

Analysis of Biodiesel Fuel Using UPLC-Xevo G2 QTof

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Abstract

This application note describes the chromatographic separation of various glycerides classes using a 15-

minute method by ACQUITY UPLC and Xevo G2 QTof.

Benefits

- Provides invaluable information for the monitoring of glycerides classes during the various biodiesel production stages.
- Rapidly separate and identify different classes of glycerides.
- Simultaneous acquisition of key data, including both low energy precursor (MS) and high energy fragment ions (MS^E).
- Achieve both qualitative and quantitative analyses of glycerides using Xevo G2 QTof.
- The analysis of FAMES and glycerides can be achieved in a single analytical run without the need for derivatization or multiple runs.

Introduction

With the increase in petroleum prices in recent years, biodiesel is gaining significant interest as an environmentally-friendly substitute since it is renewable and cleaner burning. Biodiesel is produced from renewable sources by transesterification of triglycerides (TG) from fatty acids in vegetable oils, such as canola oil and rapeseed oil, to fatty acid methyl esters (FAME). Biodiesel also has physical and chemical properties similar to conventional petroleum-based diesel; thus it can be used in current diesel cars without the need for modifications.

However, the presence of the original unconverted oil compounds in biodiesel, such as TG, diglycerides (DG), monoglycerides (MG), and glycerine can degrade engine performance due to deposition in the engine, clogging of the filters, fuel deterioration, and the formation of toxic emissions.¹⁻³ It is therefore highly important to develop a sensitive and reliable analytical method in order to monitor and quantify the level of these glycerides classes during the various production stages, especially in the final biodiesel product. Thus for a biodiesel producer, the ideal situation would be to achieve a maximum yield of FAME compounds and minimize the presence of contaminants, such as MG, DG, TG, and glycerine.

Currently, there are several established biodiesel product standards, including ASTM (American Society for Testing and Materials) Standard D6751,⁴ and European standards EN14105⁵ and EN590,⁶ that are intended to regulate and limit the presence of these compounds.

Both GC and HPLC have been used to analyze biodiesel and its contaminants. However multiple injections with different experimental conditions are required to characterize these compounds. Tedious

derivatization of glycerides is required prior to GC analysis, whereas a long analysis time (30 to 80 minutes) is necessary for conventional HPLC analysis.

This application note describes the chromatographic separation of various glycerides classes using a 15-minute method by ACQUITY UPLC and Xevo G2 QTof.

Experimental

LC conditions

LC system:	ACQUITY UPLC
Runtime:	15 min
Column:	ACQUITY UPLC HSS T3 1.8 μ m, 2.1 x 100 mm
Column temp:	55 °C
Mobile phase A:	Acetonitrile/water (40:60) with 10 mM ammonium acetate
Mobile phase B:	Acetonitrile/isopropanol (10:90) with 10 mM ammonium acetate
Flow rate:	0.40 mL/min
Injection volume:	5 μ L PLNO injection

UPLC gradients are detailed in Table 1

Gradient

	Time (min)	Flow rate (mL/min)	%A	%B	Curve
1	Initial	0.40	80	20	–
2	10.0	0.40	0	100	6
3	13.0	0.40	0	100	6
4	13.1	0.40	80	20	6
5	15.0	0.40	80	20	6

Table 1. ACQUITY UPLC gradient for 15-minute biodiesel analysis.

MS conditions

MS system:	Xevo G2 QTof
Ionization mode:	ESI+
Scan time:	0.2 s
Capillary voltage:	3.2 kV
Sampling cone:	35.0 V
Extraction cone:	4.0 V
Source temp:	120 °C
Desolvation temp:	400 °C

Desolvation gas:	800 L/hr
Cone gas:	20 L/hr
Mass range:	50 to 1200 <i>m/z</i>
MS ^E conditions	
Low energy:	6 V
High energy ramp:	20 to 35 V

LockSpray conditions

Compound:	Leucine enkephalin
Mass:	<i>m/z</i> 556.2771 (MS ^E); <i>m/z</i> 556.2771, and <i>m/z</i> 278.1141 (MS/MS)
Capillary voltage:	3.0 kV
Collision energy:	21.0 eV

Results and Discussion

A simple five-step analytical workflow was employed to identify compounds present in the biodiesel samples, as shown in Figure 1. The biodiesel sample was diluted 100-fold with acetonitrile prior to analysis with an ACQUITY UPLC System coupled to Xevo G2 QTof MS.

