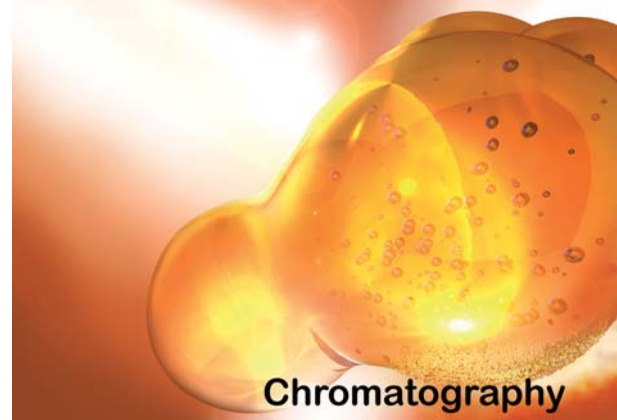


Application Note

Biodiesel Quality Control
According to DIN EN 14105
Determination of free and total glycerol and mono-, di-,
Triglyceride contents (reference method)
Part 4: Signal identification



Introduction:

Since the standards contain only one component of each group (mono-, di- and triglycerides, see fig. 1) the identification of all components must be done in a real biodiesel sample (see tab. 1).

Used naming	Alternative naming
Glycerin	"free" Glycerin
Butanetriol (ISTD1)	
Monopalmitin	1-Mono palmitoyl-rac-glycerol
Monolinolenin	1-Mono linoleoyl-rac-glycerol
Monoolein	1-Mono oleoyl-rac-glycerol
Monostearin	1-Mono stearoyl-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

Tab. 1: Naming of biodiesel components

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 (see fig. 3) starts with glycerin and the first internal standard butanetriol. After the FAME peaks (from 10 to 14.5 minutes) the monoglycerides elutes followed by the second internal standard tricaprin, and the di- and triglycerides.

From the standard chromatogram (fig. 1) the retention times of glycerin, butanetriol (see fig. 2) as well as tricaprin are known. Additionally one component of each group:

1-Mono oleoyl-rac-glycerol (monoglycerides),

1,3-Di[cis-9-octadecenoyl]glycerol

(diglycerides),

1,2,3-Tri[cis-9-octadecenoyl]glycerol

(triglycerides),

is identified by the standard chromatogram.

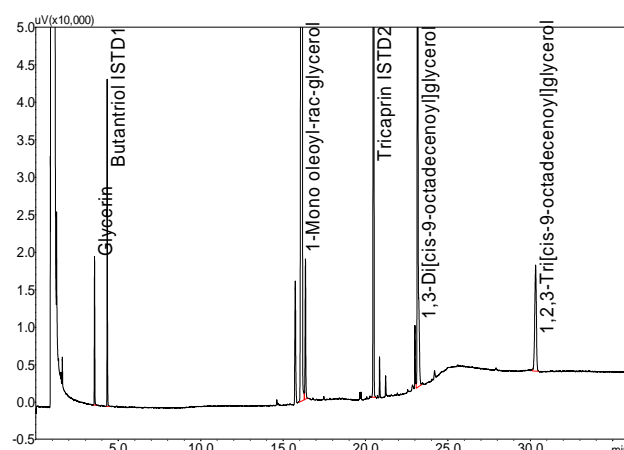


Fig. 1: Standard chromatogram measured on a 25m HT5, ID 0.32mm, df=0.1µm with retention gap.

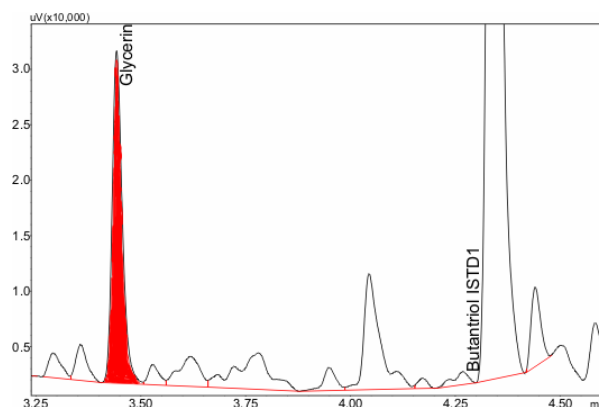


Fig. 2: Glycerin and Butanetriol peak zoomed from biodiesel chromatogram in fig 3.

In principal 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 (fig. 3) only one triglyceride was found.

