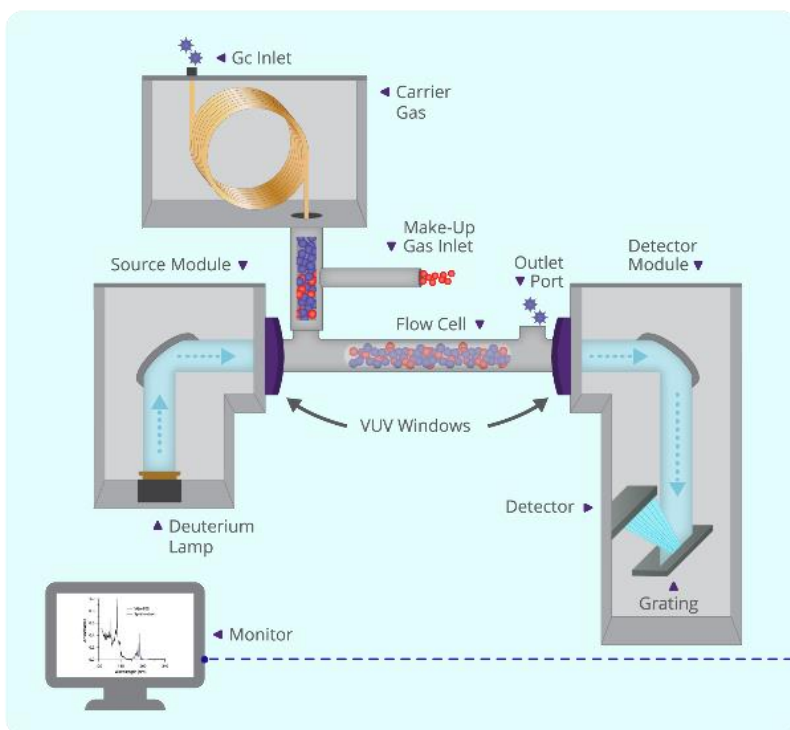
Differentiation and Determination of Fatty Acid Methyl Esters by Gas Chromatography – Vacuum Ultraviolet Spectroscopy

APPLICATION NOTE

Introduction

Gas Chromatography (GC) is a mature analytical technology used predominately for separating and quantifying the components of complex organic chemical mixtures. The addition of mass selective detectors has extended the use of GC to include compound identification. A wide range of columns and sample introduction techniques allow GC to solve most analytical problems for volatile and semi-volatile compounds. Absorption spectroscopy, although widely used in liquid chromatography, has not been widely adopted in gas chromatography due to the relative lack of absorption for most analytes in gas phase within traditional regions of the electromagnetic spectrum.

Recent scientific advances[1] have allowed for the use of very short wavelengths (120nm – 240nm), a region where essentially all chemical compounds absorb. The use of this region, which has traditionally been restricted to bright source synchrotron facilities, has been critically enabled by the use of specially coated reflective optics paired with a back-thinned coupled device (CCD) which can simultaneously assess absorption features across the spectrum for peaks eluting from a GC column. The unique, characteristic spectra that result allow for the identification or classification of unknowns as well as the capacity to deconvolute co-eluting, spectrally distinct compounds. The VGA-100 vacuum ultraviolet (VUV)



