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Structural Elucidation of an Unknown Compound in the Fatty Acid Methyl Esters (FAMEs) Extract of Avocado Using APGC-HRMS

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APPLICATION BENEFITS

- Generation of accurate mass measurements of low- and high-energy spectra across a wide *m/z* range aided in identification of known FAMEs and elucidation of the unknown compound.

- "Soft" ionization using APGC resulted in preservation of the molecular ion followed by fragmentation to provide more comprehensive molecular detail.

- Integrated elucidation tools in UNIFI® Discovery Toolset facilitated the search of hundreds of online databases, facilitating elemental composition and fragment match.

INTRODUCTION

Avocado is a major tropical fruit with various known health benefits when consumed as part of a balanced diet.[1] Its high lipid fraction in particular contains phytosterols, tocopherols, and most notably omega fatty acids.[1] Profiling this fatty acid content is often performed via gas chromatography (GC) following derivatization into fatty acid methyl esters (FAMEs). This is done to reduce the fatty acid polarity and neutralize the carboxyl functionality so that structural differences can be exploited via chromatography.[2] Chromatographic resolution is sufficient and reproducible enough for various FAMEs to be identified by retention time alone, and some methods will implement the use of non-mass selective detection such as flame ionization detection (FID). However the appearance of additional unexpected constituents in the derivitized lipid fraction can complicate the chromatogram.

Identification of unknowns in foodstuffs is a major challenge for food companies who need to determine whether an unknown compound may be harmful to consumers. In this application note, we describe a FAMEs analysis of an avocado sample that contained a previously unknown constituent resulting from an unexpected chromatographic peak. Through the use of high resolution mass spectrometry (HRMS) and atmospheric pressure chemical ionization following gas chromatography (APGC), the accurate mass of the molecular ion and generated fragments were used to propose an identification of the compound.

WATERS SOLUTIONS

Xevo® G2-XS QTof

UNIFI Scientific Information System

Atmospheric Pressure Gas Chromatography (APGC)

KEYWORDS

Avocado, fatty acids, FAMEs, HRMS, APGC, Universal Source

EXPERIMENTAL

GC conditions

GC system:	A7890
Column:	DB-5MS 30 m x 0.25 mm 0.25 µm (J&W)
Injection mode:	Splitless
Liner:	Gooseneck Splitless, deactivated (Restek)
Column pneumatics:	Constant flow
Column flow:	1.2 mL/min
Injector temp.:	280

GC oven temp. ramp:

Temp.	Temp. ramp	Hold time
100 °C		4 min
240 °C	5 °C/min	15 min

Total run time:	47 min

MS conditions

MS system:	Xevo G2-XS QTof
Ionization mode:	API+
Acquisition mode:	MS^E
Acquisition range:	50 to 1000 *m/z*
Low collision energy:	6 eV
High collision energy:	20 to 50 eV
Source temp.:	150 °C
Interface temp.:	310 °C
Corona current:	5.0 µA
Cone voltage:	30 V
Cone gas:	110 L/hr
Auxillary gas:	300 L/hr
Make-up gas:	300 L/hr

Data management

UNIFI Scientific Information System

An avocado extract (NIST, Gaithersburg, MD, described below) was diluted 1:1000 (v/v) in heptanes and 1 µL was injected for analysis on the Waters® APGC-Xevo G2-XS QTof System. For the analysis of relatively polar compounds, as described in this work, a vial of water was placed into the enclosed ionization source in order to generate protons and yield the preferential formation of [M+H]$^+$ ions upon vaporization (Figure 1). Ionization is achieved through coronal discharge in the APGC source. Alternating states of low and elevated collision energy occurred during the chromatographic run, and the resulting accurate mass spectrum is time-aligned for precursor and fragment ions (MS^E). Both spectra were used in the identification of known FAMEs and structural elucidation of the unknown peak. A search list in the analysis method of known FAMEs was generated by importing structures obtained on ChemSpider (www.chemspider.com) for the following compounds: C18:2 methyl lineoleate, C18:1 trans-methyl elaidate, C18:1 cis-methyl oleate, C16:1 methyl palmitoleate, and C16:0 methyl palmitate. Structural import provided the UNIFI Scientific Library search list with the resulting molecular formula and exact mass, which was automatically used to generate extracted ion chromatograms, as well as propose fragment structures from the high collision energy data.
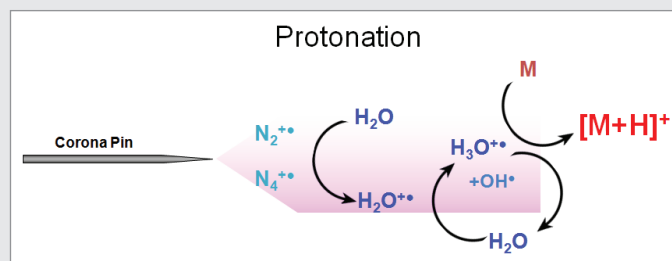