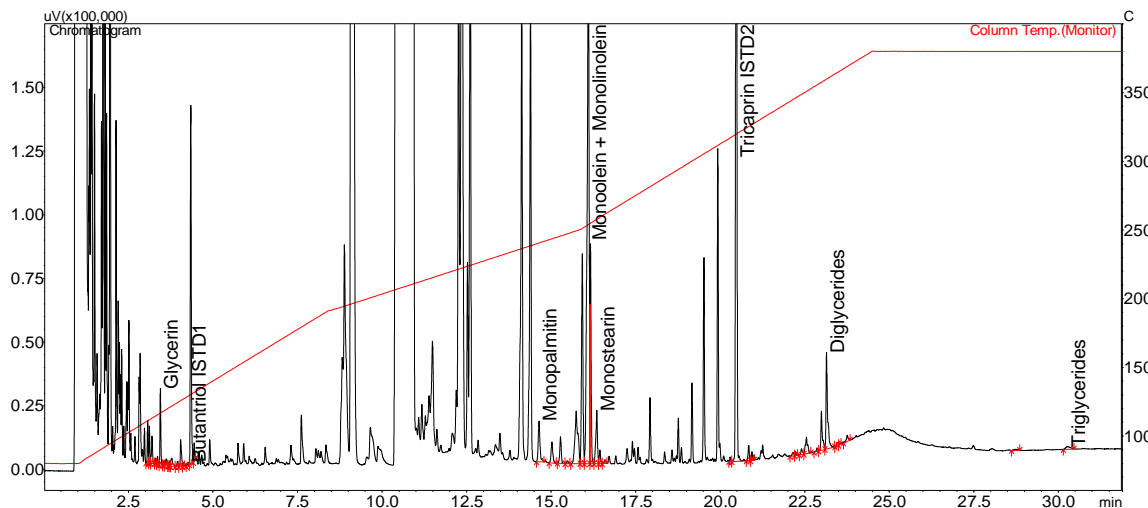


Fig. 3: Biodiesel chromatogram measured on a SGE HT5, 25m, ID 0.32mm, df 0.1µm with retention gap 0.53mm.

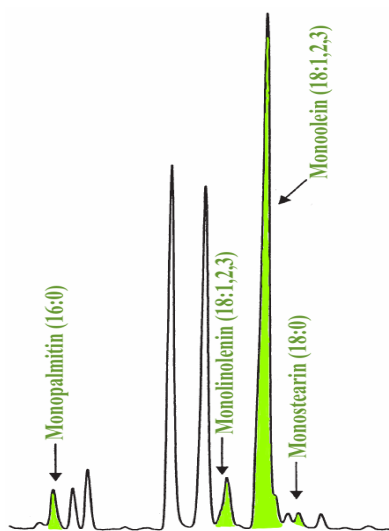


Fig. 4a: Monoglyceride pattern taken from DIN EN 14105.

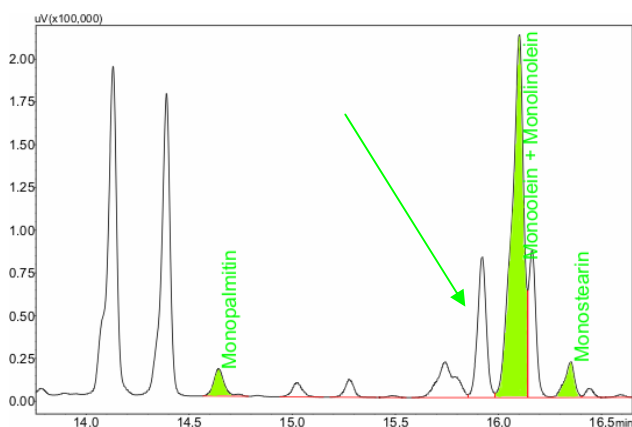


Fig. 4b: Monoglycerides zoomed from biodiesel chromatogram in fig 3.

Fig. 4a shows the monoglyceride pattern given in the DIN EN 14105. For comparison fig. 4b shows the corresponding part zoomed out of fig. 3.

According to the DIN EN 14105 the peak marked with a green arrow would be monolinolenin. In order to get a positive identification for all monoglycerides we injected a mixture from Supelco that contains all four monoglycerides. The resulting chromatogram shows three peaks only. Using the pure components we could prove a co-elution of monolinolenin and monoolein on the SGE HT-5 column. The same result was found on other non polar columns (e.g. Zebtron ZB-5ht). Therefore we recommend a positive identification by using a monoglyceride mixture - for more information see appendix A.

The diglycerides appear as one group of peaks in the chromatogram (see fig 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 (see fig. 5a and b). If you feel unsure with the identification of start and end of the group it is better to implement one peak to much than one less. Mostly the peaks are very small and do not contribute much to the final concentration.

