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

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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

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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

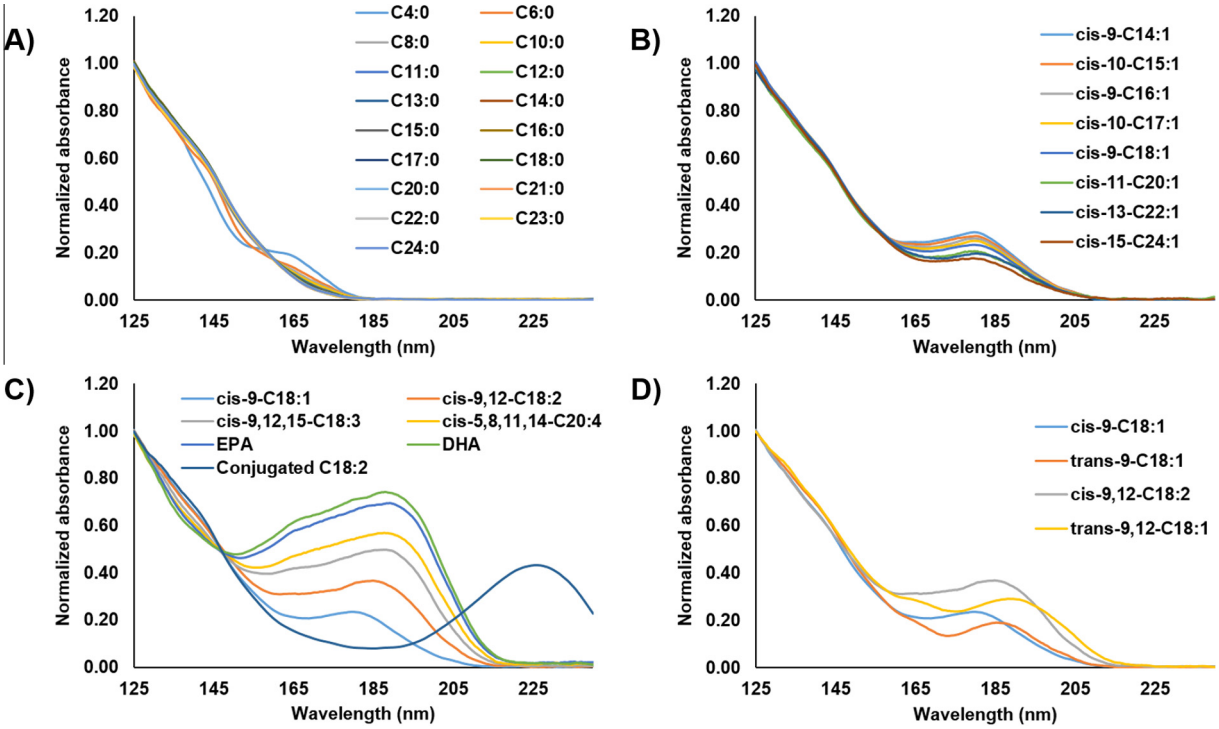


Fig. 1. VUV spectral features of (A) saturated FAMEs, (B) *cis*-mono-unsaturated FAMEs, (C) increasing degrees of unsaturation in FAMEs including conjugation, and (D) *cis*- vs. *trans*-isomerization. *Note:* These spectra were smoothed as outlined in Section 2.2.

taking place in its linear structure is a single methylene group (mass = 14 amu) which reflects in little change in the absorption spectrum. Fortunately, these SFAs are easily separated and can be identified by retention index (Kittiratanapiboon, Jeyashoke, & Krisnangkura, 1998). This similarity of spectra for SFAs reflects one of the strengths of GC/VUV which allows the user to classify SFAs by comparison with a single spectrum. It should be noted that the VUV spectra for branched SFAs have not been studied yet and may yield slightly different spectra. Some distinct VUV absorption features in comparison of linear versus branched alkanes have been reported (Schug et al., 2014).