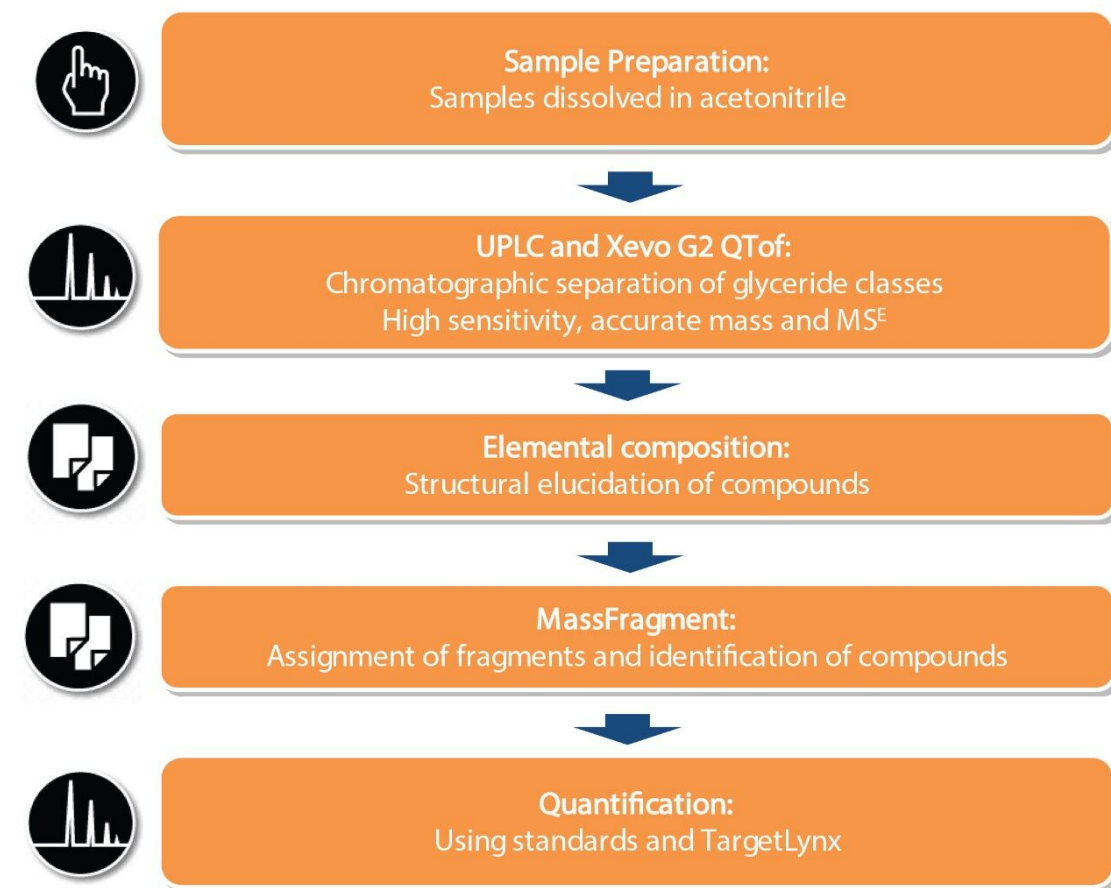


Figure 1. TOF screening workflow for biodiesel analysis.

Using the UPLC method described above, chromatographic separation of the different classes of glycerides, including FAME, MG, DG, and TG was achieved, as shown in Figure 2.