Figure 1. Schematic of protonation as it occurs with the APGC Source.

Sample description

The fat from the samples was prepared by acid hydrolysis Mojonnier extraction according to AOAC 922.06. The recovered fat was converted to FAMEs with NaOH/MeOH/BF$_3$ according to AOAC 969.33. The final FAME extract was diluted in heptanes.

Further interrogation of the fragmentation of the FAMEs in the high energy spectra yielded some key structural characteristics. Figure 3 shows the high energy spectrum for C18:1 cis-methyl oleate in an expanded and annotated view. The presence of the traditionally observed (i.e. as seen using electron ionization GC-MS) loss of the terminal ether group is seen at m/z 265.2531 (mass error -0.2 mDa, or -0.89 ppm). To the left of this fragment, we see a collection of alkyl chain breakages. Within these breakages, clusters spanning ~6 m/z indicate the process of hydride abstraction,[3] where the chain will lose two subsequent hydrogen atoms. In combining all of these fragments, the complete structure of the compound can be further confirmed.
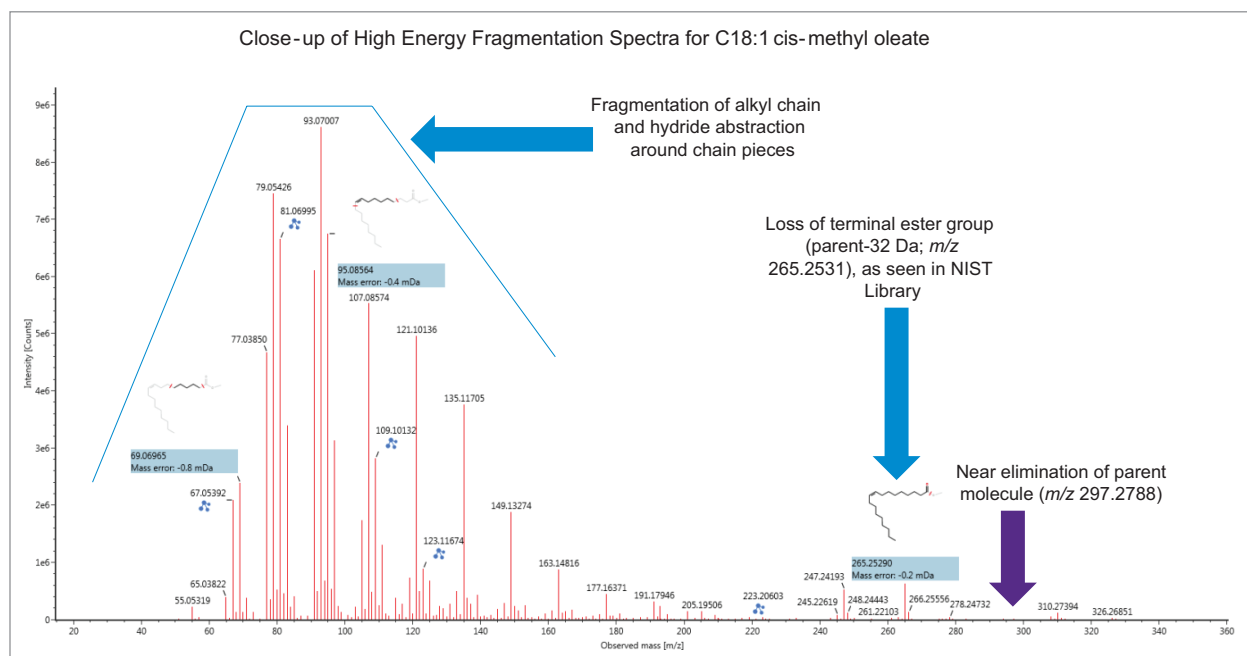

Figure 3. Accurate mass fragments observed for C18:1 cis-methyl oleate.

