerides)
- 1,3-di[*cis*-9-octadecenoyl] glycerol (diglycerides)
- 1,2,3-tri[*cis*-9-octadecenoyl] glycerol (triglycerides)

is identified in the standard chromatogram.

In principle, four monoglycerides should be found in the chromatogram. In case of the diglycerides up to 10 separate peaks are in the chromatogram but for the triglycerides seldom more than two peaks are found. In the biodiesel sample (Figure 3) only one major triglyceride peak was found.

Figure 4a shows the monoglyceride pattern stated in DIN EN 14105. For comparison Figure 4b shows the corresponding part zoomed out of Figure 3. According to DIN EN 14105 the peak marked with a green arrow is monolinolenin. In order to get a positive identification for all monoglycerides a mixture from Supelco was injected containing all four monoglycerides. The resulting chromatogram shows three peaks only. Using the pure components a co-elution of monolinolenin and monoolein on the SGE HT5 column could be proven.

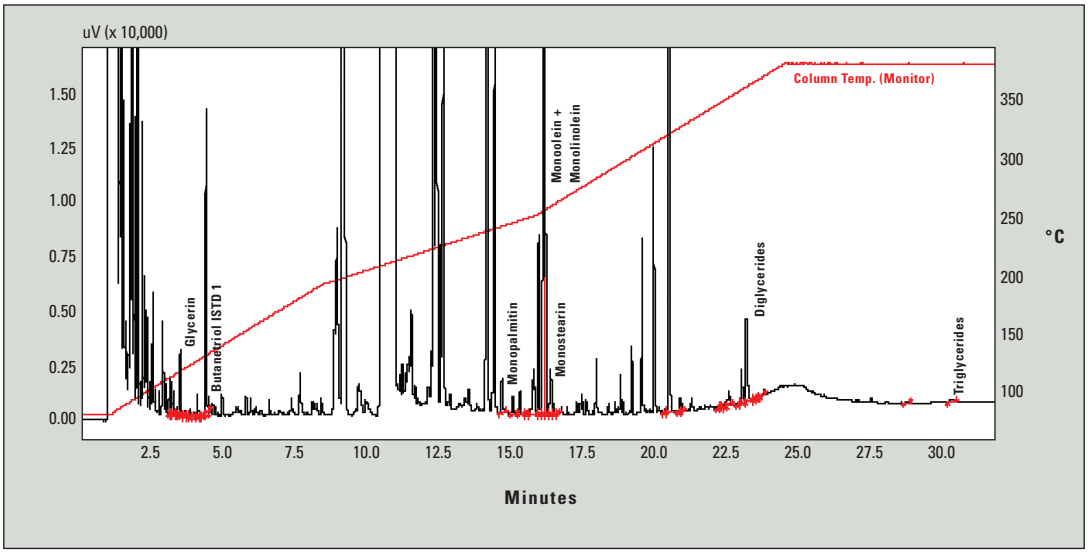


Figure 3: Biodiesel chromatogram measured on a SGE HT5, 25 m, ID 0.32 mm, df 0.1 µm with a retention gap 2 m, ID 0.53 mm

