Identification and quantification of unsaturated fatty acids using electron-activated dissociation (EAD) fragmentation on the ZenoTOF 7600 system

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ABSTRACT

Octadecenoic acid has multiple biologically relevant isomers that vary in the position and/or stereochemistry of the double bond. It is hard to distinguish them using collision-induced dissociation (CID) fragmentation. In this work, EAD generated diagnostic fragments to distinguish the 4 octadecenoic acid isomers.

INTRODUCTION

Octadecenoic acids are typically analyzed in the negative ion mode and some isomers cannot be adequately resolved by liquid chromatography. Using CID fragmentation data, the product ions derived from the individual positional and stereoisomers are indistinguishable. Here, the fatty acid isomers were derivatized using a tertiary amine that enables their detection in the positive ion mode. These products generate intense precursor and fragment ions, improving assay sensitivity. To address structural specificity, a complimentary fragmentation mode, EAD, was used to generate structurally diagnostic fragment ions. EAD enabled the characterization of each isomer by identifying the double bond position and stereochemical configuration.

MATERIALS AND METHODS

Sample Preparation:

The analyzed compounds included oleic acid (9-cis-octadecenoic acid), vaccenic acid (11-cis-octadecenoic acid), elaidic acid (9-trans-octadecenoic acid) and trans-vaccenic acid (11-trans-octadecenoic acid).

Unsaturated fatty acids were derivatized at the carboxylic acid functional group using trimethylethylenediamine.

The derivatized unsaturated fatty acids were prepared in a mixed standard and diluted with 1:1, methanol/water.

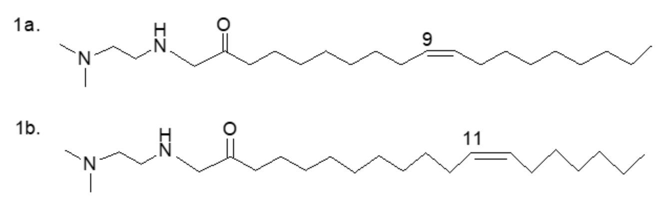

Figure 1. The structural formulas of four octadecenoic acids. 1a. oleic acid (cis-9-octadecenoic acid) and elaidic acid (trans-9-octadecenoic acid);
1b. vaccenic acid (cis-11-octadecenoic acid) and trans-vaccenic acid (trans-11-octadecenoic acid).

HPLC conditions:

A Shimadzu Prominence LC system was used with a BEH C18 (100 × 3.0 mm, 1.7 µm) column set at 50℃. A gradient was used at a flow rate of 300 µL/min. The total gradient time was 13 min. The injection volume was set to 5 µL.

MS/MS conditions:

Data were acquired using SCIEX OS software on the ZenoTOF 7600 system in positive polarity. Data were collected from a single injection, using a combination of data-dependent acquisition (DDA). To compare the information generated from CID with EAD, both fragmentation techniques were used in separate experiments. Relevant MS parameters for the EAD method are described in reference 1, MRM^{HR} parameters are included in table 1.

Compounds	Q1(Da)	Q3(Da)	DP(v)	KE(eV)
Oleic acid/Elaidic acid	367.37	253.2274	80	10
Vaccenic acid/Trans–vaccenic acid	367.37	256.2509	80	10