STRUCTURAL ELUCIDATION OF UKNOWN PEAK

Following the retention order of the known FAMEs, an unknown prominent peak was observed in the BPI (base peak intensity) chromatogram, eluting after the known FAMEs. Investigation of the spectrum under this peak resulted in a proposed molecular mass of 303.2683 m/z. Figure 4 shows the overlaid XIC of this mass, relative to the other FAMEs.
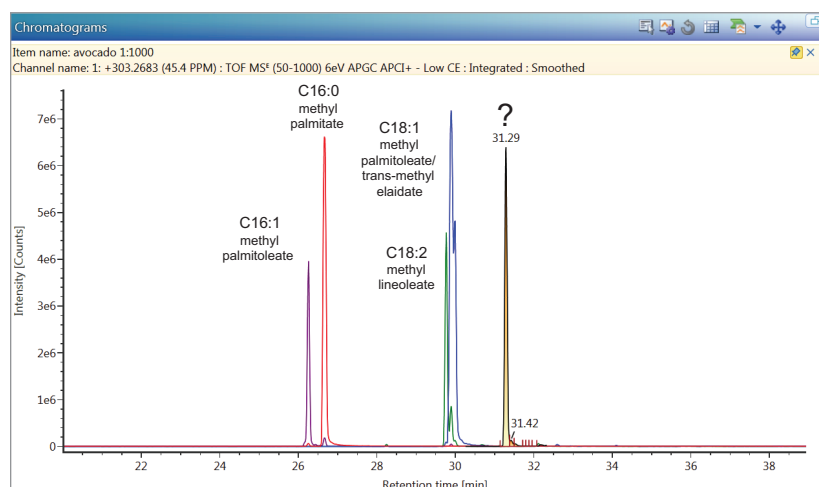

Figure 4. Known FAMEs and an unknown peak at 31.29 minutes.