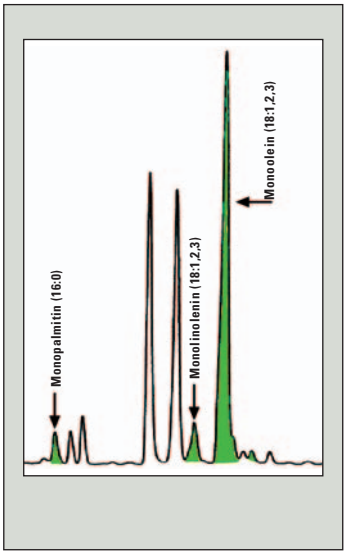


Figure 4a: Monoglyceride pattern taken from DIN EN 14105

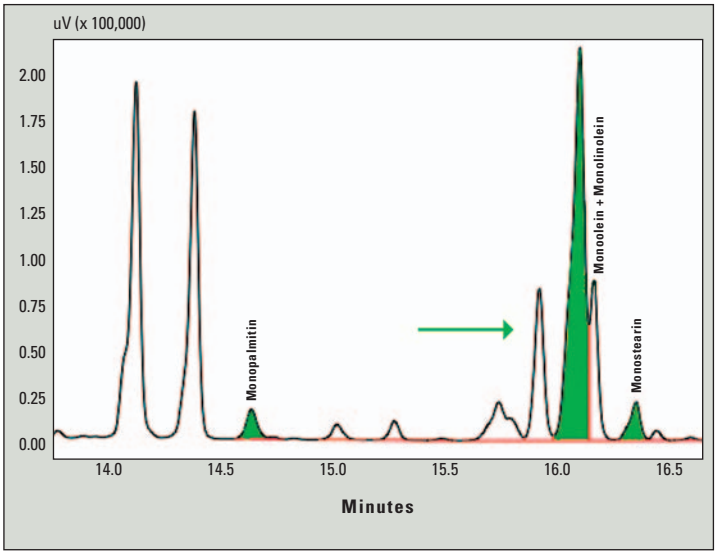


Figure 4b: Monoglycerides zoomed from the biodiesel chromatogram in Figure 3

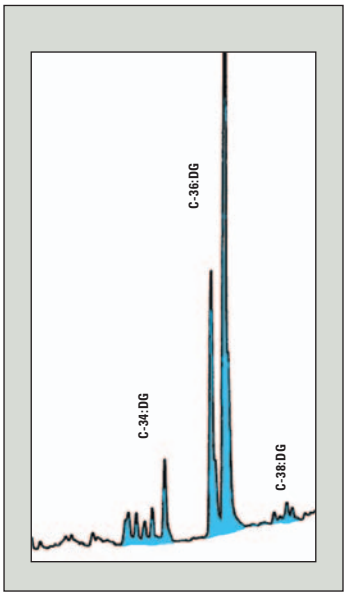


Figure 5a: Diglyceride pattern taken from DIN EN 14105

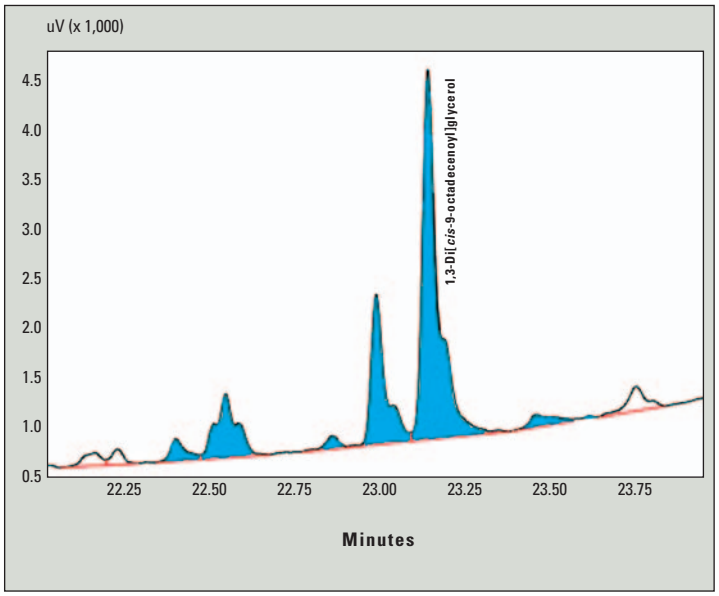


Figure 5b: Diglycerides zoomed from the biodiesel chromatogram in Figure 3

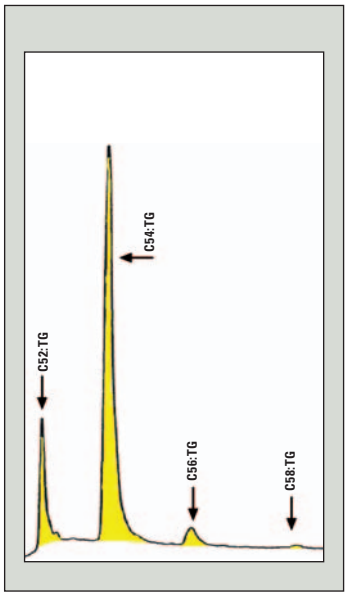


Figure 6a: Diglyceride pattern taken from DIN EN 14105

The same result was found on other non-polar columns (e.g. Zebron ZB 5HT). Therefore, a positive identification is recommended by using a monoglyceride mixture – for more information see Appendix A.

