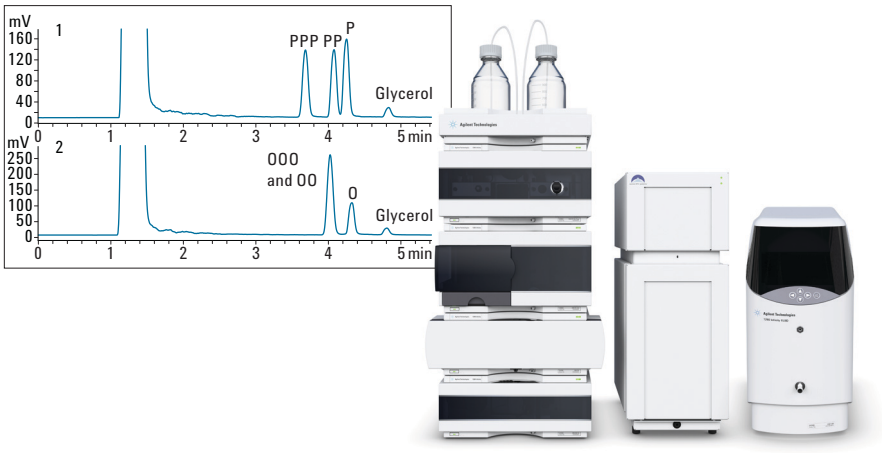
Fast screening of impurities in biodiesel using the Agilent 1260 Infinity Analytical SFC System in combination with evaporative light scattering detection

Application Note

Petrochemical



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Abstract

According to EU regulation, the concentration of residual glycerol, mono-, di- and triglycerides in biodiesel should be monitored. This analysis is normally performed by gas chromatography after derivatization of the sample. Alternatively, supercritical fluid chromatography coupled to evaporative light scattering detection (SFC-ELSD) can be applied, eliminating the need for derivatization. Using the Agilent 1260 Infinity Analytical SFC System, excellent separation repeatability and good sensitivity were obtained, while the analysis time is approximately 6 minutes. The described SFC-ELSD method can be used for screening control of biodiesel (B100) samples.

Agilent Technologies

Introduction

The interest in biodiesel is growing tremendously. Biodiesel is typically obtained from vegetable oils such as rape seed oil, sunflower oil, soybean oil, or palm oil, by transesterification, resulting in a mixture of fatty acid methyl esters (typically referred to as B100).

According to EN Norm 14214, biodiesel samples should comply with a number of criteria [1]. The EU Norm EN-14105 regulates the maximum residue level of monoglycerides (MG), diglycerides (DG), triglyceride (TG), and glycerol (Gly) in biodiesel. These maximum residue level ranges are 0.8% (w/w) for monoglycerides, 0.2% (w/w) for diglycerides and triglycerides, and 0.02% (w/w) for glycerol. The official method to determine these residues involves derivatization by silylation (MSTFA) followed by the analysis of the silylated sample by high temperature gas chromatography on a short capillary column with thin film thickness and flame ionization detection[2].

Supercritical fluid chromatography can be considered as an alternative since it is able to elute triglycerides at low temperatures, while polar solutes such as glycerol can be analyzed without derivatization. This Application Note describes a fast screening method using the Agilent 1260 Infinity Analytical SFC System. Separation is done on a ZORBAX SB-CN column (4.6 × 250 mm, 5 µm)

The cyanopropyl column used in SFC mode offers selectivity, with the bulk of the sample (FAMEs) eluting first, followed by mono-, di- and triglycerides and, finally, glycerol. This separation cannot be obtained by HPLC (on C18 or CN) or in SFC (on C18 or silica). For detection, an Agilent 1260 Infinity ELSD detector was used, resulting in good sensitivity for the detection of glycerides and glycerol. The response factors are less dependent on chain length and number of double bonds compared to UV detection.

Experimental

Solutions

Stock solutions of monopalmitin (P), dipalmitin (PP) and tripalmitin (PPP) were prepared in chloroform (20 mg/mL) and a stock solution of glycerol was prepared in ethanol (20 mg/mL). Stock solutions of mono-olein (O), diolein (OO), and triolein (OOO) were purchased in pyridine (5 mg/mL). All glyceride standards and glycerol were obtained from Sigma-Aldrich (Belgium).

Aliquots from these stock solutions were then spiked in a sample of 100% biodiesel (B100) in ethanol (10 mg/mL) to obtain two reference mixtures:

- Mix 1 contained P, PP, PPP, and glycerol in biodiesel
- Mix 2 contained O, OO, OOO, and glycerol in biodiesel

These solutions were prepared at different levels from 0.1 to 5 % (w/w biodiesel). The final mixtures contained 10 mg/mL biodiesel (consisting of C16-C18 fatty acid methyl esters) and 10–500 µg/mL glycerol and mono-, di-, and triglycerides.

