

Application Note

Biodiesel Quality Control
According to DIN EN 14105
Determination of free and total glycerol and mono-, di,
Triglyceride contents (reference method)
Part 5: Evaluation and Quantification

Introduction:

After identification of the components in a biodiesel chromatogram (see part 4 of this application manual) quantification will be the next step. According to DIN EN 14105 a calibration curve is measured for one substance of each group. The calculated response factors are adapted for all components of the respective group.

Used naming	Alternative naming
Glycerin	"free" Glycerin
Monoolein	1-Mono oleoyl-rac-glycerol
Diglyceride	1,3-Di[cis-9-octadecenoyl]glycerol
Triglyceride	1,2,3-Tri[cis-9-octadecenoyl]glycerol

Tab. 1: Biodiesel components used to determine response factors of the different groups (marked with the different colours).

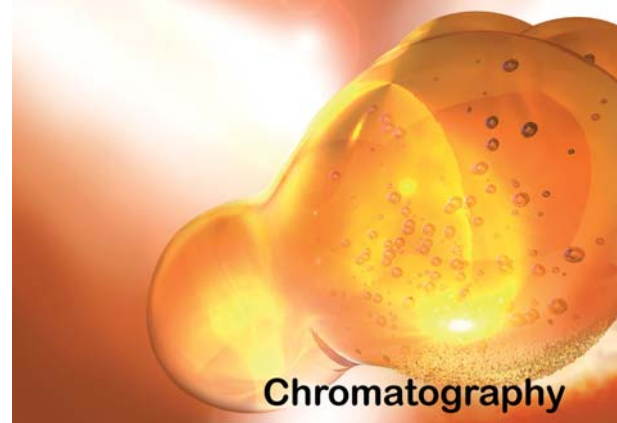
Representative for all other components the substances in table 1 are calibrated by determination of a four level calibration curve.

Quantitation settings:

The application uses 'Internal Standard' calibration with four 'Calibration Levels'. Curve Fit Type is set to 'Linear'. In first step the calibration curve is not forced through the zero point. A Weighting Method is not used and the Unit is mass-%.

For identification the 'Band' is used -means for every identified component an individual time interval, Retention Time plus minus a time ($RT \pm \Delta t$), can be selected. Dependent on the used column 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 must be typed – see Table 2 as an example. The concentrations of the calibrated components are set according to part 1 of this manual (sample preparation). Not calibrated components are just set to one.

Under 'Type' the Internal Standard components are marked as 'Internal Standard and Reference' if Relative Retention Times are used. According to the 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; see tab 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 one.

Settings for Di- and Triglycerides

In part 4 of this manual the identifications of the di- and triglycerides were discussed. For quantification of these "peak groups" GCsolution offers a special tool using the identification by band.

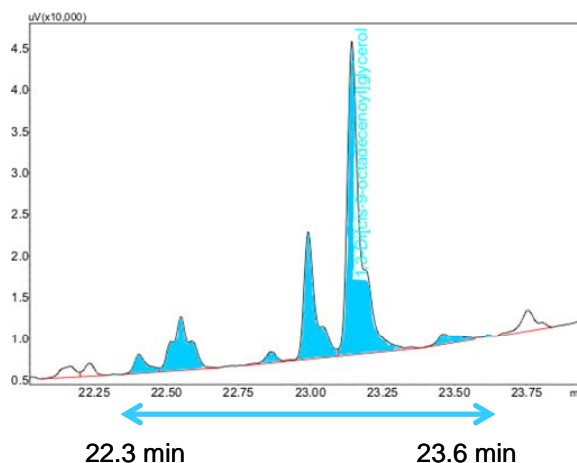


Fig. 1: Diglycerides in a biodiesel chromatogram

Name	Type	Ret.Time	Band	ISTD				Conc.1	Conc.2	Conc.3	Conc.4
				Group	Group ID#						
Glycerin	Target	3.442	Default	1	1	0.005	0.02	0.035	0.05		
Butantriol 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		

Tab. 2: Example of a compound table layout in GCsolution software. Clicking with the right mouse button upon the Compound Table releases the function 'Table Style'. This allows hiding of not used rows and definition of a convenient format.

According to the diglyceride pattern in the 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:

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

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

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

Since 1,3-Di[cis-9-octadecenoyl]glycerol is the only positively identified Diglyceride we recommend 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.