The diglycerides appear as one group of peaks in the chromatogram (Figure 5a). In most cases the biggest peak is the 1,3-di[*cis*-9-octadecenoyl]glycerol. Using this as marker the start and end of the "diglyceride peak group" can be identified according to the pattern shown in the DIN EN standard (Figures 5a and 5b).

If identification of start and end of the group is uncertain it is better to implement one peak too many than one too less. Mostly the peaks are very small and do not contribute much to the final concentration.

It may happen that no triglyceride signals can be found in a biodiesel chromatogram. Rarely four peaks can be found as shown in the pattern given in DIN EN 14105 (Figure 6a).

Often only the signal of 1,2,3-tri[*cis*-9-octadecenoyl]glycerol is found. This makes the definition of the triglyceride group difficult.

According to the DIN EN standard the middle of the group corresponds approx. with the retention time of the 1,2,3-tri-[*cis*-9-octadecenoyl] glycerol peak plus 0.4 minutes. We recommend evaluating all peaks within the range of the resulting retention time ± 1.5 minutes as triglycerides (Figure 6b). Dependent on column and temperature program used also ± 1.0 minute could be sufficient.

In Figure 6b the retention time of 1,2,3-tri[*cis*-9-octadecenoyl] glycerol is 30.27 minutes, plus 0.4 yields 30.67 minutes. Within the interval from 29.17 to 32.17 minutes all peaks were evaluated as triglycerides.

Appendix A

The mixture of monoglycerides from Sigma Aldrich (Supelco) is custom designed.

It contains:

- 1-Monopalmitoyl-rac-glycerol 25 % w/w
- 1-Monolinoleoyl-rac-glycerol 25 % w/w
- 1-Monooleoyl-rac-glycerol 25 % w/w
- 1-Monostearoyl-rac-glycerol 25 % w/w
- Total of 100 mg per ampoule

When ordering please specify custom-designed according to Lot No. DE1675 made by Sigma Aldrich Europe.

The components in the ampoules can be dissolved in heptane. It may be treated as a biodiesel sample. Just add internal standard and MSTFA for derivatization.