System configuration

Analyses were performed on a 1260 Infinity Analytical SFC System coupled to a 1260 Infinity Evaporative Light Scattering Detector. The 1260 Infinity ELSD was coupled to the SFC system using a similar procedure as used for SFC-MS [3]. The system components are summarized in Table 1. Figure 1 shows the instrumental configuration.

The 1260 Infinity ELSD was coupled to the SFC module by means of a heating device prior to the ELSD inlet. Additionally, a make-up flow was added between the UV detector and the BPR, through an Agilent Zero dead volume T-piece, as described in the Technical Note for SFC-ELSD [4]. Without an external heating device, the CO_2 exiting the BPR might cause the inlet of the ELSD to freeze, which can potentially result in a leak error.

Experimental conditions

The experimental conditions are summarized in Table 2.

Part number	Description	
G4309A	Agilent 1260 Series Analytical SFC System	
G1310B	Agilent 1260 Infinity Isocratic Pump (Make-up Flow)	
G4260B	Agilent 1260 Infinity Evaporative Light Scattering Detector	
AG1	Caloratherm	Available through RIC[1,2]
AG004	Pre-heater	Available through RIC[1,2]

[1]Contact info@richrom.com for more information.
[2]Transfer capillary heating can alternatively be performed by using the heat exchanger of G1316A.

Table 1
System modules.

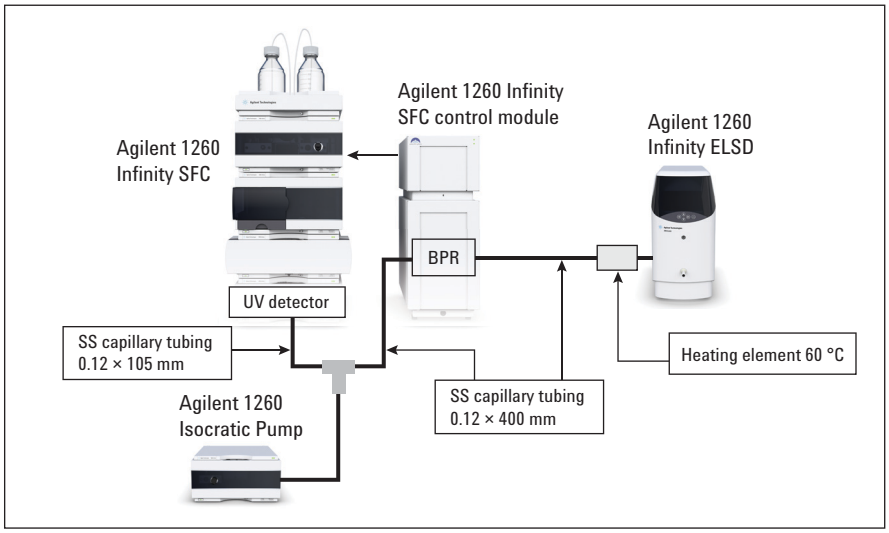

Figure 1
Schematic of the SFC-ELSD configuration.

Conditions	
Column:	Agilent ZORBAX SB-C18, 4.6 × 250 mm, 5 µm (p/n 880975-905)
Supercritical fluid:	CO_2
Modifier:	MeOH
Outlet pressure:	150 bar
Flow rate:	3 mL/min
Gradient:	0-1.3 minutes: 2% and 1.3 to 3.2 minutes: 2%-10%
Column temperature:	60 °C
Injection volume:	5 µL (15 µL syringe fill)
Make-up flow:	IPA at 0.6 mL/min (before BPR)
Transfer line heating:	60 °C
DAD:	210/4 nm, Ref. 360/100 nm
ELSD:	Evap 20 °C, Neb 30 °C, 1.60 SLM, Gain 1, Smoothing 5 seconds

Table 2
Experimental conditions.

Results and discussion

The profiles obtained for the biodiesel spiked at 5% level with P, PP, and PPP (mix 1) and with O, OO, and OOO (mix 2), containing glycerol, are shown in Figure 2. The bulk of the biodiesel matrix, consisting of C16-C18 fatty acid methyl esters, elutes between 1.1 and 1.5 minutes. Triglycerides, diglycerides, and monoglycerides are well separated. Glycerol elutes at the end of the chromatogram with good peak shape, taking into account that the compound is not derivatized.

