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Ultrahigh performance supercritical fluid chromatography of lipophilic compounds with application to synthetic and commercial biodiesel

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ABSTRACT

Ultrahigh performance supercritical fluid chromatography (UHPSFC) in combination with sub-2 μm particles and either diode array ultraviolet (UV), evaporative light scattering, (ELSD), or mass spectrometric (MS) detection has been shown to be a valuable technique for the determination of acylglycerols in soybean, corn, sesame, and tobacco seed oils. Excellent resolution on an un-endcapped single C_{18} column (3.0 mm × 150 mm) with a mobile phase gradient of acetonitrile and carbon dioxide in as little as 10 min served greatly as an improvement on first generation packed column SFC instrumentation. Unlike high resolution gas chromatography and high performance liquid chromatography with mass spectrometric detection, UHPSFC/MS was determined to be a superior analytical tool for both separation and detection of mono-, di-, and tri-acylglycerols as well as free glycerol itself in biodiesel without derivatization. Baseline separation of residual tri-, di-, and mono-acylglycerols alongside glycerol at 0.05% (w/w) was easily obtained employing packed column SFC. The new analytical methodology was applied to both commercial B100 biodiesel (i.e. fatty acid methyl esters) derived from vegetable oil and to an "in-house" synthetic biodiesel (i.e. fatty acid ethyl esters) derived from tobacco seed oil and ethanol both before and after purification via column chromatography on bare silica.

1. Introduction

The development of new technologies that afford the production of fuels that are obtained from renewable resources is driven by both environmental concerns and fossil fuel deficiency. Biofuels, such as the biodiesel produced via base catalyzed *trans*-esterification of vegetable oils and animal fats using either ethanol or methanol in the U.S. is a promising alternative fuel source [1]. Biodiesel has similar chemical structure and energy content to petro-diesel in terms of fuel quality. Compared to petroleum diesel, however, biodiesel can reduce CO_2 emissions approximately 78%. As an energy source, biodiesel (i.e. fatty acid alkyl esters) should have certain criteria [2]. The feed-stocks available for producing biodiesel are highly dependent upon the region, climate, soil conditions, and geography. For example, rapeseed is the dominant feedstock for biodiesel production in Europe, soybean in the United States and palm oil in tropical countries such as Malaysia and the Philippines. Coconut is another feedstock used for biodiesel production in the Pacific Rim region.

The advantages of biodiesel in addition to being considered more environmentally friendly than petro diesel are higher octane number and higher combustion efficiency. Biodiesel is usually commercially blended with petro-diesel to 5% biodiesel. A major problem related to the use of biodiesel is its inherent instability to oxidation. For example, biodiesel degradation can change the fuel properties by formation of gums, acids, insolubles, and other oxidation products. The level of impurities in commercial biodiesel B100 as cited in ASTM D 6751 for (a) tri-acylglycerol which is unreacted starting material, (b) di-acylglycerol and mono-acylglycerol which are reaction intermediates, and (c) free glycerol which is a by-product should be 0.02 wt% for glycerol itself and 0.24 wt% total glycerol which includes mono-, di-, and tri-acylglycerols as well as glycerol itself. It is interesting to note that each year producers of biodiesel create more than a million tons of "just" glycerol worldwide much of which goes to waste [3]. The presence and

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concentration of these impurities obviously lead to biodiesel products that vary in complexity, polarity, solubility, and volatility.

Analysis of acylglycerols typically follows two routes: (1) analysis of the whole molecule which can be a mixture of glycerol itself, mono-acylglycerol, di-acylglycerol, and tri-acylglycerol and (2) analysis of the range of fatty acids that originally esterify the glycerol molecule [4]. Analyses of whole acylglycerol molecules have been reported in the literature and in ASTM D 6584. Such techniques use GC, HPLC, TLC or a combination of these [5]. Most GC techniques employ flame ionization detection; while HPLC frequently uses evaporative light scattering [6] and mass spectrometric [7] detection. For glycerol, mono-, and di-acylglycerols, the analytical method is based on conversion of the molecular hydroxyl function into silyl derivatives in the presence of pyridine and N-trimethylsilyltrifluoroacetamide followed by high temperature GC on a short capillary column with thin film thickness using on-column injection and flame ionization detection [4]. Most techniques, on the other hand, dedicated to HPLC are designed to deal with tri-acylglycerols (TAGs). In most of these cases, the methodology employs multi-dimensional chromatography wherein argentation chromatography is one of the components designed to achieve separation of unsaturated acylglycerols

Packed column supercritical fluid chromatography (SFC), on the other hand, can be essentially considered to be a more powerful tool for lipid analyses [8] than either HRGC or HPLC. In comparison to GC, acylglycerols are separated via SFC at a much lower temperature and compared to HPLC, different selectivity and shorter analysis times are obtained with SFC. Contrary to numerous references that have appeared in the older literature, polar solutes such as free glycerol can be analyzed via SFC without derivatization. For example, TAGs have been analyzed by packed column SFC using for example 2-ethylpyridine [9] and silver-ion exchange [10] stationary phases. Using a reversed phase stationary phase such as octadecylsilica [11], the SFC separation may be based upon carbon number (i.e. total number of carbons in all fatty acids) and/or on the number of double bonds. In a more recent reversed phase separation [12], three Zorbax SB-C_{18} columns (4.6 mm × 250 mm, 5 μm) were coupled in series. Temperature was 25 °C with an acetonitrile/methanol modifier gradient. With a flow rate of 2.5 mL/min, a mixture of eight TAGs eluted between 45 and 60 min, although they were not baseline resolved. On the other hand, separation of selected unsaturated vegetable oils on the silver loaded column was mainly based on the number of double bonds. Evaporative light scattering detection results were reproducible and provided enhanced sensitivity compared to UV detection.

