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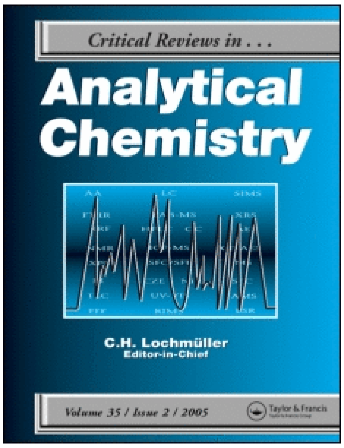

# Critical Reviews in Analytical Chemistry

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M. Holcapek; P. Jandera; J. Fischer

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# Analysis of Acylglycerols and Methyl Esters of Fatty Acids in Vegetable Oils and in Biodiesel

*M. Holčapek, P. Jandera, and J. Fischer*

**ABSTRACT:** Chromatographic techniques are compared with respect to their suitability for the analysis of acylglyerols and methyl esters of fatty acids. The identification of the individual compounds using high-performance liquid chromatography—mass spectrometry (HPLC/MS) with the atmospheric pressure chemical ionization (APCI) is discussed.

**KEY WORDS:** acylglycerols, triacylglycerols, HPLC/MS, atmospheric pressure chemical ionization, positional isomers.

## I. INTRODUCTION

Vegetable oils are comprised of various triacylglcerols (TGs) differing in the acyl groups. Individual oils have characteristic composition of TGs, and their determination allows us to find out the origin of oils or their possible adulteration in the food industry. Biodiesel, as an environmentally favorable fuel, is produced by transesterification of TGs in vegetable oils with methanol in alkaline environment yielding methyl esters of fatty acids (MEs). The concentrations of residual contaminants in biodiesel, as TGs, diacylglycerols (DGs), monoacylglycerols (MGs), fatty acids and free glycerol have to be kept as low as possible, because these contaminants can cause malfunction of the engines and formation of toxic emissions. Therefore, a sensitive analytical method is required for the determination of these contaminants in biodiesel.

## II. BACKGROUND SECTION

The liquid chromatographic system (a Model 616 pump, a Model 717+ autosampler, and a Model 996 diode-array UV detector, all from Waters, Milford, MA, USA) was connected to a Platform quadrupole mass analyzer (Micromass, England) equipped with an atmospheric pressure chemical ionization probe. The data were recorded in the mass range m/z = 35 to 1000. The APCI probe temperature was kept at 500°C and the ion source temperature at 120°C. The voltage of 20 V was applied on the sample cone. The injection volume of 10 ul, the flow rate of 1 ml/min, and the column temperature of 40°C were used in all HPLC experiments.

The HPLC method 1 (nonaqueous reversed-phase gradient elution): column Separon SGX C18 (150 × 3 mm I.D., 7 μm particle size, glass cartridge) from Tessek (Prague, Czech Republic), linear gradient from 100% methanol in 0 min to 50% methanol + 50% 2-propanol/hexane (5:4) in 15 min.

The HPLC method 2 (combined aqueous — organic and nonaqueous reversed-phase gradient elution): two NovaPack C18 columns in series (150 × 3.9 mm I.D., 7 um particle size, stainless steel cartridge) from Waters (Milford, MA, USA), linear gradient from acetonitrile-water (7:3) in 0 min to 100% acetonitrile in 20 min, hold-up step to 36 min, then to 2-propanol-acetonitrile (6:4) in 132 min.

## III. CHROMATOGRAPHIC ANALYSIS

Gas chromatography (GC) is the method of choice for the analysis of MEs, but is not very suitable for the separation of MGs and DGs, because trimethysilyl,[1] acetyl,[2] or other derivatives generally have to be prepared prior to the

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chromatographic separation. The analysis of undervatized TGs is tedious and requires short capillary columns with a good temperature stability.[2] The characterization of unknown oils by means of GC analysis of fatty acid derivatives was reviewed by Spitzer.[3] This approach to the analysis of TGs is simplified and provides only the information on the total amount of the fatty acid residues, but the information about the real composition of the individual TGs is lost.