3.1.2. Mono-unsaturated FAMEs

A comparison of Fig. 1A and B reveals that MUFAs may be easily distinguished from SFAs, due to their stronger absorbance in the 170–200 nm wavelength range. This feature was exploited in Fig. 2 to selectively extract MUFAs and PUFAs (20 in total) from the 37 component Food Industry FAME mix by using a 185–200 nm spectral filter. Fig. 1B also shows how the VUV spectra of *cis*-MUFAs change as a function of carbon number. The λ_{max} of the 170–200 nm region remains unchanged, namely 180 nm, regardless of chain length (see Table 1). The ratio of the absorption at 180 nm to that at 125 nm however becomes slightly smaller, ranging from 0.29 for *cis*-9-C14:1 to 0.18 for *cis*-15-C24:1 (Table 1). Thus, unlike with the SFAs above, MUFAs may be accurately matched with the VUV library since the spectral shape is dependent on the size of the FAME. Unfortunately, positional isomers for a given carbon number cannot be distinguished as has been shown in a separate study (Weatherly et al., 2015). It is anticipated, though, that a terminal olefin or one which is conjugated with the carboxylate moiety will afford distinct spectra.

3.1.3. The effect of increasing degrees of unsaturation

Because of the importance of MUFAs and PUFAs in health and nutrition, it would be particularly useful to have additional means to distinguish between them that complements retention index and EI fragmentation data. The VUV absorption is highly sensitive to the structure of the molecule, especially changes in bonding that introduce electron-rich units. Fig. 1C and Table 1 show how the λ_{max} exhibits an increasing bathochromic shift ranging from 180 nm for MUFAs to 190 nm for EPA and DHA. This can be explained by the decrease in the energy required by the multiple $\pi \rightarrow \pi^*$ transition in PUFAs, in comparison to the single $\pi \rightarrow \pi^*$ transition in MUFAs (Kalsi, 2007). An even greater bathochromic shift (226 nm) is observed when just two double bonds are conjugated as is the case for conjugated linoleic methyl ester. This important class of FAMEs, naturally occurring in ruminant-derived foods, may be selectively monitored by the judicious use of a spectral filter such as 220–230 nm. GC–MS on the other hand would not be able to achieve this as it would see the same molecular ion ($m/z = 294$) for all C18:2 FAMEs regardless of the positioning.

In the course of this study, an additional tool was developed to help classify chromatographic peaks. Since the 125–160 nm and 170–200 nm spectral filters are commonly used, the VUV spectra for each FAME in the Food Industry FAME mix was characterized by summing the absorption data in each range and expressing the two sums as a ratio, i.e. 170–200 nm relative to 125–160 nm. This is reflected in Fig. 3A and, as expected, the ratio increases as a function of the number of double bonds present. It was found that all SFAs exhibited a relative ratio of 2%; relative ratios for MUFAs were ~28%, PUFAs with two double bonds were ~46%, and PUFAs with three double bonds were ~72%, while remaining PUFAs were >80%.

This characterization scheme was then applied to a segment of the GC–VUV chromatogram found in Fig. 2. By integrating the peak areas under the two spectral filters and expressing them as a ratio, the results may be compared against Fig. 3A and classified accordingly (see Fig. 3B). As may be seen in the table in Fig. 3B, the scheme correctly classifies these peaks. It is clear that a relative ratio of 0.18 is almost halfway between a SFA and a MUFA and thus it is not unreasonable to suppose that a coelution of both may be