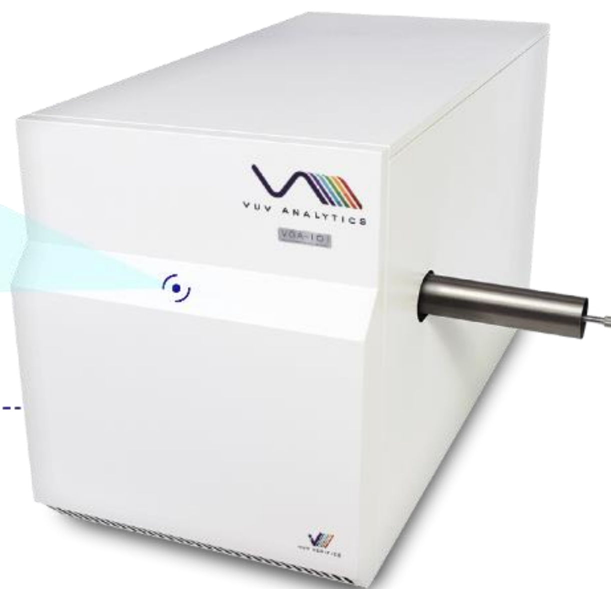


Figure 1. Schematic (not to scale) of the GC-VUV instrument. Dimensions of the VGA-100 detector are 13 in. (w) x 30 in. (d) x 17 in. (h). Flow cell volume is ~80 µL. Path length is 10 cm.

VUV ANALYTICS

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detector is the very first bench-top spectrometer capable of detection in this short-wavelength, VUV region, and can be connected to any standard GC through a heated transfer line. A schematic of the VGA-100, which is capable of capturing full spectra at a sampling rate as high as 100 Hz, is shown in Figure 1. Additionally, a selected set of VUV spectra captured with the VGA-100 are displayed in Figure 2.

In this paper, we demonstrate the use of the VGA-100 for the quantitation and identification of a test mixture of 37 saturated and unsaturated fatty acid methyl ester (FAME) compounds. These compounds are the transformation products of fatty acids, which are commonly present in biodiesel and various consumer products. The analysis of FAMEs provides serious identification challenges for modern mass selective detectors due to the prevalence of isomeric analyte species.

Conversely, VUV universal detection, which makes use of highly featured gas absorption spectra, yields powerful identification capabilities, especially when paired with the ever-growing VUV spectral libraries.

Experimental

➢ Sample Preparation

Fatty acids themselves are not amenable to GC analysis due to their limited volatility, but can be esterified to their corresponding FAME compounds, which can then be separated and analyzed via GC. A 37 component FAME standard mix was purchased at Supelco (CRM47885). A mixture of 10 FAMEs in a rapeseed oil standard mixture (Restek AOCS #3) was also analyzed.

➢ GC Separation

A Shimadzu GC-2010 gas chromatograph, coupled with a VUV Analytics VGA-100 VUV detector was utilized for the separation of the FAME mixture. An autosampler was used to deliver a 0.2 µL injection volume. The split ratio was set to 5:1 and the injection port was held at a constant 250 °C temperature throughout. The column utilized was a highly polar SLB-IL 111 capillary column (dimensions: 60 m x 0.25 mm x 0.2 µm) from Supelco and it was operated in the constant pressure mode (185 kPa) with helium carrier gas. The oven profile was set to start at 140 °C (held for 12 minutes) and then increased to 170 °C at a rate of 5 °C/min followed by 30 °C/min to 185 °C (held for 30 minutes).

➢ Detection

The transfer line and flow cell temperatures were set at 300 °C and 275 °C, respectively. The make-up gas (Argon), which can be used to alter the sample residence time in the detector cell, was set to 0.25 psi. Full spectra ranging from 120 – 240 nm were acquired at a 10Hz