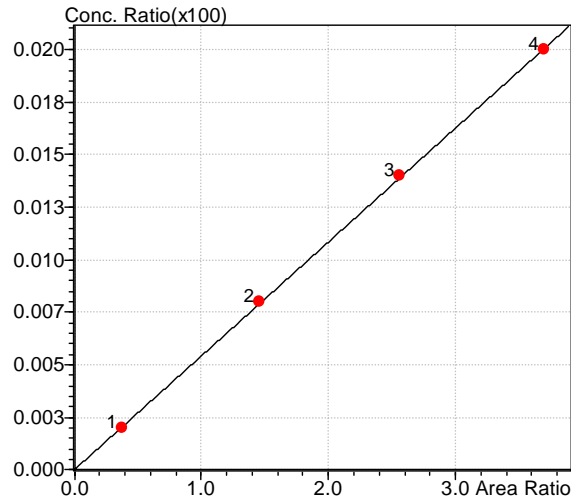
It is important that Diglyceride stands in the compound table before Diglycerides (rest).

In this case GCsolution quantifies Diglyceride first and does not implement this peak in the Diglycerides (rest) group.

Similar it works with the Triglycerides. The determined retention time interval from 29.17 to 32.67 minutes yields Retention Time RT = 30.67 minutes and Band Δt = 1.5 minutes (see tab. 2)

Calibration

After measuring the four standard samples with this method GCsolution calculates a calibration curve for each group (fig 1). It is important to check the offset 'b' of all calibration curves. If the deviation from zero is within acceptable tolerances and also the curve fit is OK the response factors can be adapted for the other group constituents.



$$Y = aX + b$$

$a = 0.5415221$
 $b = 4.398673e-003$
 $R^2 = 0.9998509$
 $R = 0.9999255$

Internal Standard
Calib.Curve:Linear
Origin:Not Forced

Fig.1: Calibration curve measured for Monoolein

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 fig. 1. Y(0) is the offset and equal to 'b'. For Glycerin, Monoolein, Di- and Triglyceride the RF and Y(0) values are set equivalent to the values determined by the calibration curves. These can be copied to the other components corresponding to Tab. 3. All offsets 'Y(0)' are set to zero.

Name	RF	Y(0)
Glycerin	0.09161	0
Butantriol 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

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

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 (tab. 2) the groups are defined under 'Group ID#'. Then the groups must be named in the Group Table (tab. 4)

Group ID#	Name	Unit
1	Glycerin	mass-%
2	Monoglycerides	mass-%
3	Diglycerides	mass-%
4	Triglycerides	mass-%

Tab. 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 mouse click upon the respective table.

The method is now finished 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.

Name	Conc.	Units	Peak#
Glycerin	0.0549	mass%	1
Butantriol 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

Tab. 5: Compound Results Table in GCsolution software.

Tab.5 shows results from a biodiesel samples that contained almost no triglycerides. Therefore 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# 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.

Group#	Name	Conc.	Units
1	Glycerin	0.0549	mass-%
2	Monoglycerides	0.3599	mass-%
3	Diglycerides	0.0859	mass-%
4	Triglycerides	0.0018	mass-%

Tab. 6: Group Results Table in GCsolution software.

The Group Results Table (Tab. 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:

$$\begin{aligned}
 &1.000 * \textit{Free Glycerol} \\
 &+ 0.255 * \textit{Monoglycerides} \\
 &+ 0.146 * \textit{Diglycerides} \\
 &+ 0.103 * \textit{Triglycerides} \\
 \hline
 &= \textit{Total Glyceride}
 \end{aligned}$$

With the results from table 6 a Total Glyceride value of 0.159 is calculated. Thus the measured biodiesel sample has a too high content of free glycerin but fulfils all other requirements including the Total Glyceride limit (see tab 7).

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

Tab. 7: Limits for the different glycerides given in the DIN EN 14214

Summary

In routine analyses the described “step by step” procedure is of course automated in GCsolution software. The batch processing functionality is a powerful tool in development of calibration curves or quantification of a sequence of unknown samples. Using export functionalities even the calculation of the Total Glyceride value can be automated.

If you have any questions concerning this or other parts of this application manual please contact you local Shimadzu representative.