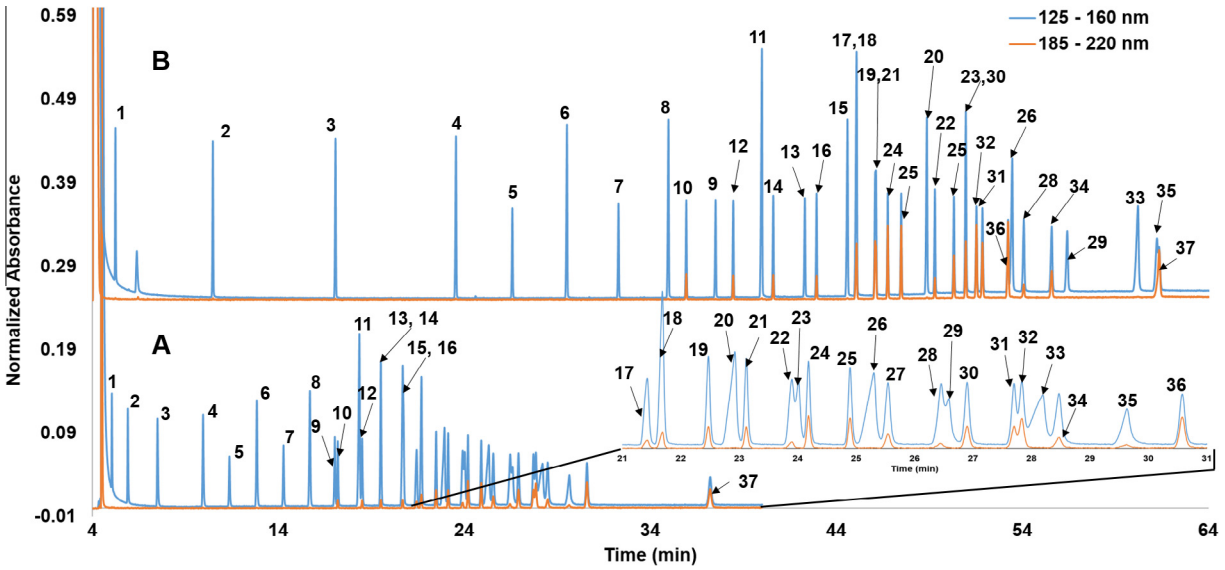


Fig. 2. A separation of the Food Industry FAME mix containing 37 components analyzed on the (A) SLB-IL111 and (B) Stabilwax-DA. The data are presented using the 125–160 nm (blue) and 185–200 nm (orange) spectral filters. Analyte: (1) C4:0, (2) C6:0, (3) C8:0, (4) C10:0, (5) C11:0, (6) C12:0, (7) C13:0, (8) C14:0, (9) C15:0, (10) *cis*-9-C14:1, (11) C16:0, (12) *cis*-10-C15:1, (13) C17:0, (14) *cis*-9-C16:1, (15) C18:0, (16) *cis*-10-C17:1, (17) *trans*-9-C18:1, (18) *cis*-9-C18:1, (19) *trans*-9,12-C18:2, (20) C20:0, (21) *cis*-9,12-C18:2, (22) *cis*-11-C20:1, (23) C21:0, (24) *cis*-6,9,12-C18:3 (GLA), (25) *cis*-9,12,15-C18:3 (ALA), (26) C22:0, (27) *cis*-11,14-C20:2, (28) *cis*-13-C22:1, (29) C23:0, (30) *cis*-8,11,14-C20:3, (31) *cis*-11,14,17-C20:3, (32) *cis*-5,8,11,14-C20:4, (33) C24:0, (34) *cis*-13,16-C22:2, (35) *cis*-15-C24:1, (36) *cis*-5,8,11,14,17-C20:5 (EPA), (37) *cis*-4,7,10,13,16,19-C22:6 (DHA). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
The λ_{max} for the spectra shown in Fig. 1 as well as the absorbance ratio of the λ_{max} ($A_{\lambda_{max}}$) to that at 125 nm (A_{125nm}).

