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Gas chromatography–vacuum ultraviolet spectroscopy for analysis of fatty acid methyl esters

Hui Fan [a,1], Jonathan Smuts [b,1], Ling Bai [a], Phillip Walsh [b], Daniel W. Armstrong [a], Kevin A. Schug [a,*]

[a] Department of Chemistry & Biochemistry, The University of Texas at Arlington, Arlington, TX, USA
[b] VUV Analytics, Inc., Austin, TX, USA

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ABSTRACT

A new vacuum ultraviolet (VUV) detector for gas chromatography was recently developed and applied to fatty acid methyl ester (FAME) analysis. VUV detection features full spectral acquisition in a wavelength range of 115–240 nm, where virtually all chemical species absorb. VUV absorption spectra of 37 FAMEs, including saturated, monounsaturated, and polyunsaturated types were recorded. Unsaturated FAMEs show significantly different gas phase absorption profiles than saturated ones, and these classes can be easily distinguished with the VUV detector. Another advantage includes differentiating *cis*/*trans*-isomeric FAMEs (e.g. oleic acid methyl ester and linoleic acid methyl ester isomers) and the ability to use VUV data analysis software for deconvolution of co-eluting signals. As a universal detector, VUV also provides high specificity, sensitivity, and a fast data acquisition rate, making it a powerful tool for fatty acid screening when combined with gas chromatography. The fatty acid profile of several food oil samples (olive, canola, vegetable, corn, sunflower and peanut oils) were analyzed in this study to demonstrate applicability to real world samples.

1. Introduction

Dietary fats and oils consist of triglycerides, which release free fatty acids through hydrolysis. Fatty acids (FAs), the building blocks of fats and oils, are aliphatic monocarboxylic acids primarily of even carbon number (4–24 carbons), although odd carbon number chains can also be found in nature, e.g. in ruminant milks (C5–C11) and the lipids of certain bacterial species (C13–C19). Depending on the number of double bonds in the hydrocarbon chain, as well as the conformation, FAs are classified as saturated (SFAs), monounsaturated (MUFAs), polyunsaturated (PUFAs), and *cis*/*trans*-FAs. A growing body of research suggests MUFAs and PUFAs decrease blood cholesterol and reduce cardiovascular risk factors (Dawczynski, Martin, Wagner, & Jahreis, 2010; Dawczynski et al., 2013), while SFAs and *trans*-FAs have opposing effects (Hunter, Zhang, & Kris-Etherton, 2010; Krauss et al., 2000; Micha & Mozaffarian, 2010; Sun et al., 2007). Many PUFAs are considered essential nutrients and can only be obtained through diet. Well-known examples of PUFAs are omega-3 and omega-6 FAs, which contain several double bonds, with the first double bond placed on the third and the sixth carbon counting from the methyl end, respectively. Mainly found in fish and plant seed oils, these PUFAs have been shown to provide a number of other potential health benefits besides cardiovascular benefits, including anti-cancer (Brown, Wahle, Cascio, Pertwee, & Heys, 2011; Stehr & Heller, 2006), anti-inflammatory (Wall, Ross, Fitzgerald, & Stanton, 2010), and antioxidant properties (Giordano & Visioli, 2014). As a result, there is a rapid growth in the demand in food chemistry and related areas for FA profiling of different food sources.

The American Oil Chemists' Society has recommended gas chromatography with flame ionization detection (GC-FID) as the standard method for FA screening. A common practice in FA GC analysis is to esterify the free acid with methanol to form methyl esters (FAMEs). While FID offers excellent quantitative performance and is suitable for routine FA screening with assistance from retention indices, it provides minimal qualitative information. Identification of FAs usually relies on additional detection methods (Viron, Saunois, André, Perly, & Lafosse, 2000). GC coupled with mass spectrometric detection (GC–MS) is a more powerful and practical method for FA screening and profiling. However, MS falls short in certain areas where isobaric analytes are prominent, especially where *cis*/*trans*-isomers need to be differentiated. Studies also show that electron impact ionization tends to cause

* Corresponding author at: 700 Planetarium Pl., Box 19065, Arlington, TX 76019-0065, USA.
E-mail address: kschug@uta.edu (K.A. Schug).
[1] These authors contributed equally to this work.

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double bond migration (Budzikiewicz & Busker, 1980), which leads to ambiguous results regarding the structure of the FAs.

Recently, a vacuum ultraviolet (VUV) detector capable of measuring absorption spectra in the 115–240 nm wavelength range has been developed and used to address many of the limitations known for GC-FID and GC–MS (Schug et al., 2014). Photons in the VUV region can probe high-energy electronic transitions and essentially all chemical bonds, rendering the VUV detector a powerful universal detector that provides both qualitative and quantitative information. It has been applied towards analyses of various small molecules and their isomers (Schug et al., 2014), multiclass pesticides (Fan, Smuts, Walsh, Harrison, & Schug, 2015), natural gas, and off-gassing from lithium-ion batteries (Bai et al., 2015). Strong absorption of these compounds and highly featured spectral patterns were observed. Isomers such as *m*- and *p*-xylene that are difficult to be separated chromatographically and differentiated by mass spectrometry can be deconvolved using VUV detection and appropriate software tools (Schug et al., 2014).

To further explore the capabilities of VUV spectroscopy, the new detector was used for analysis of fatty acid methyl esters (FAMEs). VUV absorption spectra of selected FAMEs including SFAs, MUFAs, and PUFAs were collected and evaluated. Special attention was given to demonstrating the ability of the detector to differentiate *cis*/*trans*-isomers and deconvolute the contribution of multiple components to signals obtained from co-eluting chromatographic signals. The use of spectral filters and combinations of spectral filters particularly aided compound and compound class signal discrimination. The methods were further applied for profiling the fatty acid composition in a number of commercial food oils.