An improvement in chromatographic performance for similar lipophilic analytes is reported here. Fast determination of residual glycerol, free fatty acids, and mono-, di-, and tri-acylglycerols in both synthetic biodiesel prepared “in-house” and commercial B100 biodiesel (e.g. typically referred to as fatty acid alkyl esters) is described here using ultrahigh performance supercritical fluid chromatography (UHPSFC). UHPSFC is a relatively new separation technique that uses compressed carbon dioxide as the primary mobile phase [13]. It takes advantage of sub-2 μm particle chromatography columns and advanced chromatography systems that are designed to achieve fast and reproducible separation with high efficiencies and unique selectivity. The established UHPSFC/MS approach has potential application in lipidomics and food testing as a complementary method alongside HPLC–MS and HRGC–MS, as the former can separate both polar and non-polar lipids in a single run to improve both detection limits and peak shape. Unlike HRGC/MS, low volatile and very long chain fatty acids (>24 carbon atoms) can be easily analyzed without concern for analyte degradation. In this regard, the possibilities for lipid analyses via UHPSFC were recently illustrated by Jones et al. [14] who performed the separation of neutral and amphipathetic lipids using both BEH (unbonded organic/inorganic silica hybrid) and HSS C_{18} (un-endcapped silica) columns packed with sub-2 μm particles.

Studies using UHPSFC coupled with photodiode array (DAD), mass spectrometry (MS), and evaporative light scattering (ELSD) detection for the separation of TAGs isolated from tobacco, corn, sesame, and soybean seed oils are presented here. A single un-endcapped C_{18} column with acetonitrile-modified carbon dioxide was employed for separation of all seed oils. Excellent chromatography of associated, hydroxyl-containing di-acylglycerols, mono-acylglycerols, and glycerol wherein methanol was employed as a secondary modifier in addition to acetonitrile was also achieved.

2. Experimental

2.1. Sample description

Pure fatty acid ethyl esters (C_{16}, C_{18}, $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$) were purchased from Sigma–Aldrich (St. Louis, MO) and mixed to form a model biodiesel. Pure mono-, di-, and tri-octadecylglycerol plus free glycerol and soybean oil were also obtained from Sigma–Aldrich. All standards were prepared in a 50/50 dichloromethane/methanol (DCM/MeOH) mixture. The model biodiesel was prepared in the same mixture as a 5% (v/v) solution. Tobacco seed oil was obtained from R.J. Reynolds Tobacco Co. (Winston-Salem, NC). Soybean oil, corn oil, and sesame seed oil were obtained from Sigma–Aldrich (St. Louis, MO). Five percent of the different oils was dissolved in DCM/MeOH (1/1, v/v) for both UV and ELS detection and then 0.1% for the Xevo G2 QTof MS.

2.2. Chromatographic conditions

Supercritical fluid chromatography experiments were performed using a Waters Acquity UPC^2 system (Milford, MA, USA) equipped with (a) high pressure mixing binary solvent delivery manager, (b) fixed loop design autosampler, (c) active back pressure regulator, (d) column compartment with active heating and column switching control, (e) photodiode array (PDA) and evaporative light scattering (ELS) detectors. Experiments were carried out using an ACQUITY UPC^2 HSS C_{18} SB column (150 mm × 3.0 mm, 1.8 μm) at a temperature of 25 °C. The mobile phase consisted of compressed CO_2 (component A) and either 100% acetonitrile or acetonitrile/methanol (90:10) (component B). The mobile phase flow rate was maintained at 1–2 mL/min under all different gradient conditions. Backpressure was maintained isobarically and automatically by a backpressure regulator (ACQUITY CCM) at a pressure of 1500 psi. The injection volume was varied 2–8 μL for both PDA and ELSD; while, 0.1 μL was the volume for MS detection. Photodiode array detection was monitored at a wavelength range 190–400 nm with a reference of 400–500 nm. The Waters ACQUITY ELSD detector was operated with nebulizer cooling, drift tube: 50 °C, gas pressure: 40 psi and gain 10, make up flow (isopropyl alcohol) was added at 0.2 mL/min before the ELSD. The solvent flow was split prior to the back pressure regulator for ELSD (split ratio 1:3) and MS electrospray probe.

2.3. Mass spectrometric conditions

Mass spectrometry was performed using Xevo G2 QTof (Waters Corp., Milford, MA, USA). The solvent flow was split post PDA detection using a pre-BPR flow Upchurch cross 1/16 PEEK splitter. CO_2-miscible make-up solvent (MeOH in 10 mM NH_4OAc), delivered by a HPLC 515 make-up pump (Waters Corp., Milford, MA, USA), was added at a flow rate of 0.2 mL/min and mixed with the chromatographic effluent to aid ionization. A fraction of the total flow was directed from the union to the ESI source through