The separation space between the FAMEs and the first eluting triglyceride (PPP) is important since some minor solutes (C20-C24 FAMEs) elute on the tail of the FAME fraction (1.5–3 minutes). The temperature of the SFC column played an important role in this separation. Optimum resolution was obtained between the target compounds (impurities) and matrix (biodiesel) at 60 °C.

Triolein and diolein are not separated under these conditions, but the individual separation of tri-, di-, and monoglycerides was not the aim of this analysis.

Different solvents (chloroform, toluene, methanol, ethanol, isopropanol, and pyridine) were tested to dissolve the biodiesel, mono-, di-, and triglycerides, and glycerol. Different ELSD responses were obtained for the different solvents. While chloroform and toluene yielded good detector response for mono-, di-, and triglycerides, the solubility of glycerol was too low. Therefore, ethanol was used, giving the best response for all compounds.

Table 3 shows that by using a pure biodiesel sample spiked at different levels (0.1 to 5 % (w/w)) with glycerol, mono-, di-, and triglycerides, calibration curves were constructed, and a good quadratic fit was obtained (R^2> 0.99). Table 3 also includes the limit of detection (LOD), corresponding to a signal-to-noise ratio (S/N) greater than 3.

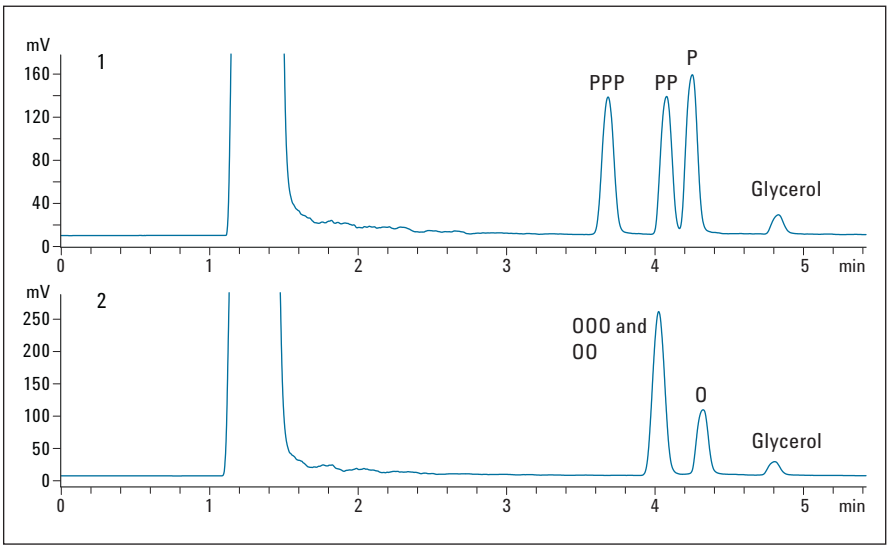

Figure 2
Biodiesel spiked with P, PP, PPP, and glycerol at 5% (w/w) (upper trace) and with O, OO, OOO, and glycerol at 5% (w/w) (lower trace).

	Linearity (R^2)[3]	0.2% (w/w) (% RSD)[4]	1% (w/w) (% RSD)[4]	5% (w/w) (% RSD)[4]	LOD % (w/w)
P[1]	0.9989	4.9	3.0	3.1	0.1
PP[1]	0.9992	4.7	3.0	4.3	0.1
PPP[1]	0.9997	3.3	1.9	5.0	0.1
O[1]	0.9994	2.3	2.4	3.3	0.1
OO and OOO[1]	0.9998	2.2	3.2	3.9	0.1
Glycerol[2]	0.9995	3.9	3.4	2.0	0.2

[1] 0.1, 0.2, 0.5, 1, 2 ,and 5% spiked level on biodiesel sample, one injection/level
[2] 0.2, 0.5, 1, 2, and 5% spiked level on biodiesel sample, one injection/level
[3] Linearity on ELSD: quadratic fit
[4] Six consecutive injections

Table 3
SFC-ELSD method performance data.

The sensitivities obtained were below the maximum residue levels set in the EN methods, except for glycerol. This is also illustrated in Figures 3 and 4, showing the ELSD chromatograms obtained for biodiesel samples respectively without spiking, spiked at 0.2% and at 1% level with mix 1 (Figure 3) and mix 2 (Figure 4).

Conclusion

The Agilent 1260 Infinity Analytical SFC System coupled to ELSD detection was successfully applied for the determination of impurities in biodiesel. Traces of monoglycerides, diglycerides, triglycerides, and free glycerol can be determined at levels down to 0.1–0.2% (w/w) in less than 6 minutes analysis time without the need for derivatization. The proposed method can be used for fast screening in quality control. In addition, SFC, known as a green technique, minimizes the consumption of organic solvents, avoiding the generation of large amounts of toxic waste and consequently inflicting minimal or no harm on the environment.