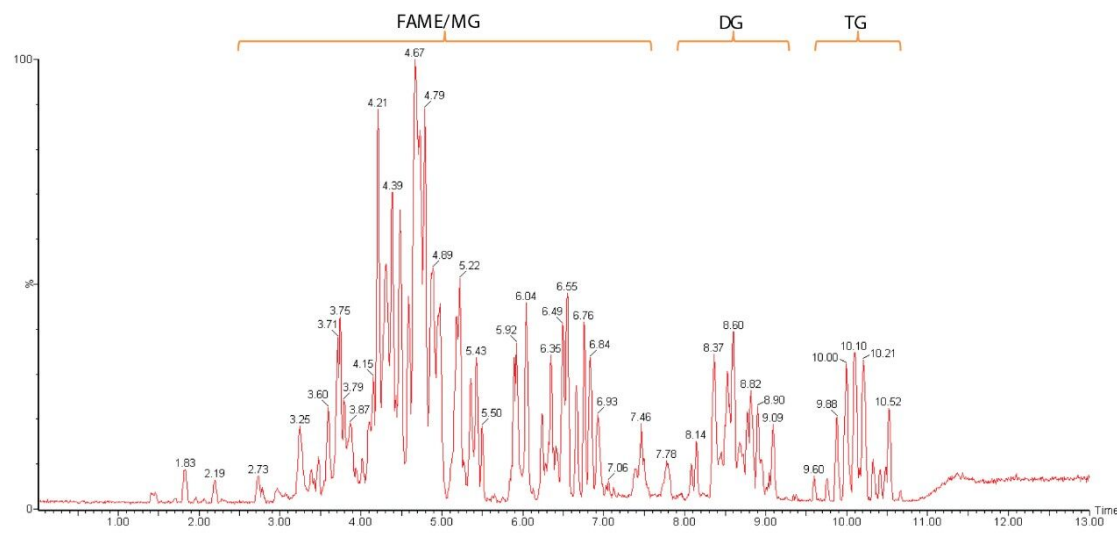


Figure 2. UPLC chromatogram of different classes of glycerides (FAME, MG, DG, and TG) present in the biodiesel sample.

All analyses were performed in MSE where molecular data (fragment ion, precursor ion, and neutral losses) were acquired in a single injection using parallel low and high collision energy MS acquisition, where low energy precursor ions and high energy fragment ions were acquired respectively.

Harnessing the high-mass accuracy of the Xevo G2 QTof, molecular formulae of both precursor and fragment ions of the unknown compounds can be determined with greater confidence and precision by the Elemental Composition Software. Elemental composition was performed on the peak with retention time 7.49 min, which consisted of three co-eluting peaks, as shown in Figure 3. Together with a lipids database (www.lipidmaps.org), the possible identities and structures of the compounds can be determined and further evaluated.

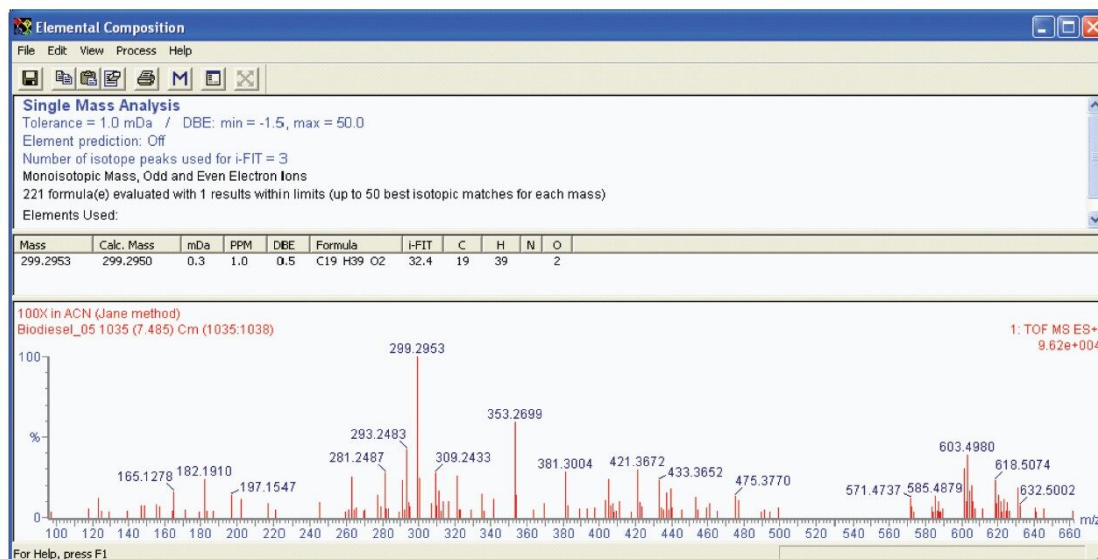


Figure 3. Elemental composition for peak m/z 299.2953 at 7.49 min. Proposed elemental composition is $C_{19}H_{39}O_2$, methyl ester of stearic acid (MeS).

Using the structures of the proposed compounds and the MS^E data acquired by the Xevo G2 QTof, MassFragment structural elucidation software was used to automatically identify and correlate the proposed product ions. MassFragment automatically identifies product ion fragments using a series of novel, chemically intelligent algorithms. This provided added confidence for the identification of the proposed compound, as shown in Figure 4. The identified compounds derived using this method are listed in Table 2.