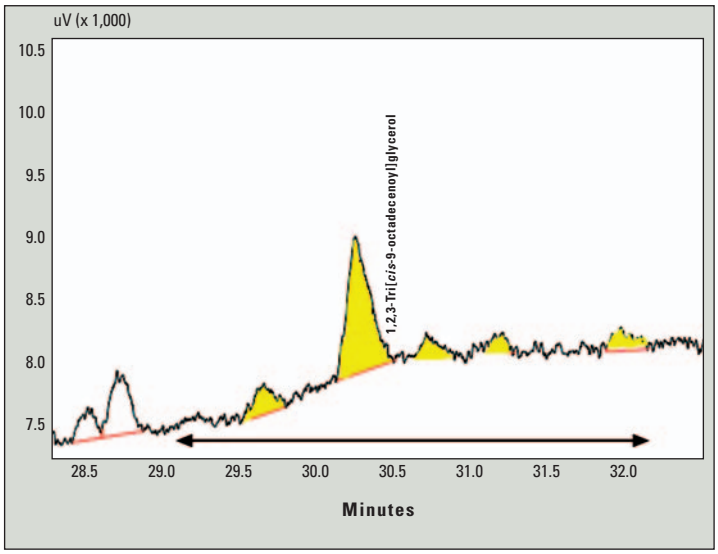


Figure 6b: Triglycerides zoomed from the biodiesel chromatogram in Figure 3

Evaluation and quantification

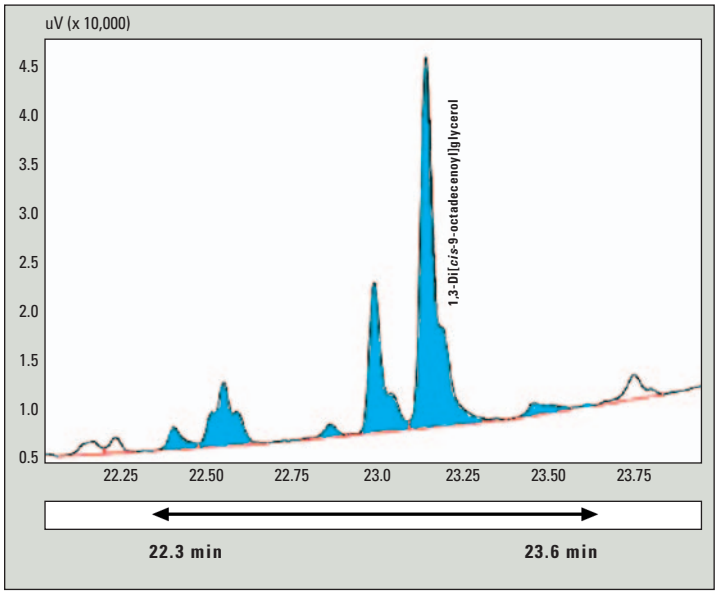


Figure 1: Diglycerides in a biodiesel chromatogram

Used naming	Alternative naming
Glycerin	"Free" Glycerin
Monoolein	1-Monooleoyl-rac-glycerol
Diglyceride	1,3-Di[*cis*-9-octadecenoyl]glycerol
Triglyceride	1,2,3-Tri[*cis*-9-octadecenoyl]glycerol

Table 1: Biodiesel components used to determine response factors of the different groups

After identification of the components in a biodiesel chromatogram (Figure 3, Part 4 on Page 23 of this application manual) quantification will be the next step. According to DIN EN 14105 a calibration curve is measured for one compound of each group. The calculated response factors are adapted for all components of the respective group.

Representative for all other components, the compounds in Table 1 are calibrated by determination of a four level calibration curve.

Quantitation parameters in GCsolution

The application uses an Internal Standard calibration with four calibration levels (concentrations). The curve fit type is set to "linear". The calibration curve is not forced through zero. A weighting method is not used and the unit is mass-%.

For identification the "Band" method is used i.e. for every identified component an individual time interval, retention time ± Δt, can be selected. Depending on the column used a "Default Band Time" between 0.05 and 0.1 min is sufficient. Relative retention times should be selected as "Identification Method" – especially if the samples are injected manually.

Under "Peak" select "All Peaks" and for grouping "Concentration Summation" is selected. In the next step the compound table has to be created – see Tab. 2 on Page 26 as an example. The concentrations of the calibrated components are set according to Part 1 (Page 15 ff) of this manual (sample preparation). Uncalibrated components are set to 1.

Under "Type" the Internal Standard components are marked as "Internal Standard and Reference" if relative retention times are used. According to DIN EN 14105 butanetriol is used as internal standard for glycerin and tricaprin for all other groups. This can be set under "Internal Standard Groups" in the compound table (ISTD groups, Table 2).

It is recommended to add the same internal standard amount to all calibration standards as well as to all unknown biodiesel samples. This would allow setting the internal standard concentrations in the compound table equal to the "Internal Standard Amount" of the unknown samples – the easiest way is to set all to 1.

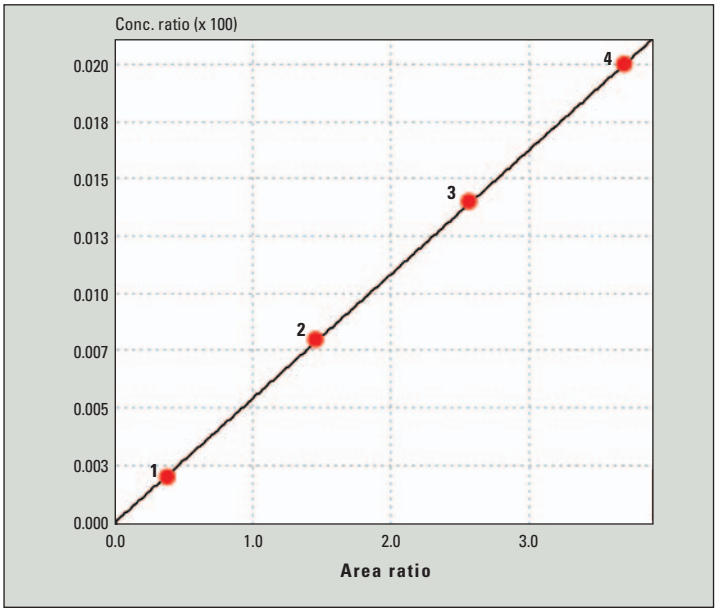
Settings for di- and triglycerides

In Part 4 (Page 22 ff) of this manual the identification of the di- and triglycerides was discussed. For quantification of these "peak groups" GCsolution software offers a special tool using the identification by band (Figure 1).

