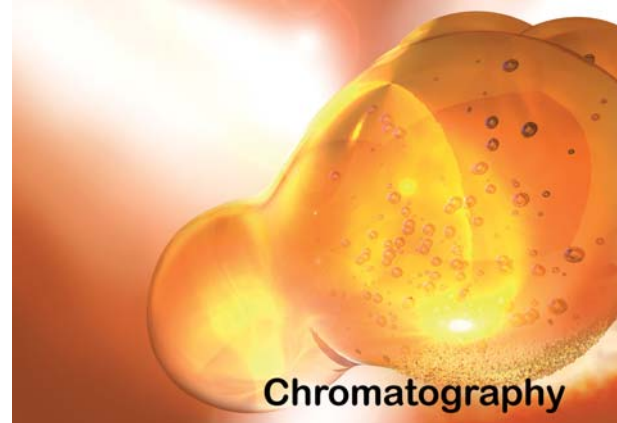


# Application Note

Biodiesel Quality Control  
According to DIN EN 14105  
Determination of free and total glycerol and mono-, di-,  
Triglyceride continents (reference method)  
Part 2: Instrument Configuration



## Introduction:

With this application the internal standard method is used for quantification therefore 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) 0.47mm - 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 0.53mm. On principal a 0.53mm 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 2m 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 (fig. 1). The preferable glass connectors can not be used with this application due to the high oven temperatures – most glass connectors have a maximum temperature of 350°C, an exception is the Restek Vu2 connector with an 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 fig 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).



Fig. 1: Mounting of retention gap kit with SGE metal connector

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

For accuracy and precision of the results it is essential that the connection of retention gap to main column is gas tight. Due to the huge oven temperature operation range the metal connector might become leaky during operation therefore it must be checked regularly.

Because of this uncertainty we do not recommend 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 0.53mm can be directly connected to the liner. The column sticks inside the liner reduction and is additionally tighten with graphite ferrule on the injector capillary adapter.

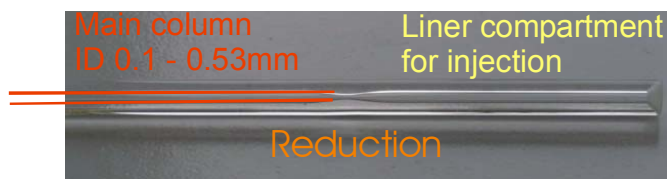


Fig. 2: Mounting of column with simple on column liner.

This simplifies the column mounting significantly and gas tightness even with hydrogen as carrier gas is no problem anymore.

Two different liners are available dependent on column inner diameter.

221-49381-02 for column ID  $\geq 0.32$ mm

980-00371 for column ID  $\leq 0.25$ mm

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

Measuring the glycerol content in biodiesel we found better precision of the results 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 20-30cm 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 this contaminants and it might be necessary to cut 20-30cm 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 in an oven. Important is that it does not get contact to 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 tri-glycerides. Generally with fused silica high temperature columns the recoveries are better.

Often used is the HT5 capillary column from SEG. It's a fused silica column with temperature limits 380/400°C – means the column can be operated at 380°C for longer times, at 400°C only short term.

Possible is also the HT8 phase from SGE but due to temperature limit 360/370°C the chromatogram time is extended.

A new fused silica capillary Zebron ZB 5HT from Phenomenex has temperature limits of 400/430°C. However, in some cases installation of this column with the “Simple On Column” liner has revealed problems with peak tailing. Often this problem was not observed after first installation but after usage and reinstallation of the column. Possibly the new designed high temperature imide layer of the Zebron columns could be the reason (see fig. 3). The outside imide layer must generate a tighten connection to the liner glass wall (see fig 2) otherwise the injected liquid does not flow quantitatively into the column. The consequences are peak tailing problems in the chromatogram. Unfortunately in several cases it was not possible to obtain a tighten connection with used Zebron columns.

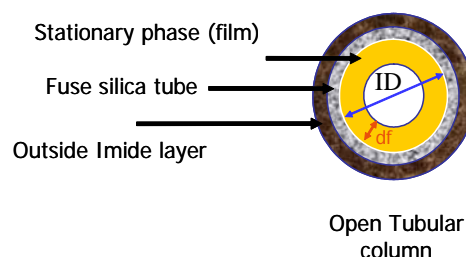


Fig. 3: Cross-section through a capillary column.

Important is also that the Zebron high temperature columns require a special conditioning procedure (recommended by

Phenomenex) before first operation. For removal of water traces from the imide layer the column should be heated slowly with a rate smaller than 5°C/min up to 400°C and kept at this temperature for approximately one hour.

Also Varian uses a special imide layer with the Factor Four high temperature columns. The same conditioning procedure should be performed with these columns even if it is not recommended by Varian. Generally we do not recommend using the Varian Factor Four column with the "Simple On Column" injection technique.

For operation with the "Simple On Column" injection technique an alternative to the SGE columns could be the new Restek high temperature column. We have tested first prototypes of the Rtx-5 biodiesel column. The column was easy to mount in the liner and first results concerning separation of Monoglycerides as well as recoveries of triglycerides looked promising.

### 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.

Fig. 4 shows a typical trend of the baseline with SGE column over more than 20 blank runs.

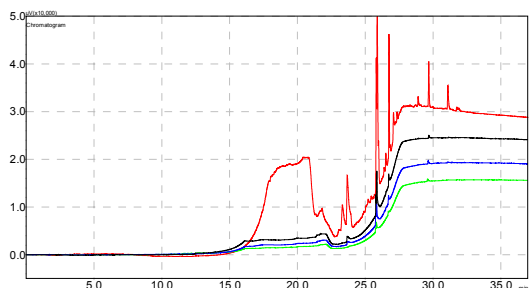


Fig. 4: Column conditioning. Between red and green line the column was cycled of about 20 times

Used was a temperature program that heating with 10°C/min from 65°C up to 380°C. At the beginning a baseline drift of about 30mV or more can be observed. After 12 hours it is reduced to 10 to 20mV. This was done with a 0.1µm film column with 0.25µm film thickness the column bleeds more and the baseline drift is more intense.

A lower baseline drift of about 5-15mV was observed with the Restek Rtx-5 high temperature column.

### Ghost peaks:

Even if the conditioning chromatogram looks good it is recommended to inject one time pure heptane before measuring biodiesel samples.

Sometimes a baseline effect as shown in figure 4 might occur.

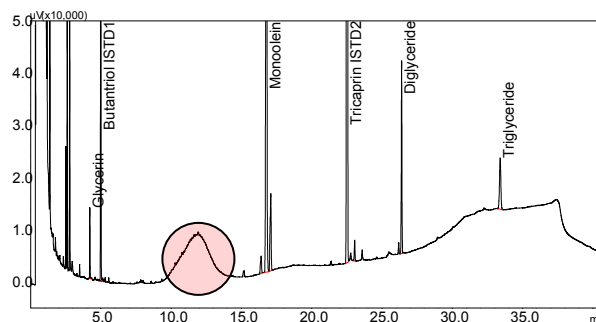


Fig. 5: Baseline drift or "ghost peak" due to liner contamination.

Most likely the liner was contaminated by contact to surfaces covered by a fatty film (for example the human skin).

If the effect occurs during operation of the system the reason might be the samples itself. For example by biodiesel additives if biodiesel from gasoline station was measured, over aged MSTFA was used for derivatisation, or the used chemicals and solvents were not water free.

Normally it is not possible to get rid of these "ghost peaks" by several injection of heptane. A new or cleaned liner must be installed and 10cm should be cut from the capillary column.

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.