Fast determination of residual glycerol and glycerides in biodiesel by SFC/MS using the Agilent 1260 Infinity Analytical SFC System

Application Note

Petrochemical

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Abstract

Supercritical fluid chromatography (SFC) in combination with mass spectrometric detection (MS) is a valuable technique for the determination of glycerol and mono-, di-, and triglyceride impurities at trace levels in biodiesel. The diluted biodiesel sample is analyzed without derivatization on a cyanopropyl silica column. Using MS detection in fast polarity switching positive and negative ion detection mode, detection limits of 0.02% (w/w) were reached for glycerol and all glycerides (mono-, di-, and tri-). Good repeatability was obtained, which allowed the system to be used for qualitative as well as for quantitative analysis, with an analysis time of less than 6 minutes.

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Introduction

The European Norm EN 14214 specifies requirements and test methods for fatty acid methyl esters (FAMEs) to be used as automotive fuel for diesel engines[1]. The FAME mixtures, referred to as B100 biodiesel, are typically produced from vegetable oils, mainly consisting of triglycerides. After conversion, the B100 biodiesel should be free from lipid contaminants. The maximum residue levels are respectively 0.8% (w/w) for monoglycerides (MG), 0.2% for diglycerides (DG) and triglycerides (TG), and 0.02% for free glycerol (Gly). The reference method for quality control EN 14105[2] is based on derivatization of glycerol and mono- and diglycerides into silyl derivates in the presence of pyridine and N-trimethylsilyltrifluoroacetamide (MSTFA), followed by high temperature gas chromatography on a short capillary column with thin film thickness, using on-column injection (or equivalent), and flame ionization detection[2].

SFC is a valuable alternative to the GC-FID method. Previously, it was demonstrated that the analysis of mono-, di-, triglycerides and glycerol in biodiesel can be performed on a cyano column in 6 minutes using the Agilent 1260 Infinity Analytical SFC System in combination with evaporative light scattering (ELSD) detection[3]. The SFC-ELSD method performs well for fast quality screening and glycerides can be detected at levels down to 0.1% (w/w). Using ELSD, glycerol cannot be detected below 0.1% level and differentiation of different glycerides (with, for instance, palmitin, olein, or linolein fatty acids) is not possible.

This Application Note used SFC in combination with mass spectrometric detection (MS) with a single quadrupole system operated in fast polarity switching mode. The nonderivatized compounds can be detected at trace concentration and the mass spectra can be used for additional confirmation and identification of the impurities.

Experimental

Solutions

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

Aliquots of these stock solutions were spiked in a B100 biodiesel sample diluted at 10 mg/mL in ethanol to obtain two series of calibration samples: Series 1 contained P, PP, PPP, and glycerol at different levels from 0.05–5% (mass versus biodiesel mass) in the biodiesel solution, and Series 2 contained O, OO, OOO, and glycerol (0.05–5% mass versus biodiesel mass) in biodiesel solution.

Instrumentation

Analyses were performed on an Agilent 1260 Infinity Analytical SFC System coupled to an Agilent Agilent 6130 Single Quadrupole LC/MS (G6130A) equipped with an APCI source. The MSD detector was coupled to the SFC module by means of a heating device (Caloratherm) prior to the APCI ionization source. Additionally, a make-up flow was added between the UV detector and the BPR, through an Agilent Zero dead volume T-piece, as previously described[4]. Table 1 summarizes the system components, and Figure 1 shows the instrumental configuration.

Part number	Description	
G4309A	Agilent 1260 Infinity Analytical SFC System	
G1310B	Agilent 1260 Infinity Isocratic Pump (make-up flow)	
G6130A	Agilent 6130 Single Quadrupole LC/MS	
AG1	Caloratherm	Available through RIC [5]
AG004	Pre-heater	Available through RIC [5]

[5] Contact info@richrom.com for more information

Table 1
Agilent 1260 Infinity Analytical SFC System – System modules.

Experimental

Separation is done on an Agilent ZORBAX SB-CN, 4.6 × 250 mm, 5-µm column (p/n 880975-905) using methanol as modifier. Detection was performed by MS in scan/SIM mode using fast polarity switching. Three signals were acquired: scan positive mode is used for detection and identification of triglycerides, scan negative mode is used for detection and identification of mono- and diglycerides, and the SIM negative ion detection mode is used for the selective and sensitive detection of free glycerol. This compound is measured as the [M-CH_3OH]- adduct (m/e 124). The experimental conditions are summarized in Table 2.