Structural elucidation of the unknown peak was initialized with UNIFI by selecting the parent ion mass from the list of *m/z* from which an assignment was not made against the screening list of FAMEs (Figure 5). The candidate mass of interest (303.2683 *m/z*) was selected, and following a right-mouse click, and the option to *Elucidate* was chosen, launching the Discovery Toolset. The Discovery Toolset within UNIFI utilizes a combination of elemental composition calculation, isotope fidelity computational scoring, ChemSpider database searching, and fragment matching of high energy data.
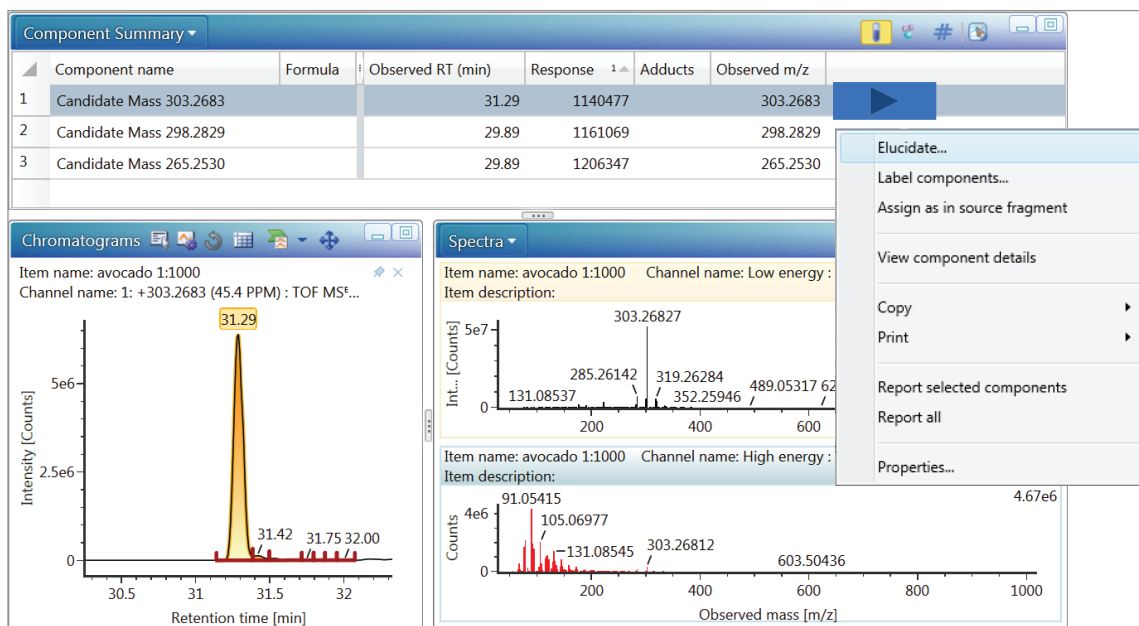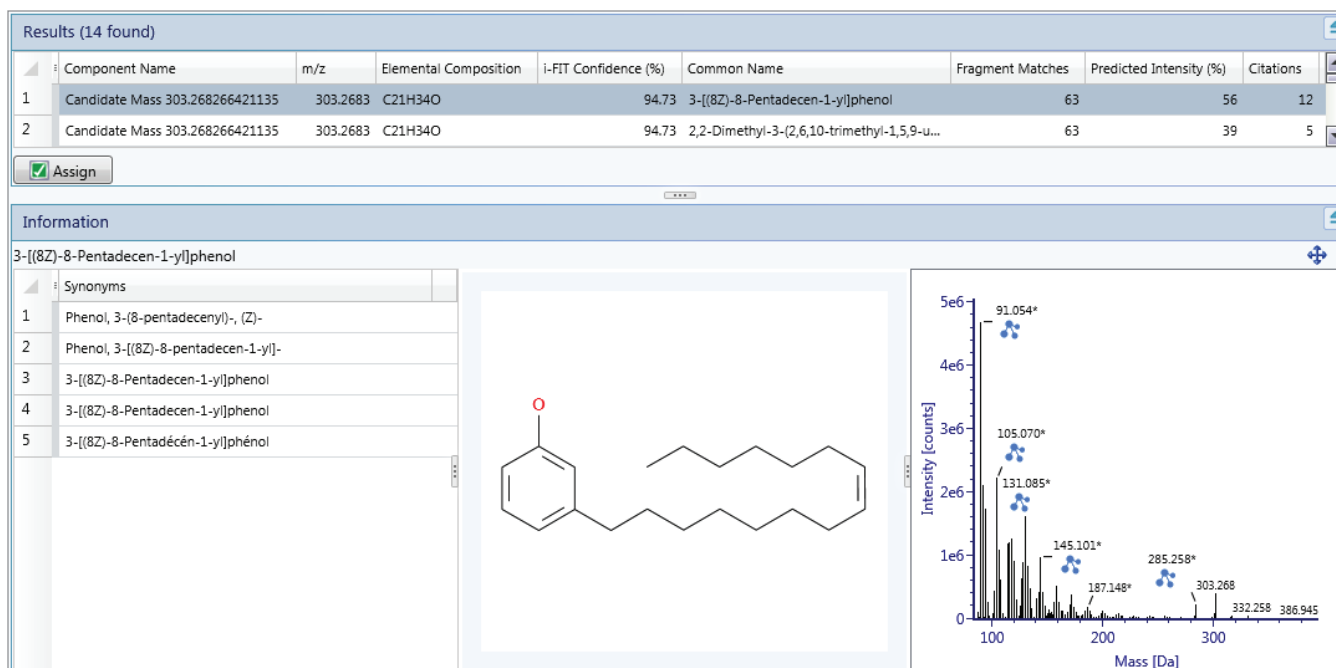

Figure 5. Candidate mass at 303.2683 m/z as it appears prior to structural elucidation. Lock mass correction has already been applied during the initial automated data processing, allowing for elucidation tools in the Discovery Toolset to be immediately applied.