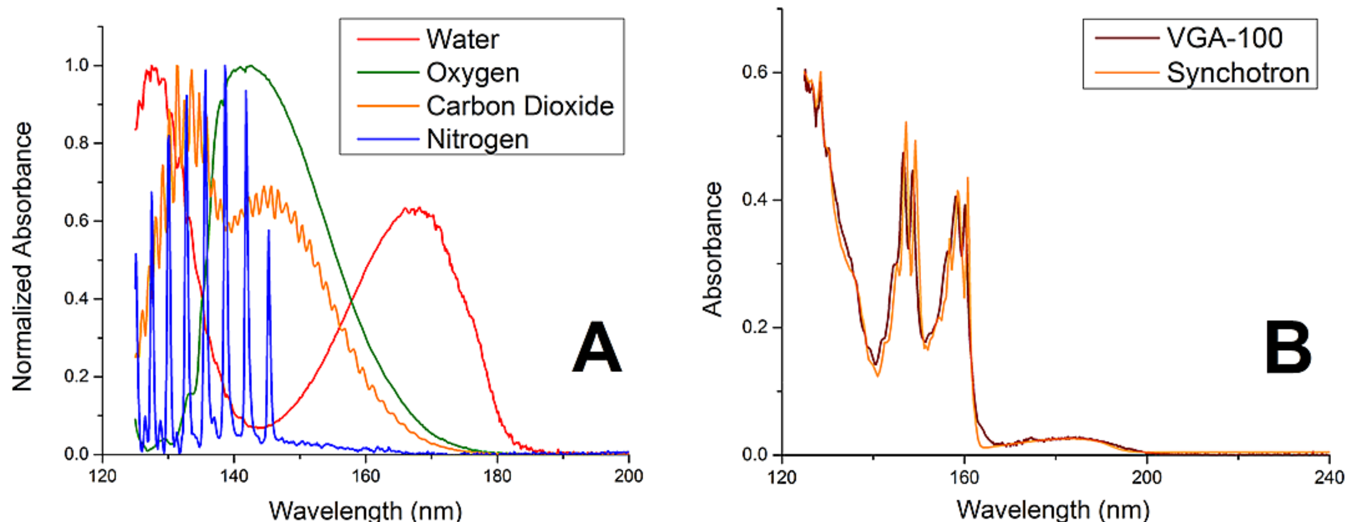

Figure 2. Absorption spectra (A) for a variety of small molecules. Measured spectra show good correlation with synchrotron data (B), as shown for methanol.

sampling rate. Spectral filters were additionally applied post- run, in some cases, to enhance specificity for compounds of interest. Compounds of similar structure exhibit similar spectral features at similar wavelengths; therefore, extracted wavelength chromatograms (i.e. the result of applying spectral filters, which is somewhat analogous to ion-extraction in Gas Chromatography Mass Spectrometry [GC-MS]) were used to selectively analyze specific groups of compounds – in this case the saturated versus unsaturated fatty acids.

Results and Discussion

The absorption spectra of the saturated and unsaturated FAMEs possessed markedly distinct characteristics, which enabled the simple classification of eluted peaks into one of the two groups. As shown in Figure 3, the absorption profiles of the saturated compounds displayed virtually no absorbance at wavelengths longer than 180 nm. Whereas by contrast, all of the unsaturated fatty acids exhibited strong absorbance features above 180 nm, as shown in Figure 4.

This difference in spectral response allows for the use of spectral filters to then 'extract' the unsaturates from the full mixture. In Figure 5, a simple 185 – 200 nm spectral filter was applied to the full 37 component chromatogram – the result is an 'extracted' chromatogram featuring peaks only from the 20 unsaturated FAMEs. The distinct absorbance spectra between saturated and unsaturated FAME compounds also enabled precise quantitation of co-eluting peaks, obviating the need for complete chromatographic separation of all compounds.