Reversed-phase HPLC makes possible the analysis of all methyl esters of fatty acids, tri-, di-, and monoacylglycerols without derivatization. The separation of TGs differing in the equivalent carbon number (ECN is the number of carbon atoms in all acyls minus twice the number of double bonds) can be easily achieved using either isocratic[4,5] or gradient[6,7] nonaqueous reversed-phase HPLC, but the separation within the class of compounds with the same ECN is more tedious and requires careful optimization of the chromatographic conditions.[8,9] Silver ion HPLC is a common method of choice for the separation of lipids differing in the number and the positions of the double bonds and can be successfully coupled with APCI-MS.[10] Normal-phase HPLC on a cyanopropyl silica column provides the separation of lipids into the classes, such as alkyl esters, free fatty acids, TGs, 1,3-DGs, 1,2-DGs, and MGs, without the resolution of various species within the individual classes.[11]

The separation of TGs with different ECN can be achieved by nonaqueous reversed-phase HPLC with mobile phases containing methanol, acetonitrile, 2-propanol, dichloromethane, or hexane. Figure 1 illustrates the separation of a mixture of rapeseed oil and biodiesel under the gradient conditions of the HPLC method 1. The TGs with the same ECN values are coeluted under these conditions. In addition to the TGs marked on the chromatogram, TGs containing the acyls of palmitic or steric acids are present as minor constituents in the rapeseed oil. The acylglycerols and methyl esters of palmitic acid (C16:0) are coeluted with these species containing the same number of oleic acid (C18:1), because these two acids have the same ECN. The HPLC method 1 was used for the monitoring of the production of biodiesel from rapeseed oil and for the quantitative determination of TGs differing in the ECN.[12]

The combined aqueous-organic gradient as the first step followed by a nonaqueous gradient of the mobile phase (the HPLC method 2) allows the separation of some TGs, DGs, MGs, and MEs with the same ECN number (Figure 2). This method can provide detailed information about the acylglycerols composition in various vegetable oils.[9]

APCI is a soft ionization technique and makes possible both molecular mass determination, the structure assignment and identification from the characteristic fragment ions in the positive-ion APCI mass spectra of the individual TGs, DGs, MGs, and MEs listed in Figure 3. The quazimolecular ions $[M+H]^+$ were observed for all compounds. In addition to these ions, the series of the aliphatic ions similar to the conventional electron ionization mass spectrometry were observed in the spectra of MEs and MGs under the ionization conditions used.