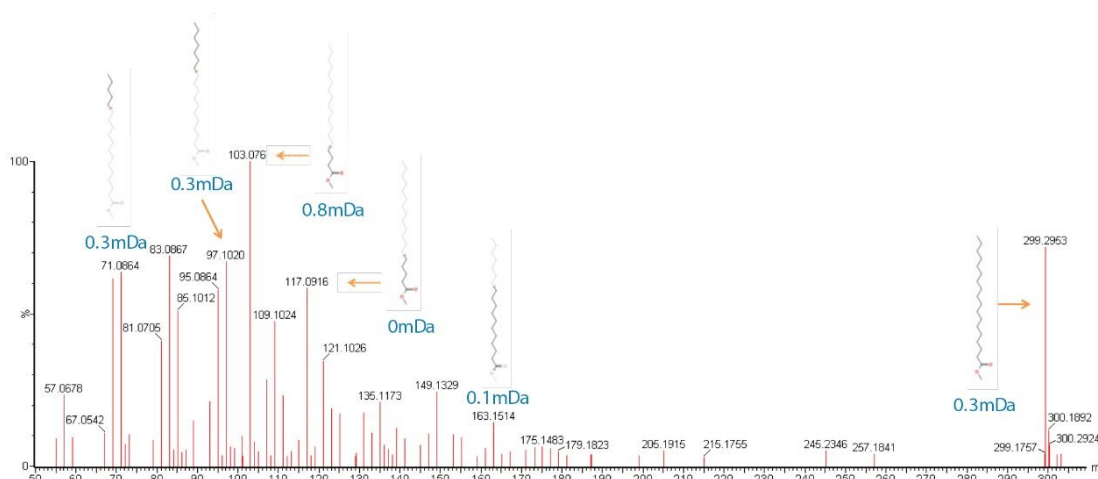


Figure 4. MassFragment Software's assignment for MS^E product ions of methyl ester stearic acid.

Retention time (min)	Measured mass (m/z)	Exact mass (m/z)	Molecular formula	Ionization	Mass accuracy (mDa/ppm)	Identity
Fatty acid methyl esters (FAME)*						
3.87	291.2323	291.2324	C ₁₉ H ₃₀ O ₂	[M+H] ⁺	-0.1/-0.3	MeSt
4.31	293.2478	293.2480	C ₁₉ H ₃₂ O ₂	[M+H] ⁺	-0.2/-0.7	MeLn
4.59	295.2637	295.2637	C ₁₉ H ₃₄ O ₂	[M+H] ⁺	0.0/0.0	MeL
5.89	325.3107	325.3107	C ₂₁ H ₄₀ O ₂	[M+H] ⁺	0.0/0.0	MeG
6.84	271.2640	271.2637	C ₁₇ H ₃₄ O ₂	[M+H] ⁺	0.3/1.1	MeP
6.93	297.2795	297.2794	C ₁₉ H ₃₆ O ₂	[M+H] ⁺	0.1/0.3	MeO
7.49	299.2953	299.2950	C ₁₉ H ₃₈ O ₂	[M+H] ⁺	0.3/1.0	MeS
Diglycerides (DG)*						
8.14	632.5260	632.5254	C ₃₉ H ₆₆ O ₅	[M+NH ₄] ⁺	0.6/0.9	LLn
8.37	634.5411	634.5411	C ₃₉ H ₆₈ O ₅	[M+NH ₄] ⁺	0.0/0.0	LL/OLn
8.60	636.5569	636.5567	C ₃₉ H ₇₀ O ₅	[M+NH ₄] ⁺	0.2/0.3	OL
8.82	638.5727	638.5723	C ₃₉ H ₇₂ O ₅	[M+NH ₄] ⁺	0.4/0.6	OO
Triglycerides (TG)*						
9.76	894.7549	894.7551	C ₅₇ H ₉₆ O ₆	[M+NH ₄] ⁺	-0.2/-0.2	LLLn
9.88	870.7557	870.7551	C ₅₅ H ₉₆ O ₆	[M+NH ₄] ⁺	0.6/0.7	PLLn
9.88	896.7708	896.7707	C ₅₇ H ₉₈ O ₆	[M+NH ₄] ⁺	0.1/0.1	LLL
10.00	872.7693	872.7707	C ₅₅ H ₉₈ O ₆	[M+NH ₄] ⁺	-1.4/-1.6	LLP/POLn
10.00	898.7864	898.7864	C ₅₇ H ₁₀₀ O ₆	[M+NH ₄] ⁺	0.0/0.0	LLO
10.10	848.7706	848.7707	C ₅₃ H ₉₈ O ₆	[M+NH ₄] ⁺	-0.1/-0.1	PPL/PPoO
10.10	874.7867	874.7864	C ₅₅ H ₁₀₀ O ₆	[M+NH ₄] ⁺	0.3/0.3	POL
10.10	900.8014	900.8020	C ₅₇ H ₁₀₂ O ₆	[M+NH ₄] ⁺	-0.6/-0.7	OOL
10.21	850.7864	850.7864	C ₅₃ H ₁₀₀ O ₆	[M+NH ₄] ⁺	0.0/0.0	PPO
10.21	876.8026	876.8020	C ₅₅ H ₁₀₂ O ₆	[M+NH ₄] ⁺	0.6/0.7	OOP
10.21	902.8182	902.8177	C ₅₇ H ₁₀₄ O ₆	[M+NH ₄] ⁺	0.5/0.6	OOO
10.21	824.7711	824.7707	C ₅₁ H ₉₈ O ₆	[M+NH ₄] ⁺	0.4/0.5	PPP
10.32	878.8184	878.8177	C ₅₅ H ₁₀₄ O ₆	[M+NH ₄] ⁺	0.7/0.8	PSO
10.32	904.8334	904.8333	C ₅₇ H ₁₀₆ O ₆	[M+NH ₄] ⁺	0.1/0.1	OOS
10.32	852.8016	852.8020	C ₅₃ H ₁₀₂ O ₆	[M+NH ₄] ⁺	-0.4/-0.5	PPS