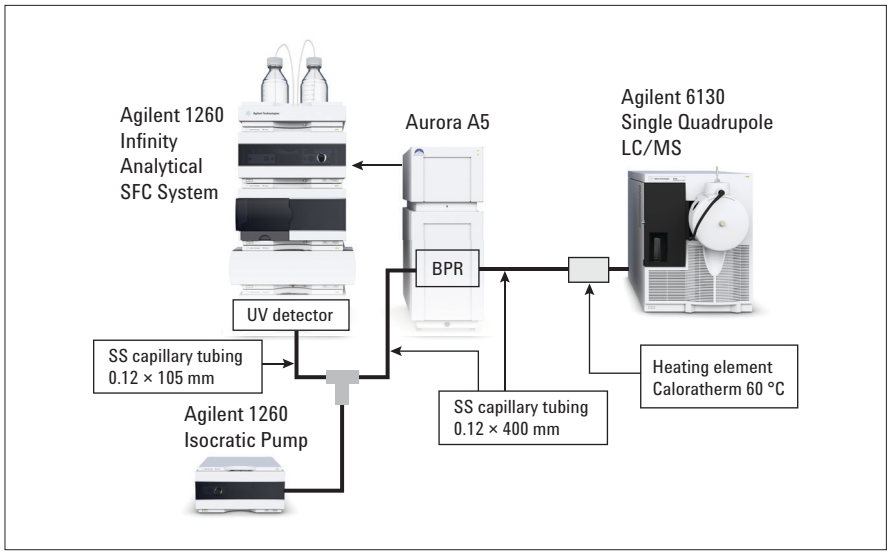

Figure 1
1260 Infinity Analytical SFC/6130 MSD configuration.

Conditions	
Column:	Agilent ZORBAX SB-CN, 4.6 × 250 mm, 5 µm
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%
Temperature:	60 °C
Injection volume:	5 µL
Make-up:	IPA at 0.6 mL/min (before BPR)
Caloratherm:	60 °C
APCI:	Capillary V ± 4000 V
	Corona I: 4.0 µA (+), 20 µA (–)
	Drying gas: 6.0 L/min at 325 °C
	Nebulizer: 55 psig
	Vaporizer: 350 °C
APCI:	Scan pos (200–1,000) Frag 70
	Scan neg (50–800) Frag 70
	SIM neg (ion 124.2) Frag 70

Table 2
Experimental conditions.

Results and discussion

It has been demonstrated that, by using SFC on a cyano column, a very interesting separation is obtained with the FAMEs (matrix) eluting first, following by triglycerides, diglycerides, monoglycerides, and finally, free glycerol[3]. Column choice and temperature are important parameters to obtain this separation. At 60 °C, optimum separation was obtained between the FAME fraction and the first eluting triglycerides (tripalmitin, PPP). Figure 2 illustrates this, showing the separation of a biodiesel sample spiked at 1% (w/w) with tripalmitin (PPP), dipalmitin (PP), monopalmitin (P), and glycerol. The highest response for triglycerides is obtained in positive ion detection mode, while di- and mono-glycerides give higher response in negative ion detection mode. Glycerol is clearly detected at 4.9 minutes in the SIM trace. Interestingly, the FAME matrix is hardly detected in any of these traces. Similar profiles are obtained for biodiesel spiked with triolein (OOO), diolein (OO), mono-olein (O), and glycerol, as illustrated in Figure 3.