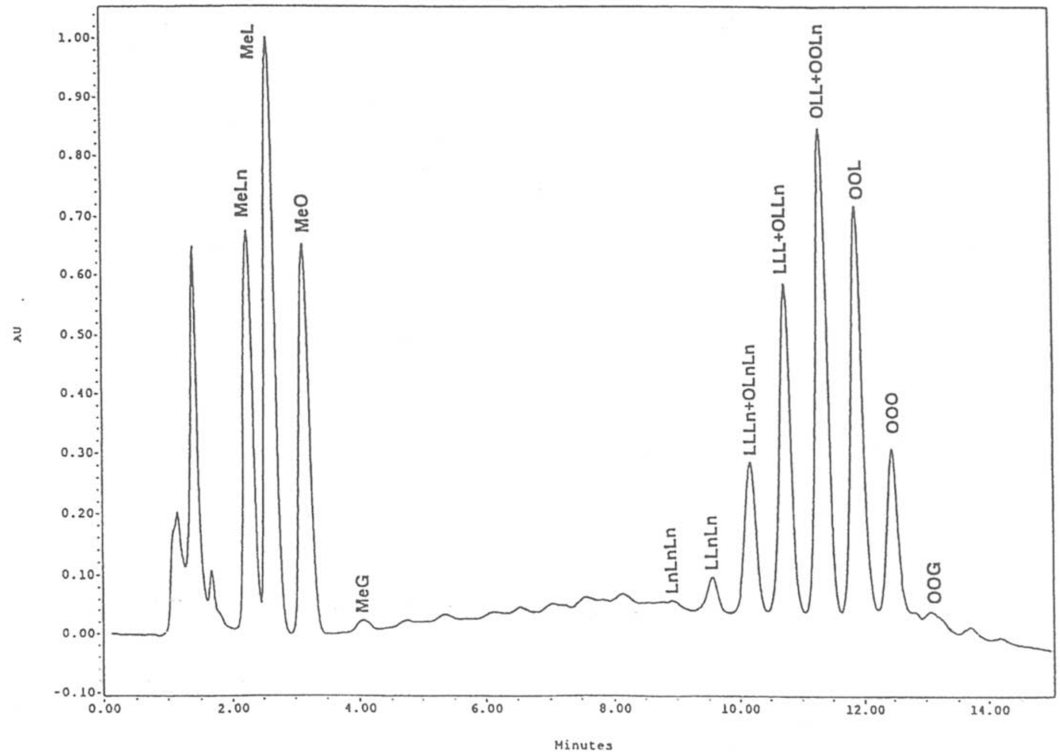

**FIGURE 1.** The chromatogram of a mixture of rapeseed oil and biodiesel under the conditions of the HPLC method 1.

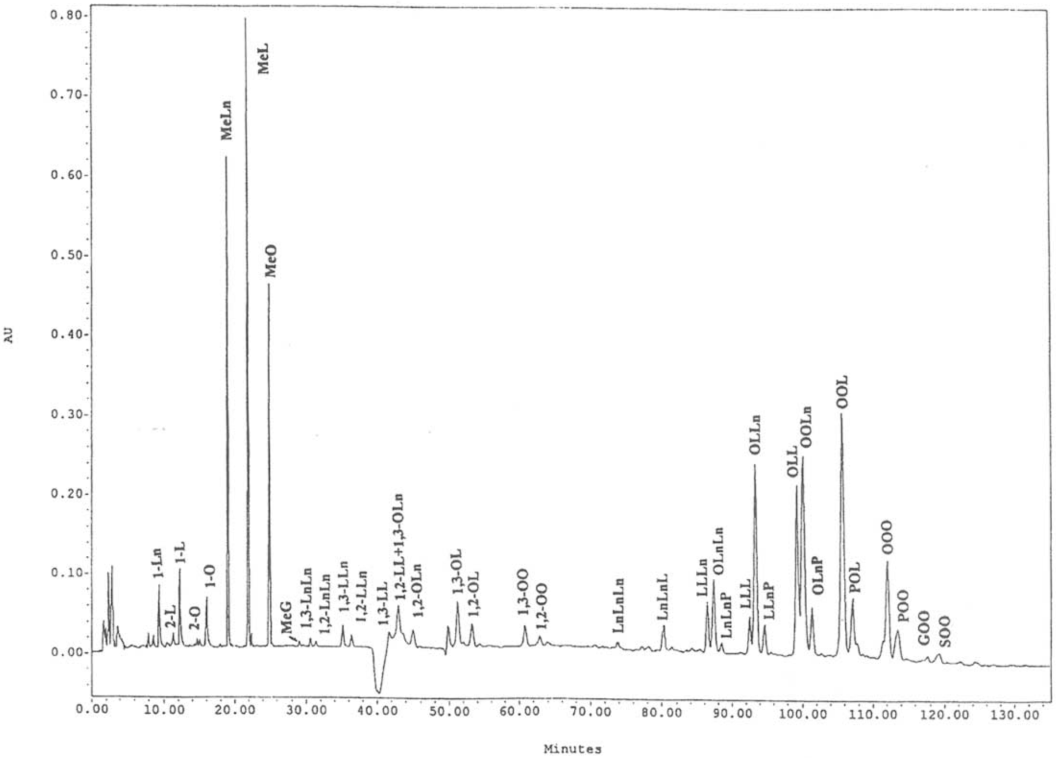

**FIGURE 2.** The chromatogram of rapeseed oil partially transesterified with methanol under the conditions of the HPLC method 2.

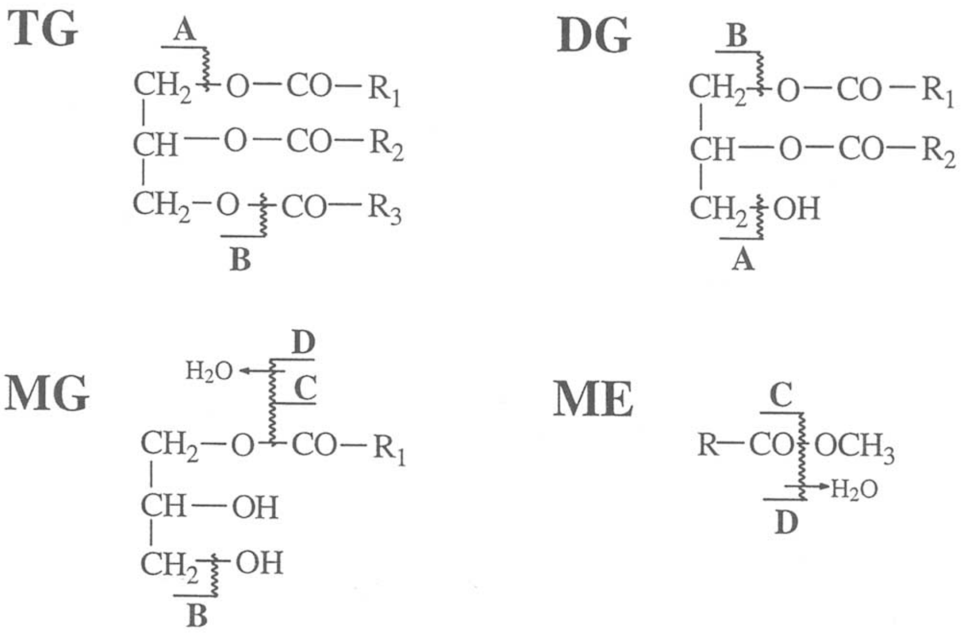

**FIGURE 3.** The characteristic fragment ions of tri- (TG), di- (DG), and monoacylglycerols (MG) and methyl esters of fatty acids (ME) using atmospheric pressure chemical ionization mass spectrometry.

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