For the unknown peak, the top result for a proposed identification was a 3-pentadecenylphenol species, shown in Figure 6. The proposed structure was found to have a high number of fragment formulas suggested, further supporting the likelihood of this identification. Further interrogation of the high energy spectra (Figure 7) shows similar alkyl chain breakage and hydride abstraction, as seen with the FAMEs – the proposed structure also contains an alkyl chain "tail", increasing confidence in the identification. However, the ester loss was seen for C18:1 cis-methyl oleate (Figure 3). Instead, the loss of H_2O (parent-18 *m/z*; 285.2582 *m/z*) can be seen off of the ring portion of the structure, and most importantly, the intact benzene ring itself is apparent as a fragment (Figure 7).

Online searching of this compound against FooDB (http://foodb.ca/), the world's largest compendium of food related compounds, showed that this compound has been found in the lipid portion of cashew nuts and *Gingko biloba* fruit (Figure 8).[4] Although not indicated to be in avocado explicitly, the nature of the compound identification with highly confirmed spectral matching, combined with the presence of this compound in lipid portions of other fruits supported a tentative match. Assignment of the previously unknown mass with the name from ChemSpider was performed within UNIFI by selecting *Assign* below the ChemSpider result view (Figure 6). Though not commercially available at the time of this writing, final confirmation would typically be performed through the analysis of a pure chemical standard.

Figure 5. Candidate mass at 303.2683 m/z as it appears prior to structural elucidation. Lock mass correction has already been applied during the initial automated data processing, allowing for the elucidation tools in the Discovery Toolset to be immediately applied.
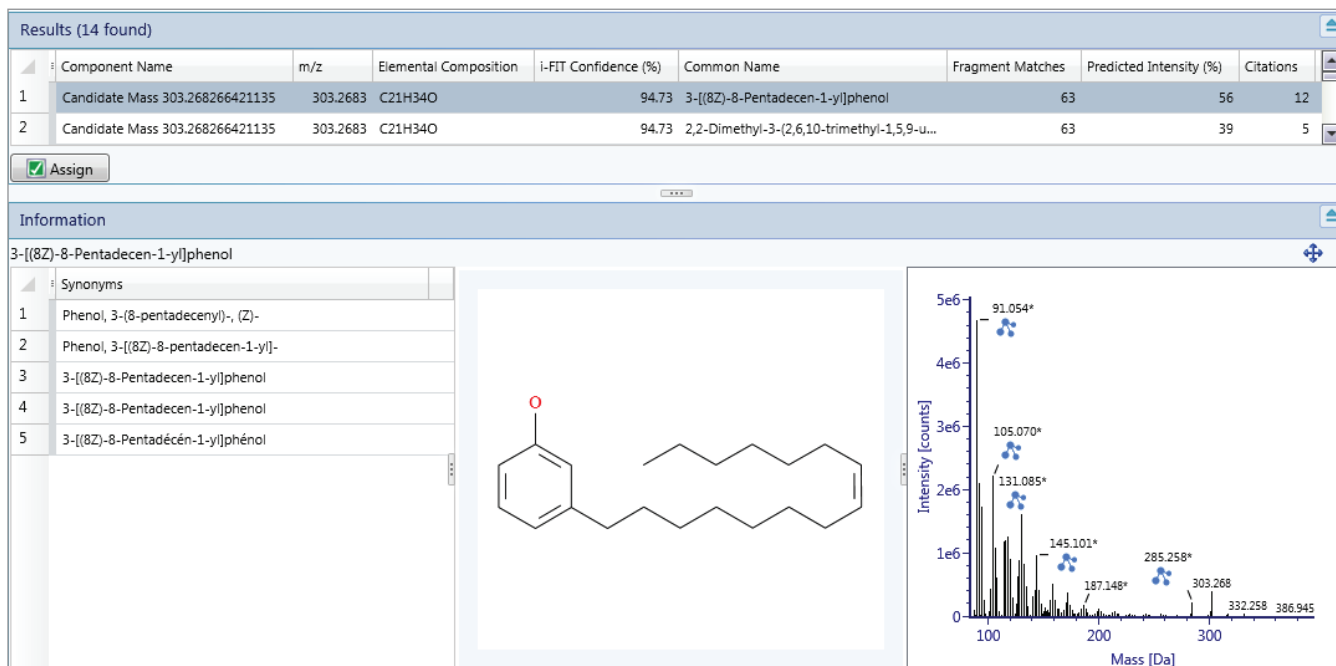