2. Materials and methods

2.1. Materials

A 30 mg/mL Food Industry FAME mix (P/N 35077) of 37 FAME components in dichloromethane was purchased from Restek Corporation (Bellefonte, PA). A conjugated linoleic acid methyl ester mixture was purchased from Sigma–Aldrich (St. Louis, MO). Where co-elutions occurred, the pure reference compounds (*cis*-9-C16:1, C17:0, C18:0 and *cis*-10-C17:1) were purchased from Sigma–Aldrich (St. Louis, MO). All standards were analyzed as received. The Food Industry FAME mix was further diluted to lower concentrations in dichloromethane (ACS reagent, 99.6%, Aldrich Chemical Co., Inc., Milwaukee, WI) for linearity determination.

Three brands of olive oil as well as a brand each of canola, vegetable, corn, sunflower and peanut oil were purchased from the local grocery store. These oil samples were transesterified using 3 N methanolic HCl (Supelco, Bellefonte, PA) before analyzing by GC. Briefly, ~40 mg of the oil was weighed into a 2 dram glass vial on analytical scale. To this was added 3 mL of 3 N methanolic HCl. The vial headspace was briefly flushed with argon and then sealed with a PTFE lined cap. The mixture was then heated overnight at 50 °C. Upon completion of the reaction, the methanol was rotary evaporated off and 1 mL of hexanes was added to the vial and vortexed thoroughly. A 100 μL aliquot was then transferred to a low-volume insert in a GC vial and analyzed by GC/VUV.

2.2. Methods

The VUV absorption spectra for the FAME standards were recorded on a VGA-100 VUV detector (VUV Analytics, Inc., Austin, TX), which was coupled to a Shimadzu GC-2010 gas chromatograph (Shimadzu Scientific Instruments, Inc., Columbia, MD). In some cases, for the sake of clarity by way of noise reduction, VUV spectra were smoothed with a moving average of ±37 points. The legitimacy of this smoothing is shown in the Supplementary information where comparisons are made between the raw and smoothed data. A SLB-IL111 (60 m × 0.250 mm × 0.20 μm), from Supelco, Bellefonte, PA, and a Stabilwax-DA (30 m × 0.25 mm × 0.25 mm) from Restek Corporation were used as GC columns in this study. The SLB-IL111 column is a new high polarity stationary phase technology based upon ionic-liquid chemistry. The new stationary phase exhibits unique selectivity in the separation of FAMEs (Anderson, Ding, Ellern, & Armstrong, 2004; Anderson, Ding, Welton, & Armstrong, 2002; Armstrong, He, & Liu, 1999) and many other analytes (Huang, Han, Zhang, & Armstrong, 2007; Payagala et al., 2009; Qi & Armstrong, 2007; Seeley, Seeley, Libby, Breitbach, & Armstrong, 2008). A Shimadzu AOC-20i Auto Injector was used to inject 1.0 μL of sample. The temperature of the GC injector was 250 °C with a split ratio of 10:1; for concentrated samples a split ratio of 50:1 was used. The temperatures of the VUV transfer line and the flow cell were 300 °C. The pressure of makeup gas (nitrogen) was 0.25 psi. Helium was used as the GC carrier gas at a constant velocity of 28 cm/s. The oven profile for the SLB-IL111 column was as follows: 100 °C (hold for 5 min); ramp at 5 °C/min to 185 °C and hold for 18 min. The oven profile for the Stabilwax-DA column was as follows: 100 °C (hold for 5 min); ramp at 5 °C/min to 240 °C and hold for 20 min.

2.3. VUV deconvolution

The capability to deconvolve co-eluted signals is a powerful feature. Quantification in VUV spectroscopy follows the well-established Beer–Lambert Law, which indicates that VUV absorbance is additive in multicomponent absorption analysis. The critical information is the absorption cross section (directly related to the molar extinction coefficient). An absorption cross section, expressed in units of cm^2/molecule, is the ability of a molecule to absorb a photon of a particular wavelength. The wavelength dependence of an analyte's absorption cross section is determined by simply measuring a reference absorbance of the pure analyte. An absolute absorption cross-section for an analyte of known concentration can be determined by using an appropriate internal standard of known concentration whose cross-section is also known.

VUV reference spectra and absorption cross sections are stored in the VUV Spectral Library. During a deconvolution process, the measured absorption spectra contained in the chromatographic peak are fit by a linear combination of the reference spectra of the co-eluting analytes using linear regression (Press, 1992). The result is a distinct peak for each of the co-eluting analytes. These new peaks are the individual contributions of analytes to the original response, and can be used in standard peak area/height quantification procedures.

3. Results and discussion

3.1. VUV spectral features of FAMEs

3.1.1. Saturated FAMEs

Fig. 1A shows the VUV absorption spectra of all SFAs in the Food Industry FAME mix standard. The spectra are dominated by absorption in the shorter wavelength range (120–160 nm), due to the high-energy $\sigma \rightarrow \sigma^*$ transition in the carbon–carbon single bonds. While the lower molecular weight FAMEs exhibit distinct curvature (C4:0–C10:0), the C10:0+ FAMEs exhibit strong similarity. It is not uncommon to find a search for C12:0 rendering a match to C16:0 or even C24:0. Essentially the molecule reaches a size, in this case 200 amu for C11:0 FAME, where the only change