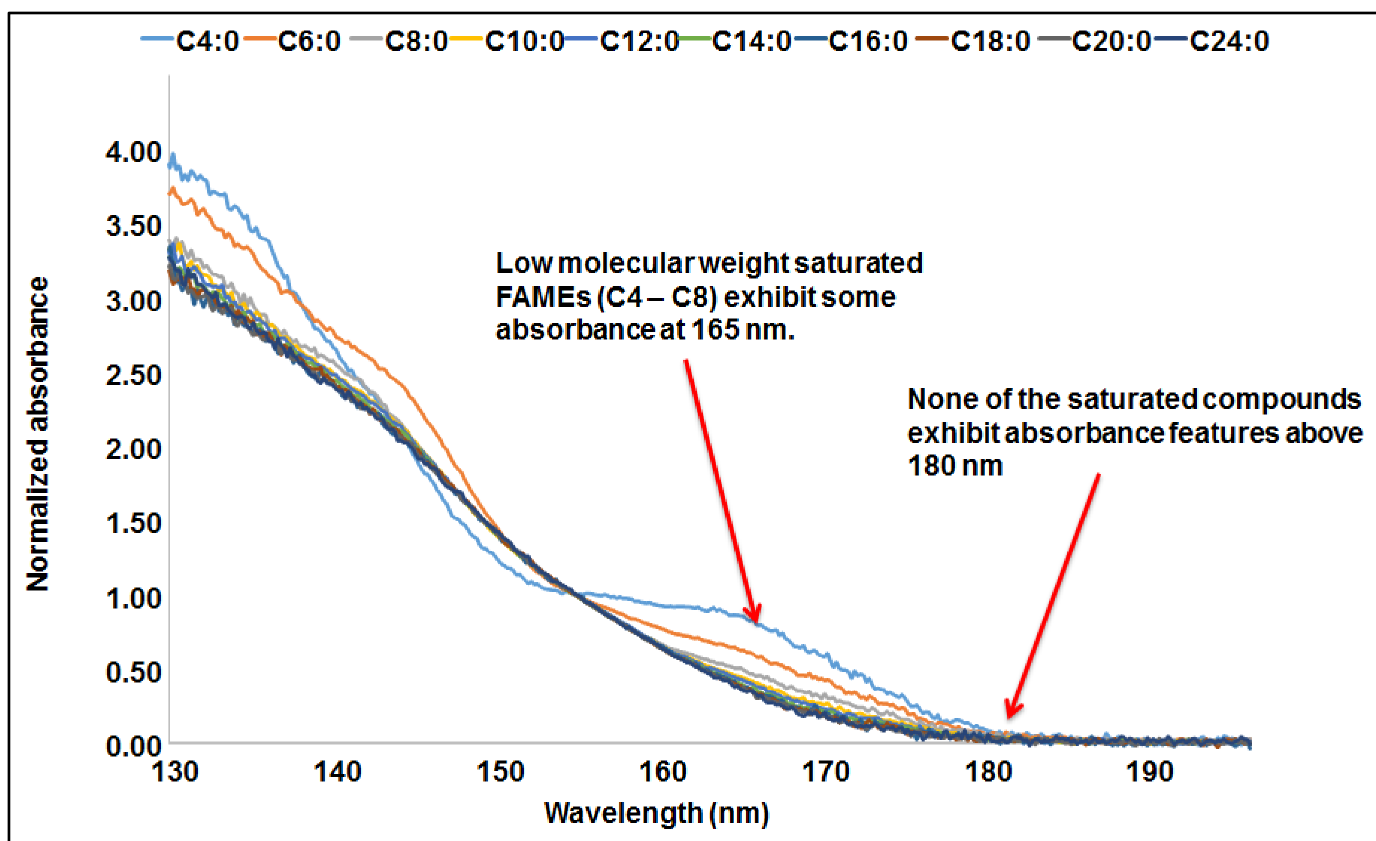

Figure 3. VUV absorption spectra for saturated FAME compounds.