- **GCsolution "Band Method" for easy quantification of di- and triglycerides**
- **GCsolution "Grouping Functionality" calculates the required total glyceride content**
- **Easy handling of multiple internal standards**

Name	Type	Ret. time	Band	ISTD group	Group ID No.	Conc. 1	Conc. 2	Conc. 2	Conc. 2
Glycerin	Target	3.442	Default	1	1	0.005	0.02	0.035	0.05
Butanetriol ISTD1	ISTD&Ref.	4.281	Default	1	0	1	1	1	1
Monopalmitin	Target	14.642	Default	2	2	1	1	1	1
Monolinolein	Target	15.921	Default	2	2	1	1	1	1
Monoolein	Target	16.102	Default	2	2	0.2	0.8	1.4	2
Monostearin	Target	16.35	0.02	2	2	1	1	1	1
Tricaprin ISTD2	ISTD&Ref.	20.488	Default	2	0	1	1	1	1
Diglyceride	Target	23.154	Default	2	3	0.05	0.2	0.35	0.5
Diglycerides (rest)	Target	22.95	0.65	2	3	1	1	1	1
Triglyceride	Target	30.3	Default	2	4	0.05	0.2	0.35	0.5
Triglycerides (rest)	Target	30.67	1.5	2	4	1	1	1	1

Table 2: Example of a compound table layout in GCsolution software. Clicking with the right mouse button on the compound table shows the function "Table Style." This allows hiding of unnecessary columns and definition of a convenient format.



Y = aX + b
a = 0.5415221
b = 4.398673e-003
R^2 = 0.9998509
R = 0.9999255

Internal standard
Calibration curve: Linear
Original: Not forced

Figure 2: Calibration curve for monoolein

According to the diglyceride pattern in DIN EN 14105 all peaks in the retention time range from 22.3 to 23.6 minutes belong to the diglyceride group. The 1,3-di[*cis*-9-octadecenoyl]glycerol is positively identified by calibration. The set retention time (RT) in the compound table is the middle of this interval:

$$RT = \frac{RT_1 + RT_2}{2} = \frac{22.3 + 23.6}{2} = 22.95\ min$$

The band (Δt) is the difference divided by two:

$$\Delta t = \frac{RT_2 - RT_1}{2} = \frac{23.6 - 22.3}{2} = 0.65\ min$$

Since 1,3-di[*cis*-9-octadecenoyl] glycerol is the only positively identified diglyceride it is recommended to quantify it separately. Therefore, the diglycerides have two entries in the compound table: diglyceride (1,3-di[*cis*-9-octadecenoyl]glycerol and diglycerides (rest) representing all other components of this group.

It is important that diglyceride ranges in the compound table before diglycerides (rest). GCsolution will then quantify diglyceride first and does not implement this peak in the diglycerides (rest) group.

The calculation of the triglycerides is performed in a similar way. The determined retention time interval from 29.17 to 32.67 minutes yields a retention time RT = 30.67 minutes and band Δt = 1.5 minutes (Table 2).

Name	RF	Y(0)
Glycerin	0.09161	0
Butanetriol ISTD1	0	0
Monopalmitin	0.54152	0
Monolinolein	0.54152	0
Monoolein	0.54152	0
Monostearin	0.54152	0
Tricaprin ISTD2	0	0
Diglyceride	0.62761	0
Diglyceride (rest)	0.62761	0
Triglyceride	0.8165	0
Triglyceride (rest)	0.8165	0

Table 3: Response factor settings in the compound table after switching to Curve Fit Type: Linear RF (Linear)

Group ID No.	Name	Unit
1	Glycerin	mass %
2	Monoglycerides	mass %
3	Diglycerides	mass %
4	Triglycerides	mass %

Table 4: Group table definition in GCsolution. Like all other tables in GCsolution the layout of the group table can be changed using the "Table Style" function available by right mouseclick on the respective table.