FAME	λ_{max} (nm)	$A_{\lambda_{max}}/A_{125nm}$
cis-9-C14:1	180	0.29
cis-10-C15:1	180	0.27
cis-9-C16:1	180	0.26
cis-10-C17:1	180	0.25
cis-9-C18:1	180	0.23
cis-11-C20:1	180	0.21
cis-13-C22:1	180	0.20
cis-15-C24:1	180	0.18
trans-9-C18:1	185	0.19
cis-9,12-C18:2	185	0.37
trans-9,12-C18:2	188	0.29
cis-11,14-C20:2	185	0.33
cis-13,16-C22:2	185	0.29
Conjugated C18:2	226	0.43
cis-9,12,15-C18:3 (ALA)	188	0.51
cis-6,9,12-C18:3 (GLA)	188	0.50
cis-11,14,17-C18:3	188	0.46
cis-8,11,14-C20:3	188	0.45
cis-5,8,11,14-C20:4	188	0.58
cis-5,8,11,14,17-C20:5 (EPA)	189	0.70
cis-4,7,10,13,16,19-C22:6 (DHA)	188	0.75

occurring. This scheme tends to fail when trace MUFAs or PUFAs coelute with large concentrations of SFAs, the relative ratio becoming spectrally diluted. In such a case, a tailor made spectral filter, as is shown in Fig. 2, would be better. Use of this combined spectral filter allows for a quick relative assessment of the fatty acid composition of an oil sample.

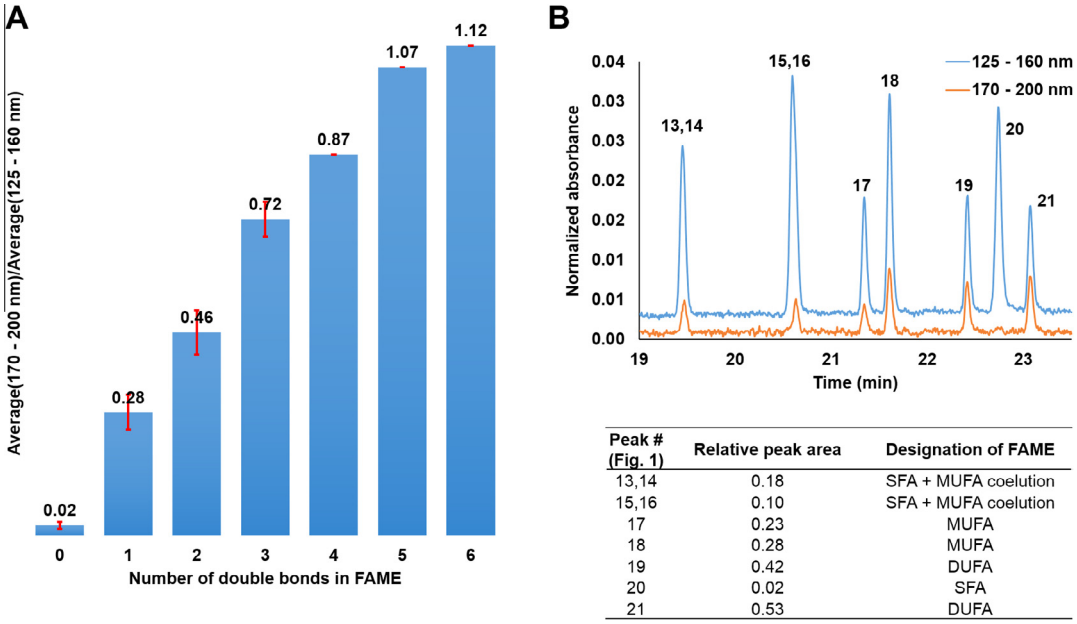
3.1.4. Determination of cis/trans-isomers

Mass spectrometry instruments have difficulty discriminating isomeric and isobaric compounds. While high-end mass analyzers such as triple quadrupole instruments are capable of differentiating isobaric species operated under selected reaction monitoring mode (SRM), it is costly and complex to implement these instruments in routine analysis. Nevertheless, isomer differentiation remains a challenge even for costly mass analyzers with high resolving power and tandem mass spectrometry capabilities (Croley, White, Callahan, & Musser, 2012). Specialty columns are usually necessary to chromatographically resolve these compounds (Croley et al., 2012; Delmonte, Hu, Kia, & Rader, 2008).