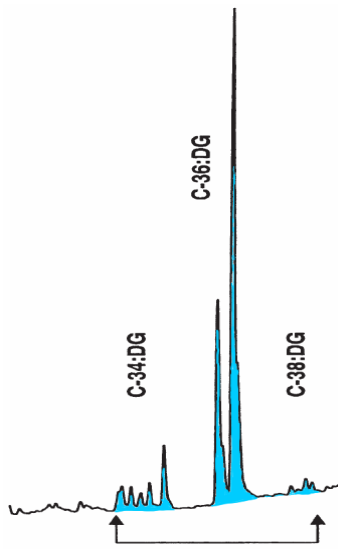


Fig. 5a: Diglyceride pattern taken from DIN EN 14105.

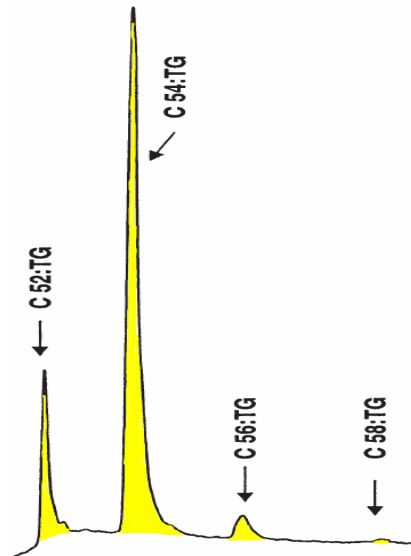


Fig. 6a: Diglyceride pattern taken from DIN EN 14105.

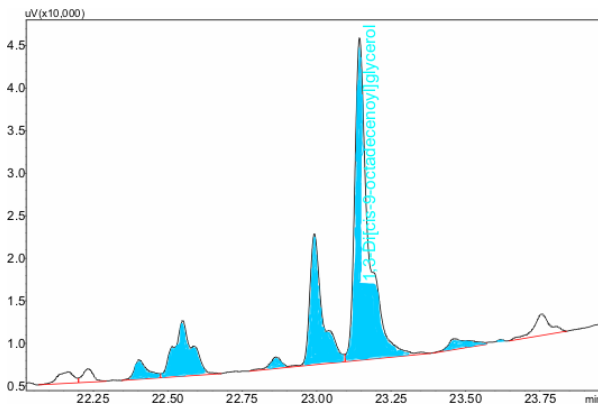


Fig. 5b: Diglycerides zoomed from biodiesel chromatogram in fig 3.

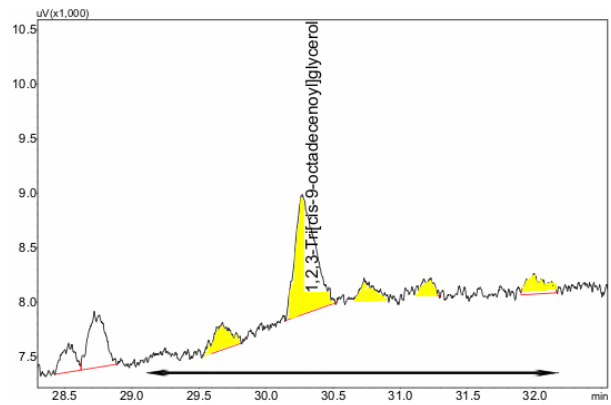


Fig. 6b: Triglycerides zoomed from biodiesel chromatogram in fig 3

It might happen that no triglyceride signals are in a biodiesel chromatogram. Seldom four peaks can be found as shown in the pattern given in the DIN EN 14105 (see fig 6a).

Often only the signal of 1,2,3-Tri[cis-9-octadecenoyl]glycerol is found. That makes the definition of the triglyceride group difficult. According to the DIN EN standard the middle of the group is approximately 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 (see fig 6b). Dependent on the used column and temperature program also ± 1.0 minute could be sufficient.

In fig 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.

The given specifications serve purely as technical information for the user. No guarantee is given on technical specification of the described product and/or procedures.

Appendix A:

The mixture of Monoglycerides from Sigma Aldrich (Supelco) is a custom designed:

It contains:

1-Mono palmitoyl-rac-glycerol 25% w/w

1-Mono linoleoyl-rac-glycerol 25% w/w

1-Mono oleoyl-rac-glycerol 25% w/w

1-Mono stearoyl-rac-glycerol 25% w/w

Total 100mg per ampoule

If you order it please mention that it is a custom designed mixture according to Lot No. DE1675 made by Sigma Aldrich Europe.

The components in the ampoules can be solved in heptane. It can be handled like a biodiesel sample. Just add internal standard and MSTFA for derivatisation.