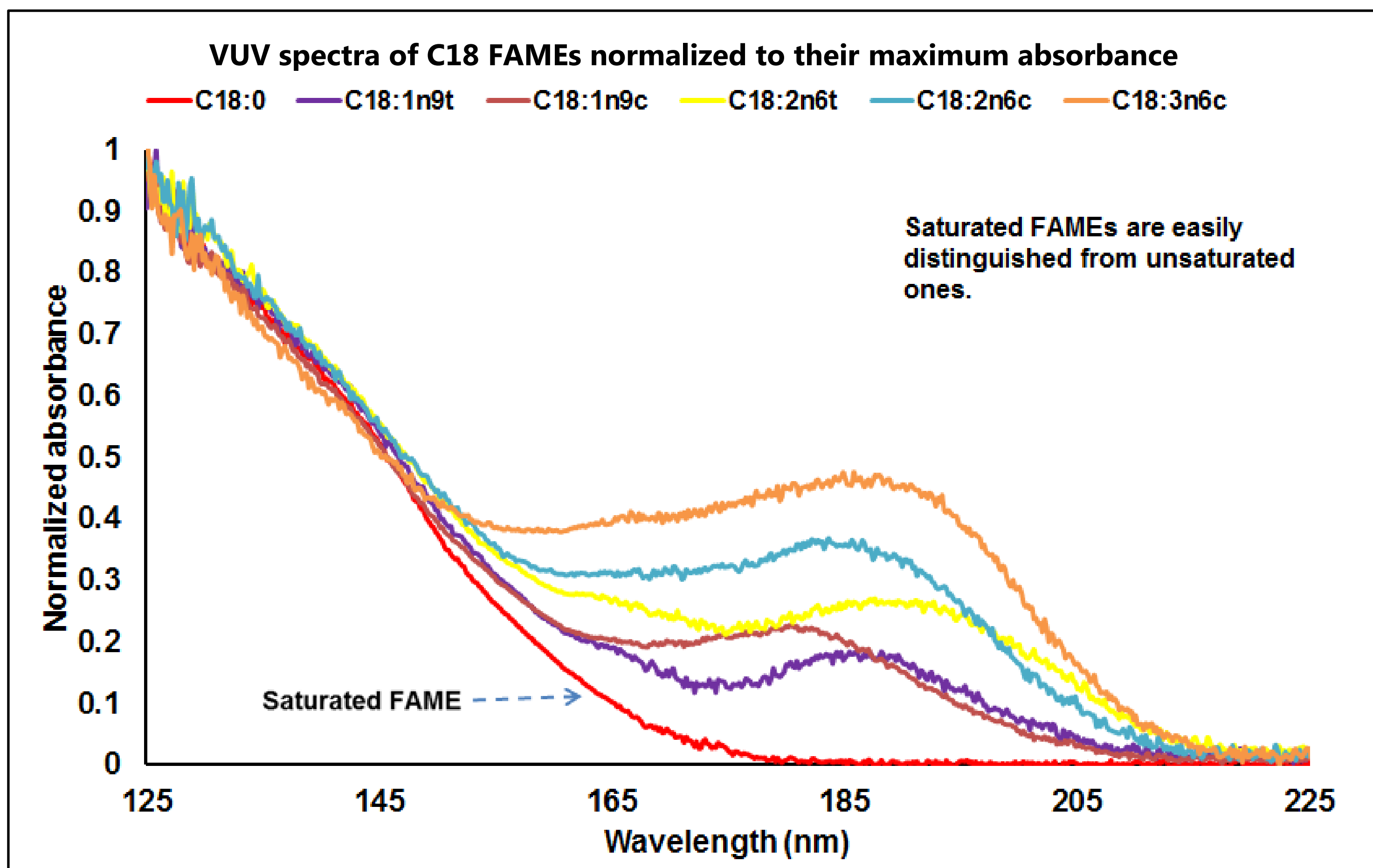

Figure 4. VUV absorption spectra for unsaturated FAME compounds.

In the rapeseed oil mixture, two compounds, methyl linolenate (C18:3) and methyl behenate (C22:0), displayed nearly identical retention times, as shown in Figure 6. However, because of the disparity in their absorption spectra (the first compound being unsaturated, while the second saturated), the VGA-100 analysis software is able to deconvolute these two peaks.

Specific identification of FAME compounds was made possible by comparing the spectral response of analyte peaks to the spectra of known, pure compounds available in VUV spectral libraries. Spectra generated on the VGA-100 closely match spectra generated at synchrotron facilities around the world (see Figure 2B), granting confidence in the optical precision of these matches. A fitting algorithm in the VUV software automates this process. In the case of the unsaturated FAMEs, 90% of the compounds could be identified exactly using the VUV spectral library. Identification of saturated FAMEs was poor due to the spectral similarity of these compounds; however, they could be identified through other discriminative means, such as retention time. Classification of all of the FAMEs as saturates, mono-unsaturates or poly-unsaturates was achieved in 100% of cases.