VUV spectroscopy provides a niche solution to this problem unlike any other detector. The degree of spectral broadening in solution phase absorption often masks important spectral features in closely related anlaytes. In gas phase VUV, however, not only are all the common carrier gases used in GC (helium, hydrogen, and nitrogen) essentially transparent (no UV cut off consideration necessary), but they also do not interact with the analyte to mask/modify its spectra. As a result, closely related isomeric compounds can be differentiated. This is demonstrated in the determination of *cis*/*trans*-FA distributions in oils and fats, an essential process for assessing their potential health impacts. Fig. 1D shows that VUV absorption spectra for methyl oleate (C18:1n9c), methyl elaidate (C18:1n9t), methyl linoleate (C18:2n6c), and methyl linoelaidate (C18:2n6t) are unique and easily distinguishable from each other. The λ_{max} of the *trans* isomers clearly exhibit a longer bathochromic shift than the *cis* isomers, namely 185 nm vs. 180 nm for C18:1 and 188 nm vs. 185 nm for C18:2 (see Table 1). The λ_{valley} for the *trans* isomer exhibits an analogous shift with respect to the *cis* isomer. Interestingly, the ratio of the $A_{\lambda_{max}}/A_{125nm}$ for the *trans* isomer is smaller than that for the *cis* isomer, namely 0.19 vs. 0.23 for C18:1 and 0.29 vs. 0.37 for C18:2 (see Table 1). The result is that these isomers can be distinguished and deconvoluted as has been explored in a separate study (Weatherly et al., 2015). It must be noted that multiple bond configurations of *cis* and *trans* isomers present together in the same molecule have not yet been studied.

3.2. GC–VUV analysis of food oils

Food oils are an important commodity in our daily living. The monitoring of *trans* fats is only one aspect of food oil quality control. Another important quality parameter not often thought of by the consumer is genuineness. In particular, it should be noted



Peak # (Fig. 1)	Relative peak area	Designation of FAME
13,14	0.18	SFA + MUFA coelution
15,16	0.10	SFA + MUFA coelution
17	0.23	MUFA
18	0.28	MUFA
19	0.42	DUFA
20	0.02	SFA
21	0.53	DUFA

Fig. 3. (A) Spectral filter ratios (170–200 nm)/(125–160 nm) calculated from the VUV spectra of the 37 Food Industry FAME mix components (17 saturated, 9 mono-unsaturated, 4 di-unsaturated and tri-unsaturated and 1 each of tetra-, penta- and hexa-unsaturated). (B) A portion of the Food Industry FAME mix chromatogram (Fig. 1) classified based upon the relative peak area ratios of the two spectral filters, i.e. the peak area in the 170–200 nm filter divided by the 125–160 nm peak area. *Note:* SFA = saturated fatty acid, MUFA = mono-unsaturated fatty acid and DUFA = di-unsaturated fatty acid. For peak identification see Fig. 2.