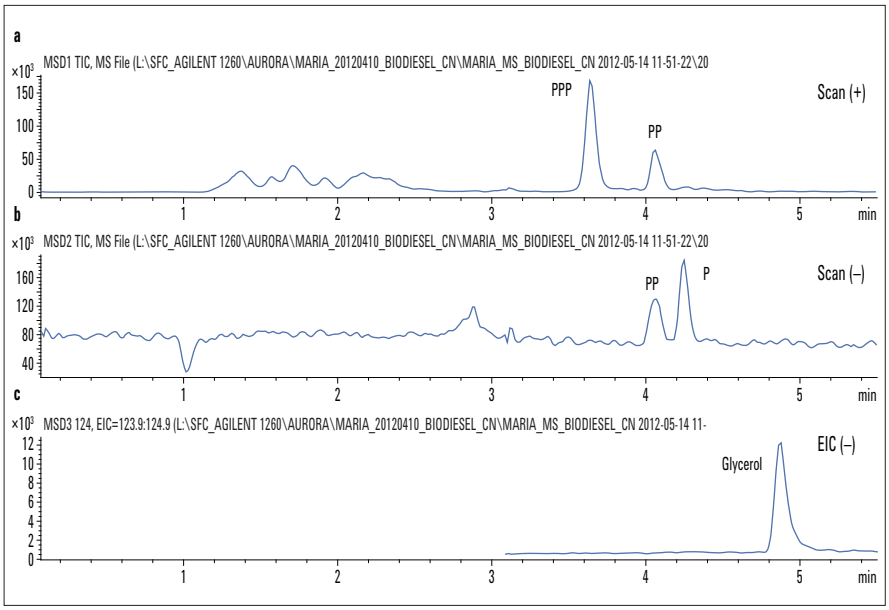

Figure 2
Biodiesel (10 mg/mL) spiked with P, PP, PPP, and glycerol at 1% (w/w). (a) Positive ion TIC, (b) Negative ion TIC and (c) EIC from SIM NEG (m/e 124.2 for Glycerol).

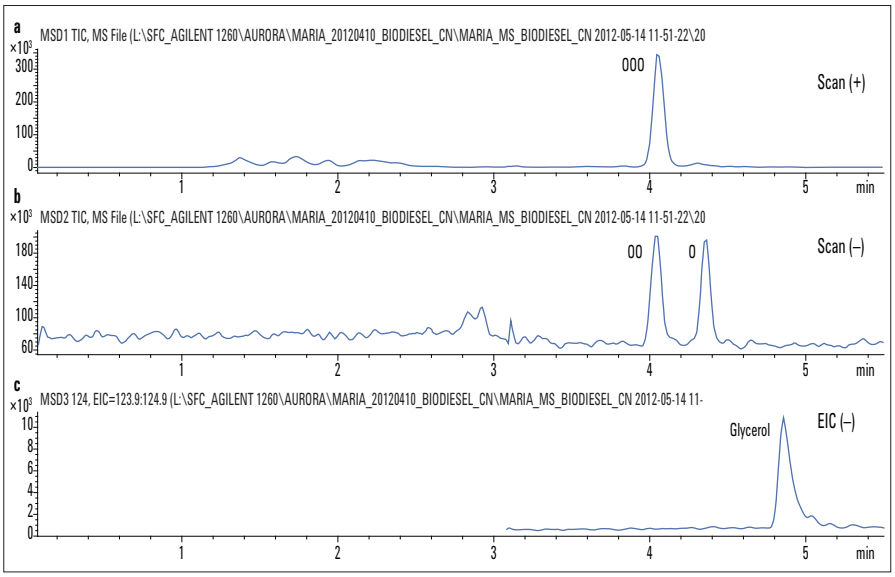

Figure 3
Biodiesel (10 mg/mL) spiked with O, OO, OOO, and glycerol at 1% (w/w). (a) Positive ion TIC, (b) Negative ion TIC and (c) EIC from SIM NEG (m/e 124.2 for Glycerol).

The performance of the SFC-MS method was evaluated by analyzing dilution series and performing a repeatability test. Table 3 summarizes the results. Good linearity was obtained in a concentration range from 0.05 to 5% spiked level. Repeatability is in the order of 5% RSD (obtained on extracted ion signals from scan TICs) for the glycerides and is better than 2% for glycerol (SIM acquisition). The detection limit was below 0.05% for all mono-, di-, and triglycerides, and 0.02% for glycerol. Figure 4 illustrates the sensitivity obtained for trace detection of free glycerol showing the analysis of a blank (glycerol free) biodiesel and a sample spiked at 0.05% level (normative level). The glycerol peak can easily be detected and quantified.

	Linearity (R^2)[1]	Range % (w/w)	Repr. (% RSD)
P	0.9986	0.05-2	5.2[2]
PP	0.9952	0.05-2	4.5[2]
PPP	0.9919	0.05-2	6.2[2]
O	0.9924	0.05-5	5.6[2]
OO	0.9972	0.05-2	7.2[2]
OOO	0.9930	0.05-2	5.1[2]
Glycerol	0.9986	0.05-5	1.4[3]

[1] 0.05, 0.1, 0.2, 0.5, 1, 2, and 5% spiked level on biodiesel sample, one injection/level

[2] Six consecutive injections of 1% (Scan)

[3] Six consecutive injections of 0.2% (SIM)

Table 3
Method performance data.