Table 2. The retention time, masses, molecular formula, and mass accuracy of each elucidated glyceride obtained using Xevo G2 QTof.

* Me: refers to methyl ester of a fatty acid.

St: Stearidonic acid; Ln: Linolenic acid; L: Linoleic acid; G: Gadoleic acid; P: Palmitic acid; O: Oleic acid; S: Stearic acid; Po: Palmitoleic acid.

+ Refers to the different type of fatty acid bound to the glycerol backbone (C₃H₅(OH)₃).

For LLn, a molecule of linoleic acid and a molecule of linolenic acid is bound to a glycerol backbone.

The identity of several FAME compounds were further confirmed using commercially purchased standards. Methyl ester standards of linolenic acid (MeLn), linoleic acid (MeL), oleic acid (MeO), palmitic acid (MeP), and stearic acid (MeS) were successfully matched with the biodiesel sample using both the retention time and MS/MS spectra. An illustration of this workflow is shown in Figure 5, where the retention time of the MeS standard was identical to the retention time in the biodiesel sample. MS/MS was then performed on

these peaks to provide added confidence to the identity of the compound with a retention time of 7.49 min – which was the methyl ester of stearic acid.

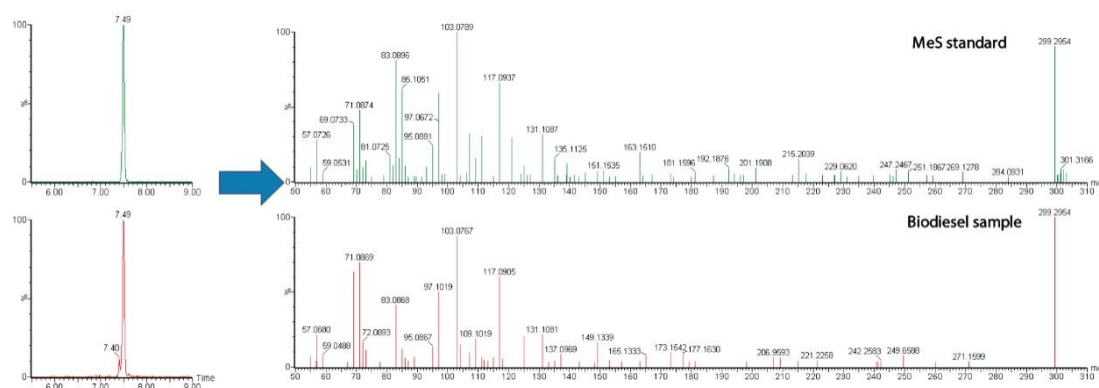


Figure 5. Extracted ion chromatogram (XIC) and MS/MS spectra (CE 20 V) of MeS at 7.49 min in both MeS standard and biodiesel sample.