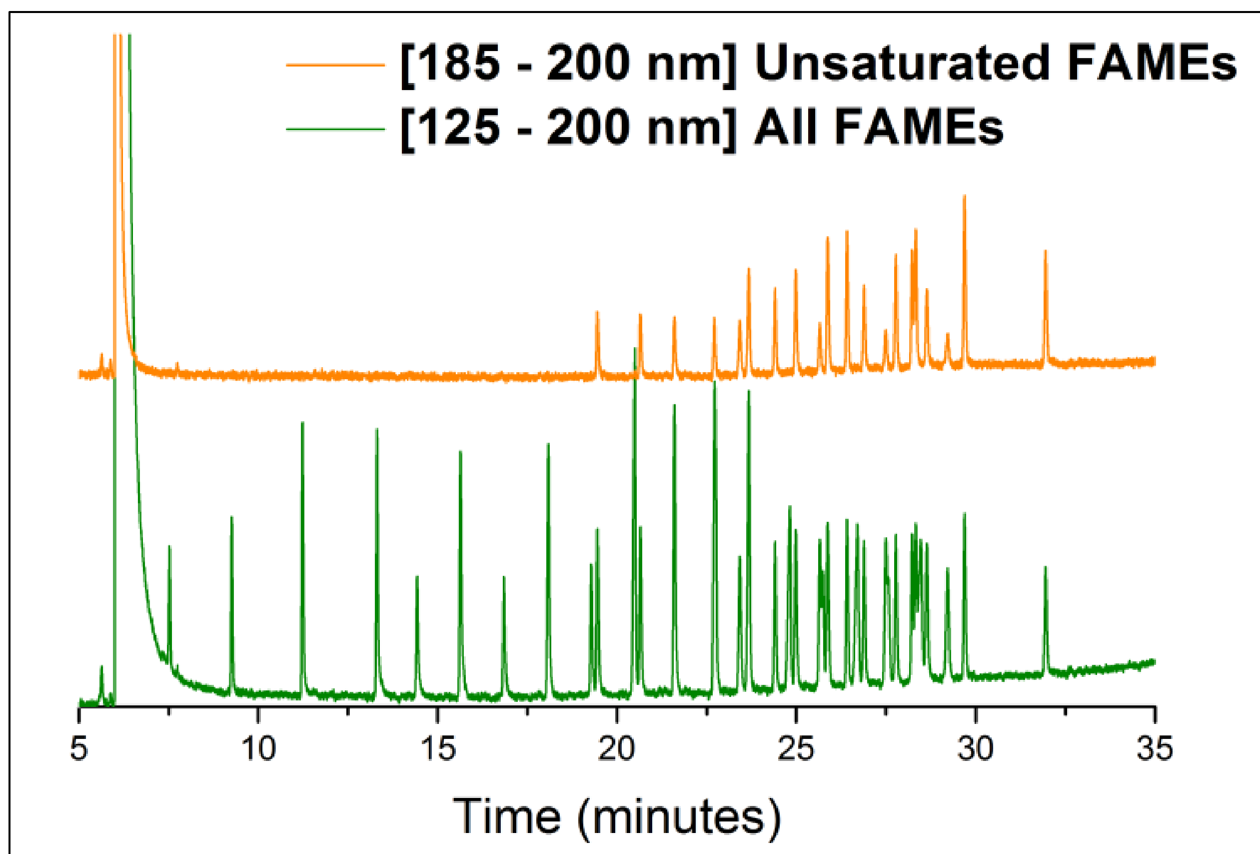

Figure 5. The selectivity of VUV spectroscopically distinguishing unsaturated FAMEs (orange)

Conclusion

A new, commercially available VUV wavelength detector, the VGA-100, was applied to the separation and analysis of several FAME mixtures. This separation was facilitated immensely by the detector's ability to segregate saturates and unsaturates based on spectral wavelength (in addition to time), thus providing additional degrees of freedom for the chromatographer. Classification of analytes using the spectral response was successful in 100% of cases, and VUV spectral matching provided identification of unsaturates with a 90% success rate. A case of co-eluting saturated and unsaturated FAMEs was successfully deconvoluted, thus affording an efficient separation. The VGA-100 provides a worthy alternative for FAME analysis.