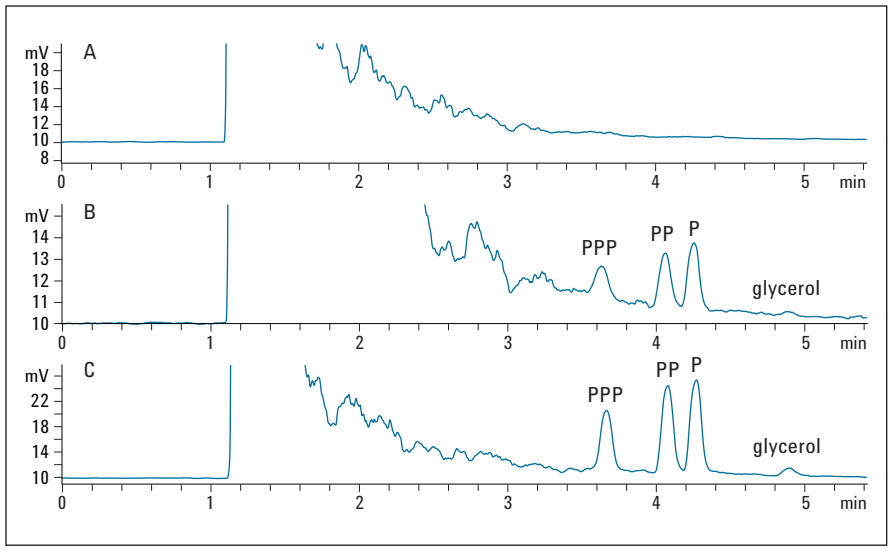

Figure 3
Biodiesel not spiked A), spiked at 0.2% B), and spiked at 1% C) with P, PP, PPP, and glycerol.

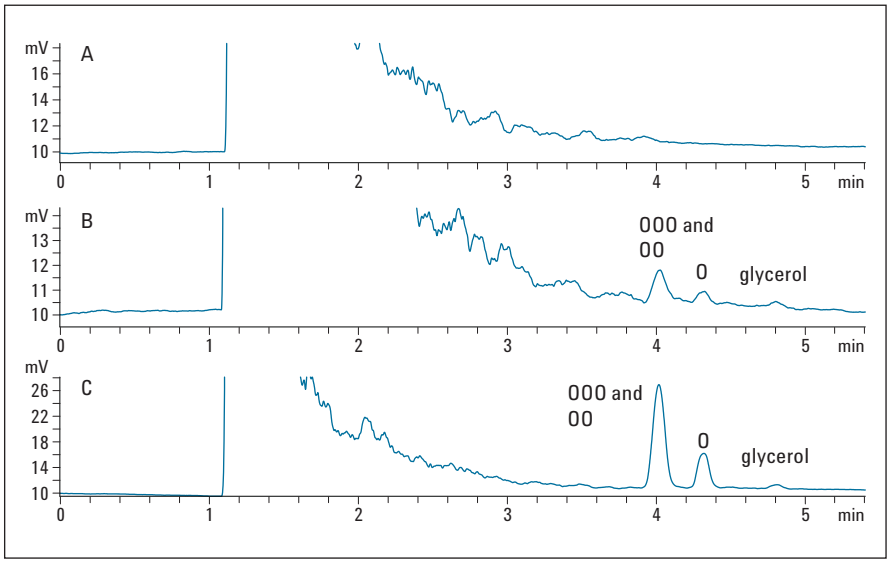

Figure 4
Biodiesel not spiked A), spiked at 0.2% B), and spiked at 1% C) with O, OO, OOO, and glycerol.

References

1.
Automotive fuels-fatty acid methyl esters (FAME) for diesel engines- Requirements and test methods (prEN 14214:**2002** E) (Brussels, Belgium).

2.
Fat and oil derivatives – Fatty Acid Methyl Esters (FAME) - Determination of free and total glycerol and mono-, di-, triglyceride content (Reference method). Norm EN 14105:**2011**

3.
M. Dunkle, G. Vanhoenacker, F. David, P. Sandra, M. Vollmer, The Agilent 1260 Infinity SFC/MS Solution, Agilent Technologies Publication Number 5990-7972EN, **2011**.

4.
M Rambla-Alegre, M. Dunkle, F. David, P. Sandra. The Agilent 1260 Infinity SFC/ELSD solution, Agilent Technologies (in preparation).

5.
The Caloratherm (p/n AG1) and pre-heater (p/n AG004) are products from Sandra Selerity Technologies and are available through RIC. For more information, contact info@richrom.com.

www.agilent.com/chem/sfc

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