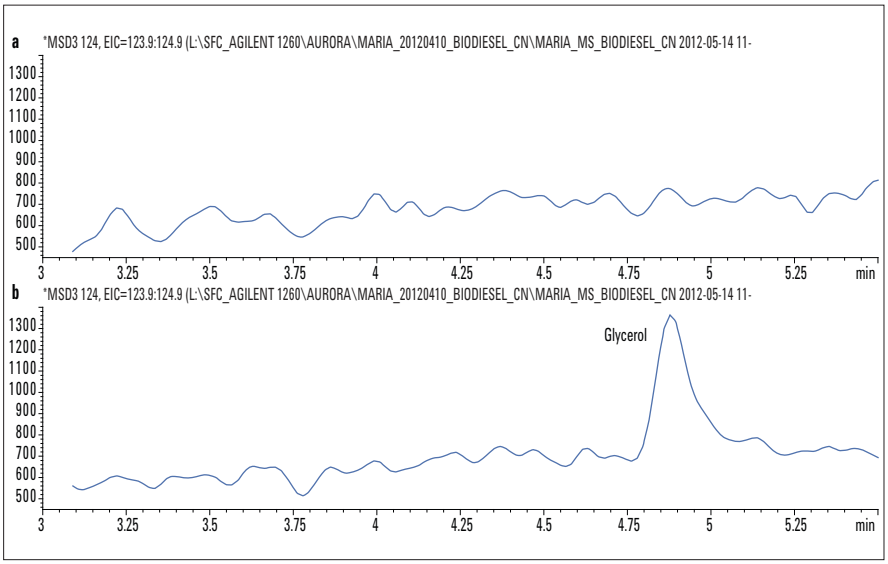

Figure 4
SIM NEG trace (m/e 124) of (a) biodiesel blank and (b) biodiesel spiked glycerol at 0.05% (w/w) in biodiesel (10 mg/mL).

As seen in Figures 2 and 3, complete separation of different triglycerides, diglycerides and monoglycerides is not achieved under these conditions. Triolein and diolein, for instance, co-elute (Figure 3). For quality control of biodiesel, this separation is, however, less important compared to the separation between FAMEs and glycerides. Moreover, since detection is done by MS in scan mode, further elucidation and characterization of glyceride contaminants is possible. Figure 5 illustrates this, showing the extracted ion chromatograms from the analysis of the biodiesel sample spiked with olein-glycerides. Using selective ions, triolein, diolein, and monolein can easily be differentiated.

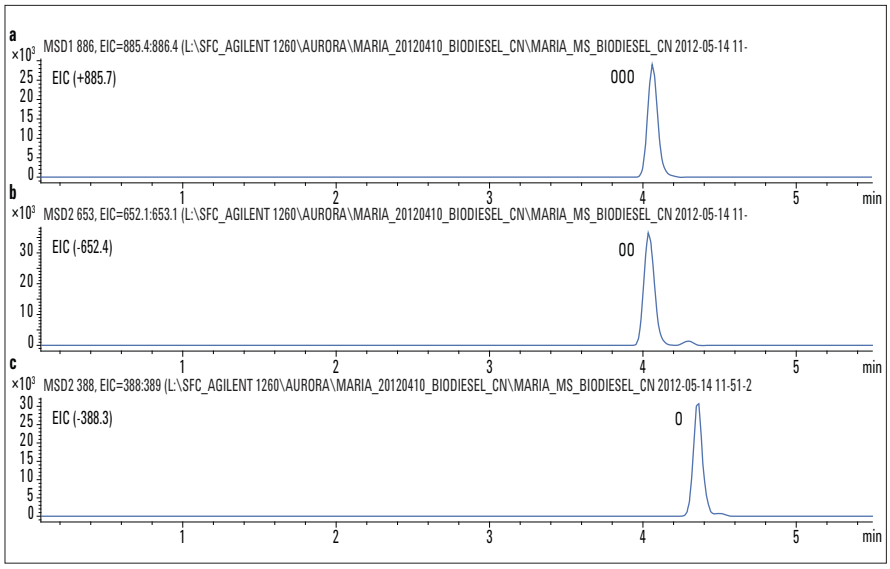

Figure 5
Biodiesel (10 mg/mL) spiked with O, OO, OOO, and glycerol at 1% (w/w). (a) EIC from TIC POS for OOO detection, (b) EIC from TIC NEG for OO detection, (c) EIC from TIC NEG for O detection.

Conclusion

The Agilent 1260 Infinity Analytical SFC System, in combination with the Agilent 6130 LC/MS Single Quadrupole System was successfully used for the analysis of impurities in biodiesel. Trace levels of free glycerol, mono-, di-, and triglycerides can be determined in biodiesel samples without the need for derivatization. Good sensitivities, complying with EU regulation, are obtained for all solutes using scan/SIM mode and fast polarity switching. Both quantification and identification of the compounds is possible within an analysis time of 6 minutes.

References

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3.
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4.
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5.
The Caloratherm (p/n AG1) and pre-heater (p/n) are products from SandraSelerity Technologies and are available through RIC. For more information, contact info@richrom.com.

www.agilent.com/chem/sfc

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