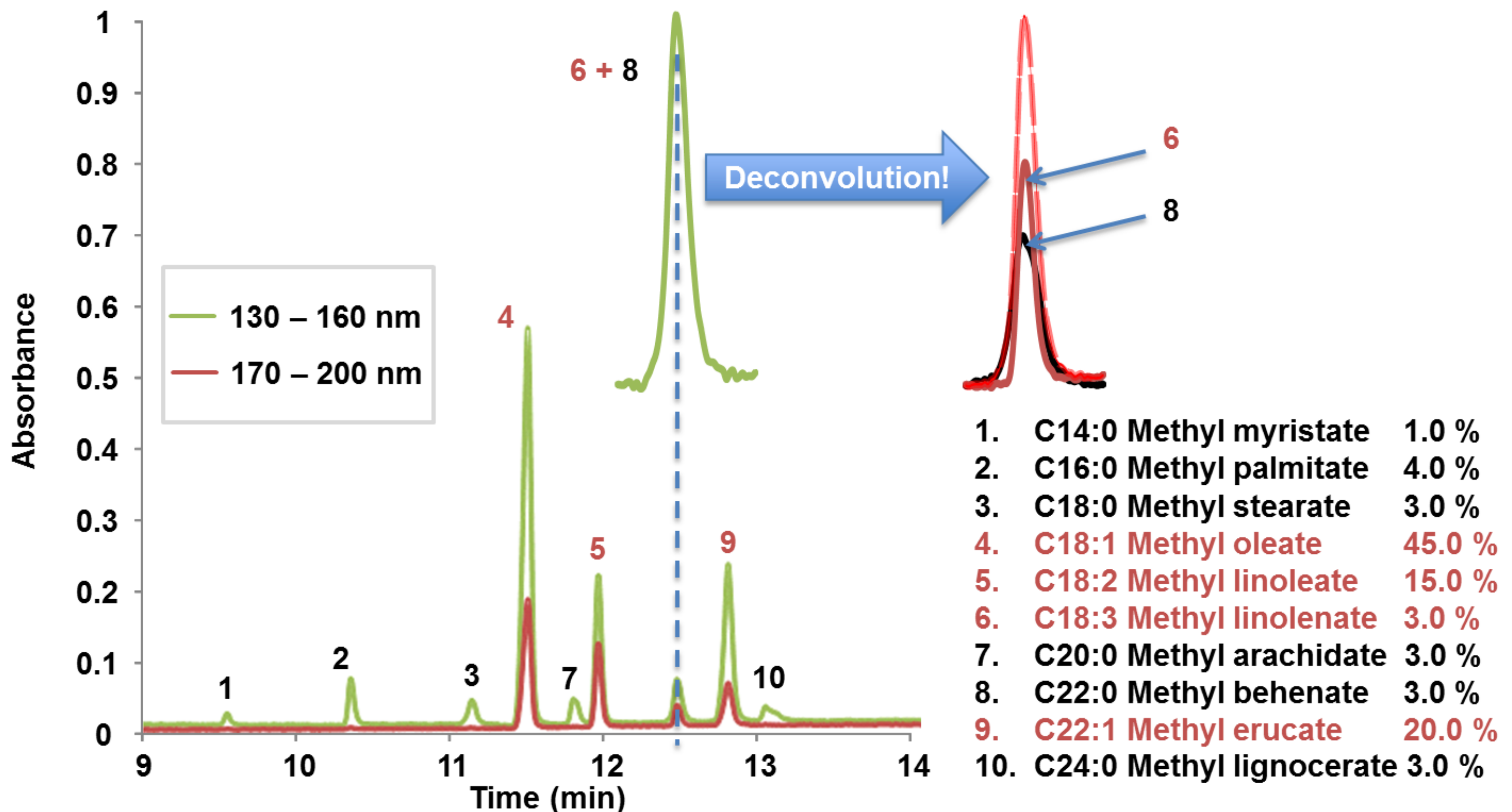

Figure 6. Rapeseed oil mixture chromatogram. Peaks 6 and 8 co-elute, but can be deconvoluted using the two wavelength range filters: 130 – 160 nm and 170 – 200 nm.

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