Figure 6. Discovery Toolset results for a proposed structure as found based on elemental composition of spectral peak at 303.2683, ChemSpider search of this composition, and comparison of high energy spectra to possible fragments from the structure. Blue molecule icons in spectral view (high energy spectra only) indicate a fragment match for that mass. A total of 63 proposed fragments were found for the spectra against this structure.
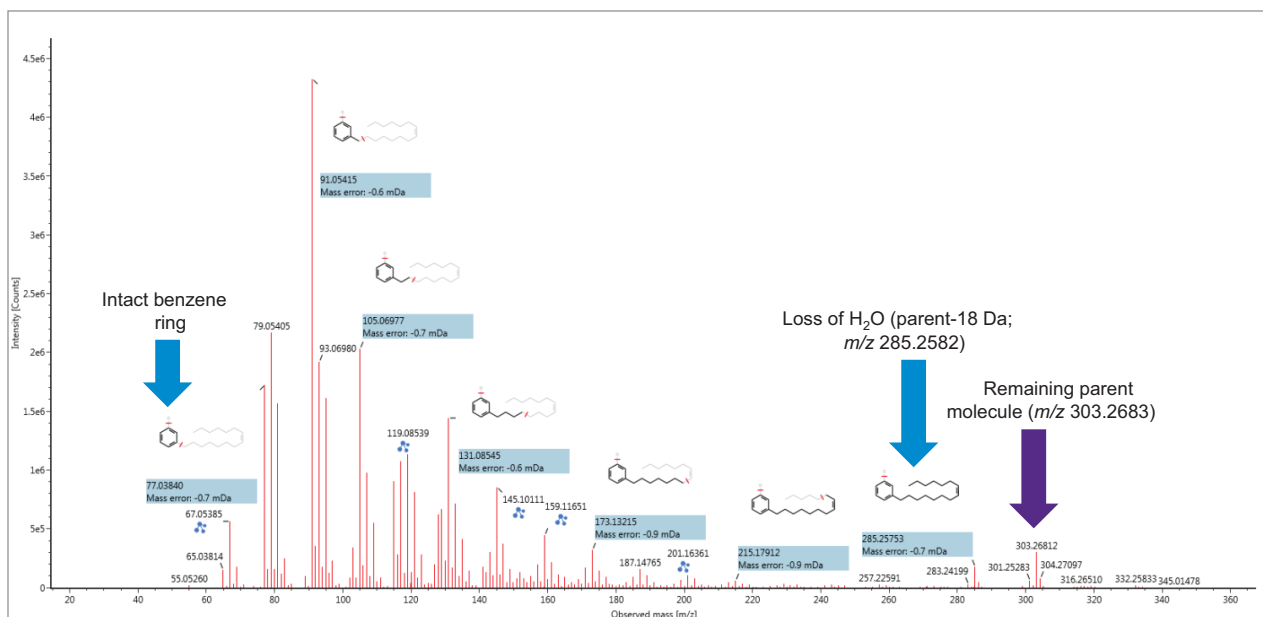
Figure 7. High energy spectrum close-up for unknown peak with proposed structure and fragment assignments.

Figure 8. FooDB search result for 3[(8Z)-8-pentadecen-1-yl]phenol.[4]

CONCLUSIONS

A lipid extract of avocado was found to contain an unexpected and unknown component. The extract was subsequently analyzed for both known FAMEs and for additional compounds. The use of full spectral acquisition, with alternating collision energy states (MS^E), afforded the ability to both interrogate the data for known compounds using fragment matching, as well as searching for any additional masses of interest. Elucidation of the unknowns was made possible by interrogating the high quality accurate mass data into the easily accessible UNIFI Discovery Toolkit. A proposed identification was made for the unknown peak based on the use of the spectra and assigned within the analysis. Future profiling of avocado samples can be performed with this additional compound in mind, and any future unknown peaks can be elucidated using this same basic approach.

Acknowledgment

References

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