Quantification of MeS was further carried out by ion extraction of the MeS peak (299.295 m/z) from the MS data using TargetLynx Application Manager. A calibration curve with a correlation coefficient of 0.99 was observed, as shown in Figure 6. The MeS level in the biodiesel sample was 4.28 mg/mL.

Compound name: MeS
 Correlation coefficient: $r = 0.998756$, $r^2 = 0.997513$
 Calibration curve: $0.166666 \cdot x + -3.31646$
 Response type: External Std, Area
 Curve type: Linear, Origin: Exclude, Weighting: Null, Axis trans: None

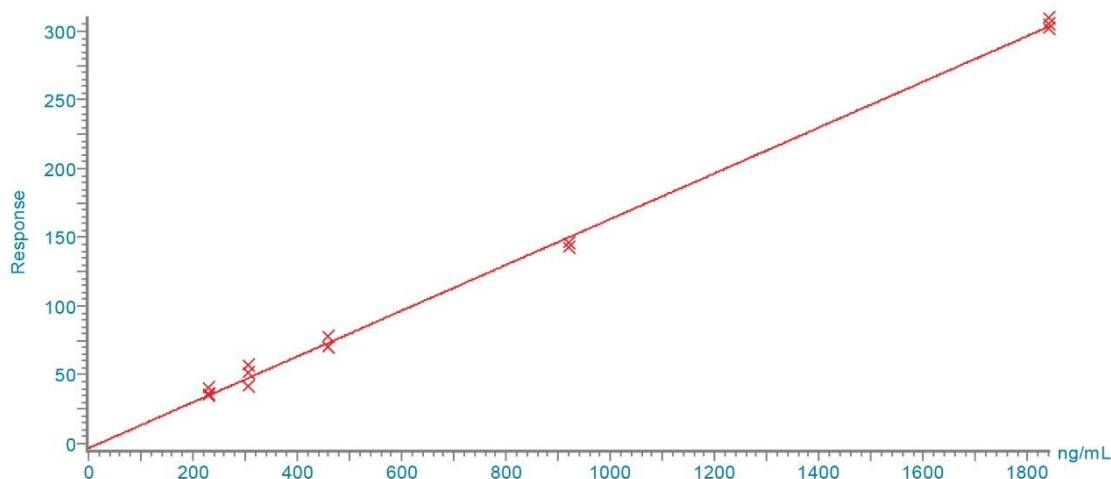


Figure 6: Calibration curve of MeS standard.

Conclusion

The experimental combination of ACQUITY UPLC and Xevo G2 QTof provides an excellent tool for the separation and identification of the various methyl esters and glycerides classes, such as MG, DG, and TG.

- The analysis of FAMEs and glycerides can be achieved in a single analytical run without the need for derivatization or multiple runs.
- The MS^E functionality of Xevo G2 QTof enables the acquisition of both low energy precursor (MS) and high energy fragment ions (MS^E) in a single rapid screening run.
- Reproducible retention times by the ACQUITY UPLC, together with the use of commercial available standards, provide added confidence in the identification of the compounds.
- Qualitative and quantitative analysis of glycerides can be achieved using a single system.
- This method allows sample to be analyzed in 15 minutes providing invaluable information for the monitoring of glycerides classes during the various biodiesel production stages.

References

1. G Knothe. Analyzing biodiesel: Standards and other methods. JAOCS. 83: 823-833, 2006.
2. G Knothe. Analytical methods used in the production and fuel quality assessment of biodiesel. ASAE. 44: 193-200, 2001.
3. M Holcapek, P Jandera, J Fischer. Analysis of acylglycerols and methy esters of fatty acids in vegetable oils and in biodiesel. Crit Rev Anal Chem. 31: 53-56, 2001.
4. American Society for Testing and Materials (ASTM) standard D6751. Standard specification for biodiesel fuel blend stock (B100) for middle distillate fuels.
5. European Committee for Standardisation. Standard EN 14105. Fat and oil derivatives - Fatty acid methyl esters (FAME) – Determination of free and total glycerol and mono-, di- triglyceride content.
6. European Committee for Standardization. Standard EN 590. Automotive fuels – diesel – requirements and test methods.

Acknowledgements:

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