Name	Conc.	Units	Peak No.
Glycerin	0.0549	mass %	1
Butanetriol ISTD1	0	mass %	3
Monopalmitin	0.024	mass %	7
Monolinolein	0.0122	mass %	15
Monoolein	0.3007	mass %	16
Monostearin	0.0229	mass %	17
Tricaprin ISTD2	0	mass %	22
Diglyceride	0.0301	mass %	38
Diglyceride (rest)	0.0558	mass %	33, 34, 35, 36, 37, 39, 40
Triglyceride	0.0018	mass %	45

Table 5: Compound results table in GCsolution software

Calibration

After measuring the four standard samples with this method GCsolution calculates a calibration curve for each group (Figure 2). It is important to check the offset "b" of all calibration curves. If the deviation from zero is within acceptable tolerances and the curve fit is also good, the response factors can be adapted for the other group constituents.

Quantitation method

In the GCsolution "Calibration Curve Window" the calculated response factors can be finally applied to the respective components. Under "Quantitative" the "Curve Fit Type" is changed to "Manual RF (Linear)." The calibration curves disappear and in the compound table the "Conc." rows are replaced by the rows "RF" and "Y(0)." RF is the response factor and equal to "a" in Figure 2. Y(0) is the offset and equal to "b." For glycerin, monoolein, di- and triglyceride the RF values are set equivalent to the values determined by the calibration curves. These can be copied to the other components respectively (Table 3). All offsets "Y(0)" are set to zero.

To calculate the total glyceride value according to DIN EN 14105 the sum concentrations for all groups are needed. These are obtainable using another grouping functionality of GCsolution software. In the compound table (Table 2) the groups are defined under "Group ID No." The groups must then be named in the group table (Table 4). The method is now completed and can be used for the evaluation of biodiesel samples.

Results

After evaluation of a biodiesel sample GCsolution provides three different result tables. The peak table lists all detected peaks – identified and not identified. The compound results table provides concentrations for all components listed in the compound table.

Table 5 shows results from a biodiesel sample containing almost no triglycerides. Only one peak was detected for triglyceride but no peak was detected in the triglyceride (rest) retention time interval.

A nice control option offers the Peak No. row in this table. It shows that the peaks with the numbers 33 to 40 are added under diglyceride (rest) with the exception of peak number 38 which was already quantified as diglyceride.

The group results table (Table 6) finally contains the information required for total glycerol calculation according to DIN EN 14105.

The total glyceride value is calculated according to the equation:

1.000* Free glycerol
+ 0.255* Monoglycerides
+ 0.146* Diglycerides
+ 0.103* Triglycerides
= Total glyceride

Group No.	Name	Conc.	Units
1	Glycerin	0.0549	mass %
2	Monoglycerides	0.3599	mass %
3	Diglycerides	0.0859	mass %
4	Triglycerides	0.0018	mass %

Table 6: Group results table in GCsolution software

With the results from Table 6 a total glyceride value of 0.159 is calculated. Thus, the content of free glycerin in the biodiesel sample measured is too high but all other requirements are met including the total glyceride limit (Table 7).

Summary

In routine analyses the "step by step" procedure described is, of course, automated in GCsolution software. The batch processing functionality is a powerful tool in the development of calibration curves or quantification of a sequence of unknown samples. Using export functionalities the calculation of the total glyceride value can also be automated.

Controlled contents in biodiesel	Dimension	Max. value	Determination according to
Monoglycerides	% (m/m)	0.8	DIN EN 14105
Diglycerides	% (m/m)	0.2	DIN EN 14105
Triglycerides	% (m/m	0.2	DIN EN 14105
Free glycerin	% (m/m)	0.02	DIN EN 14105
Total glyceride	**% (m/m)**	**0.25**	**DIN EN 14105**

Table 7: Limits for the content of the different glycerides and free glycerin according to DIN EN 14214

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