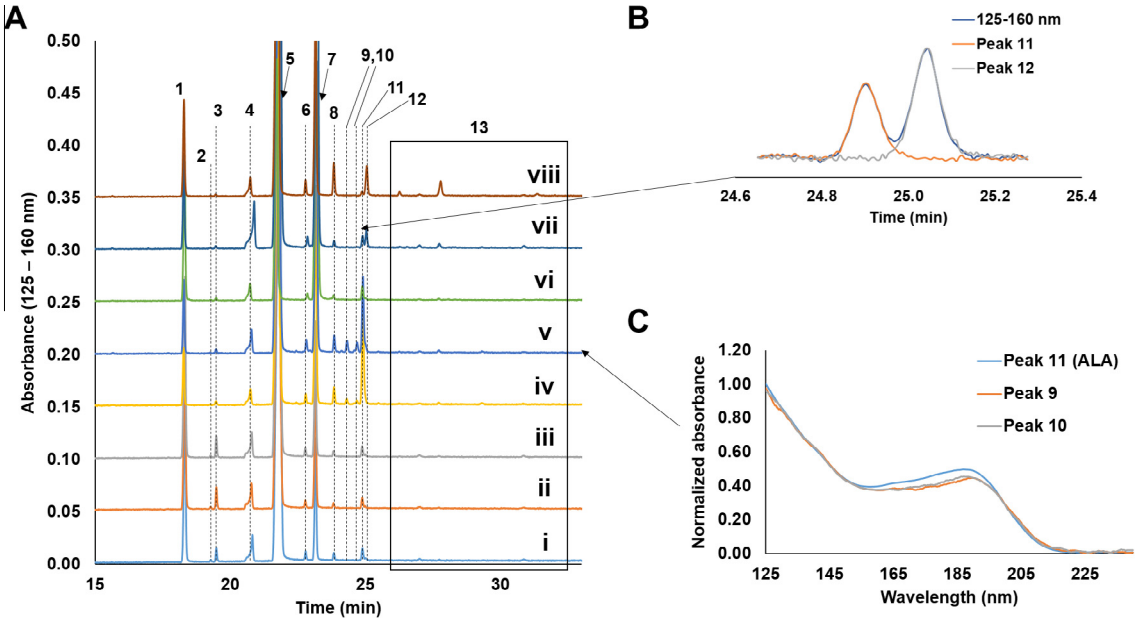


Fig. 4. (A) Overlay of GC–VUV chromatograms for several food oils: (i) olive oil brand 1, (ii) olive oil brand 2, (iii) olive oil brand 3, (iv) canola oil, (v) vegetable oil, (vi) corn oil, (vii) sunflower oil and (viii) peanut oil. (B) The VUV deconvolution of peak 9 from peak 10 for sunflower oil. (C) The smoothed VUV spectra for peak 9 and 10 compared with peak 11. For peak identification see Table 2.

that olive oil is the most adulterated food commodity on the market due to its price. This adulteration most often takes the form of dilution with an inferior food oil. In this study we analyzed three brands of olive oil ranging from $4 to $34 per bottle by GC–VUV. Additionally we analyzed five other food oils (canola, vegetable, corn, sunflower and peanut) which are most often used to adulterate olive oil. The GC–VUV method, as described in Section 2.2, was shown to be linear over three orders of magnitude with $R^2 > 0.99$. The LOD under these conditions was approximately 0.01% by weight, but an order of magnitude increase could be attained in the splitless mode (LOD of 0.002–0.004 wt.% were determined as shown in Supplementary information Table S1). Linearity were all greater than $R^2 = 0.99$ and linear range was three orders of magnitude. The slopes of the calibration curves were used as response factors for the quantification of the FAME composition. Integrated peak areas were corrected by dividing by the response factor followed by normalizing the total to 100%.

An overlay of the chromatograms for each oil is shown in Fig. 4A and results are tabulated in Table 2. At first glance, the reader should see that many peaks are in common in each oil and this is possibly why they are used as adulterants for olive oil. Upon closer examination, it should become evident that the relative

Table 2
The FAME composition for all the food oils reported in Fig. 4 reported in terms of area% using the 125–160 nm spectral filter.

Analyte	Peak #	Weight%							
		Olive 1	Olive 2	Olive 3	Canola	Vegetable	Corn	Sunflower	Peanut
Palmitic acid methyl ester	1	11.73	13.74	14.06	4.12	4.52	12.30	4.28	7.84
Unknown MUFA (C15–C16)	2	0.10	0.12	0.11	0.04	0.04	0.03	0.03	0.03
cis-9-Hexadecenoic acid methyl ester	3	0.57	1.15	1.05	0.25	0.21	0.16	0.07	0.09
Stearic acid methyl ester	4	2.68	2.98	2.73	2.04	2.51	2.05	4.05	2.23
cis-9-Octadecenoic acid methyl ester	5	75.42	70.27	71.34	62.15	59.73	29.89	62.05	62.49
Arachidic acid methyl ester	6	0.48	0.48	0.53	0.80	0.65	0.44	0.16	1.14
cis-9,12-Octadecadienoic acid methyl ester	7	7.38	9.76	8.64	19.31	23.95	53.35	27.05	18.01
cis-11-Eicosenoic acid methyl ester	8	0.42	0.34	0.35	1.19	0.63	0.41	0.31	2.41
C18:3 A	9	<0.01	<0.01	<0.01	0.34	0.71	<0.01	<0.01	<0.01
C18:3 B	10	<0.01	<0.01	<0.01	0.33	0.70	<0.01	<0.01	<0.01
cis-9,12,15-Octadecatrienoic acid methyl ester	11	0.62	0.73	0.57	8.49	5.41	1.08	0.52	0.25
Behenic acid methyl ester	12	0.19	0.07	0.23	0.40	0.52	0.09	0.87	3.01
C22 + saturated FAMEs	13	0.39	0.36	0.40	0.52	0.45	0.18	0.61	2.49
Total area		100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

concentrations are what matter. While the composition of these oils are discussed in terms of FAMEs, the reader must bear in mind that in the actual oil, these fatty acids are present as components of triglycerides.

Olive oil consists primarily of methyl oleate and here it is shown to be consistently >70% while all of the other oils are <62% (Table 2). The largest methyl oleate concentration (olive oil 1) was attributed to the most expensive brand. The next most significant component in olive oil is methyl palmitate followed by methyl linoleate. Here, the cheaper brands of olive oil (brands 2 and 3) exhibited relatively higher concentrations compared to brand 1. The only other oil with as high a value for methyl palmitate was corn oil while all of the oils exhibited significantly higher methyl linoleate concentrations compared to olive oil (>18%).

An example of deconvolution is presented in Fig. 4B for sunflower oil. Here, α-methyl linolenate (ALA) and methyl behenate (11 and 12) were partially co-eluting and could be baseline resolved using spectral deconvolution. This deconvolution was applied to all of the chromatograms and allowed for their separation for wide range of compositions (Table 2). The amount of ALA in olive oil was on average about 0.64% but was significantly higher for canola (8.49%) and vegetable oil (5.41%). The label on the vegetable oil bottle indicated that it consisted of canola and sunflower oil, but gave no indication of the ratio. In addition to ALA, two other peaks present in canola and vegetable oil matched C18:3 spectra. These have been assigned as C18:3 A and C18:3 B respectively and their spectra are compared with ALA (Fig. 4C). The overall shape is similar to ALA as is the positioning of the λ_{max} (188 nm), while the relative ratio scheme discussed above yielded values of 0.746 for ALA, 0.703 for C18:3 A and 0.666 for C18:2 B. Furthermore, the components do not match the retention time for GLA (see peak 24 in Fig. 2A) nor for any of the other PUFAs with three double bonds. We therefore speculate that these are additional C18:3 isomers, either all *cis* configuration or mixtures of *cis* and *trans* isomers within a given molecule.

Lastly, all the oils exhibited similar C22+ SFA values (0.20–0.60%) except for peanut oil which was 2.49%. This value was almost completely dominated by methyl lignocerate. In addition to this, peanut oil exhibited an equally large value for methyl behenate, significantly higher than all the other oils (3.01%). These two SFAs are peculiar to peanut oil and can serve as markers for its identification.

4. Conclusions

VUV absorption spectroscopy has been successfully applied to FAME analysis and the analysis of several food oil samples. VUV absorption spectra for unsaturated FAMEs are unique and easily distinguished from saturated FAMEs. The gas phase VUV absorption spectra are highly sensitive to the molecular structure of the analyte, such as the degree of unsaturation and presence of conjugation. As a result, *cis*/*trans*-isomers can be easily differentiated in VUV due to their distinct spectra and conjugated isomers may be easily distinguished from non-conjugated isomers.

Although many official methods recommend GC-FID and GC–MS for FAME analysis, GC–VUV has been demonstrated to be a novel and powerful combination with niche selectivity. In analyses of complex samples using GC–VUV, chromatographic separation can be buttressed by deconvolution capabilities of the VUV detector and software. This may be especially useful in fast GC applications, where co-elution of target compounds is often inevitable.

Conflict of interest

Disclosure: K.A.S. is a member of the scientific advisory board for VUV Analytics, Inc.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2015.08.004.

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