Table 1. MRMHR method parameters

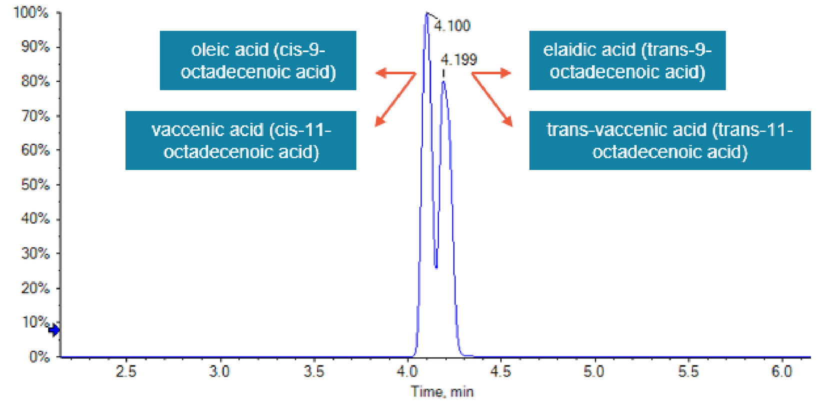

Figure 2. Extracted ion chromatograms(XICs) of four compounds

RESULTS

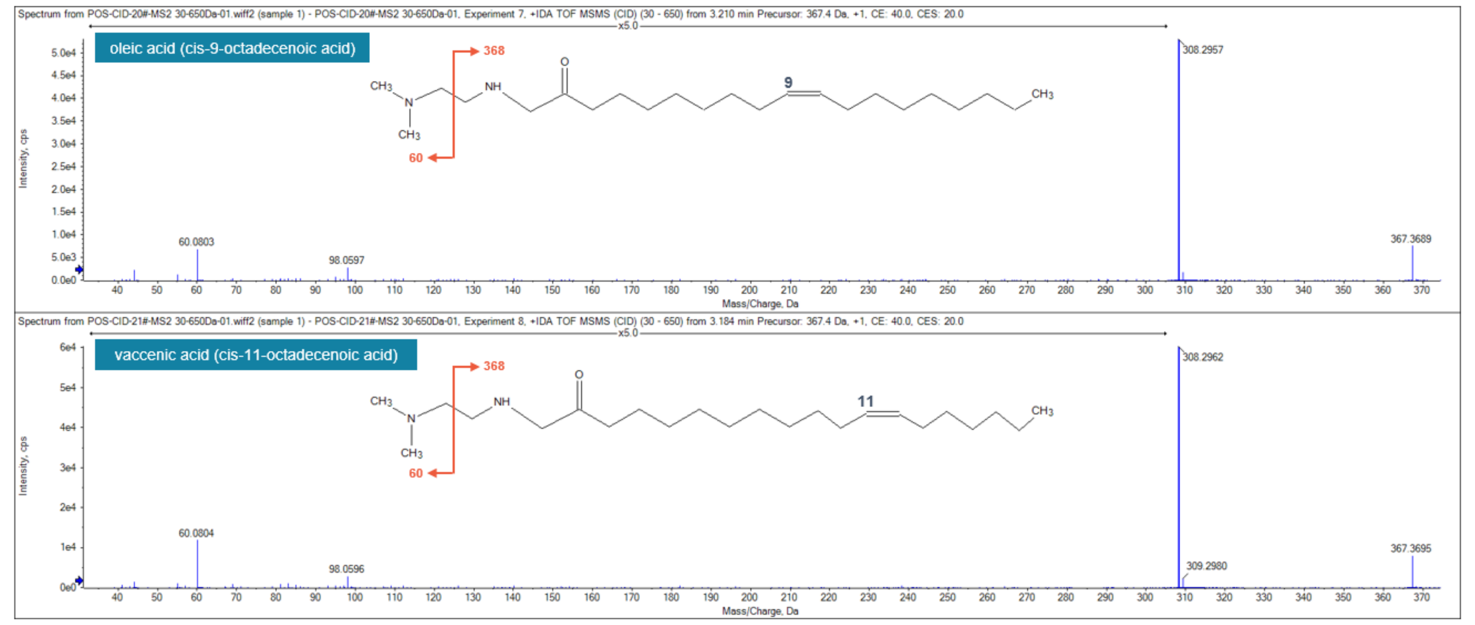

Figure 3. CID fragments of oleic acid and vaccenic acid.

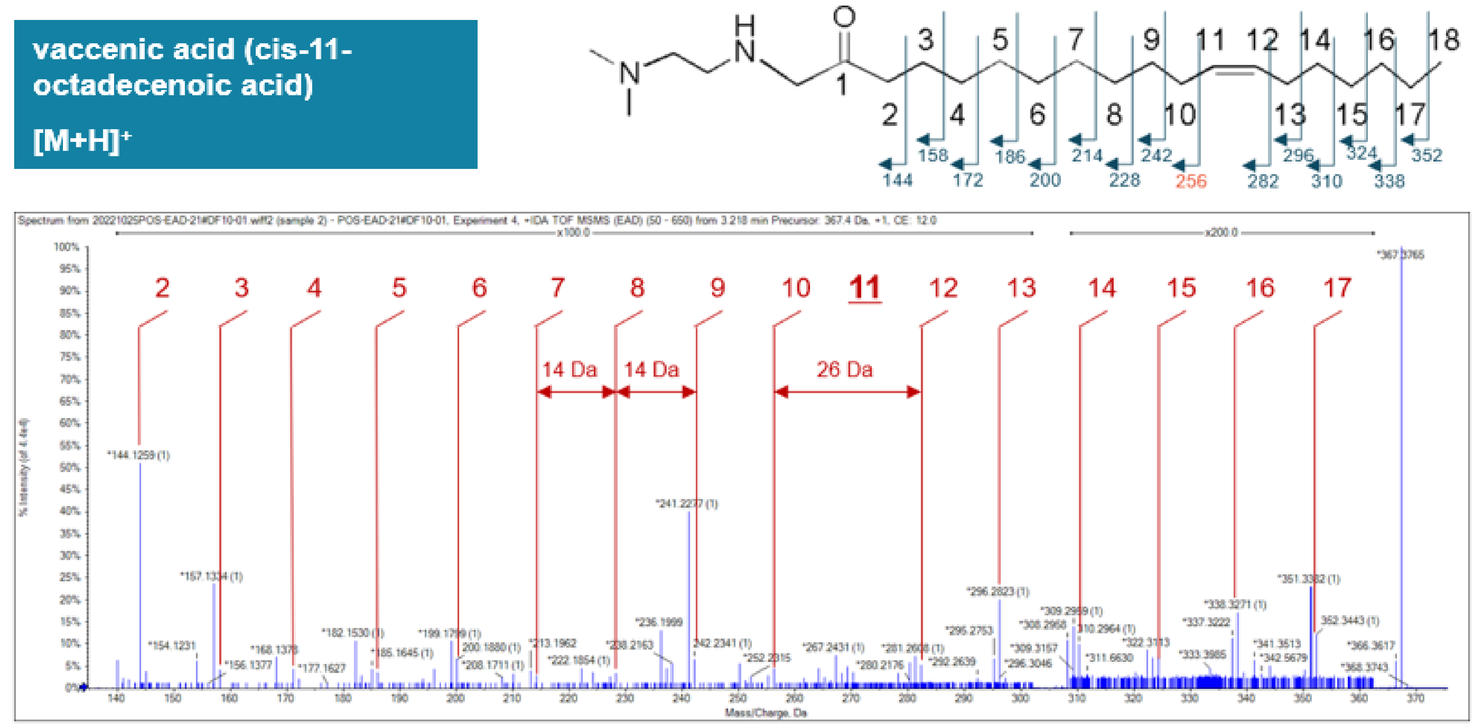

Figure 4. EAD fragments of vaccenic acid. EAD generated diagnostic fragments that could distinguish the octadecenoic acid isomers.

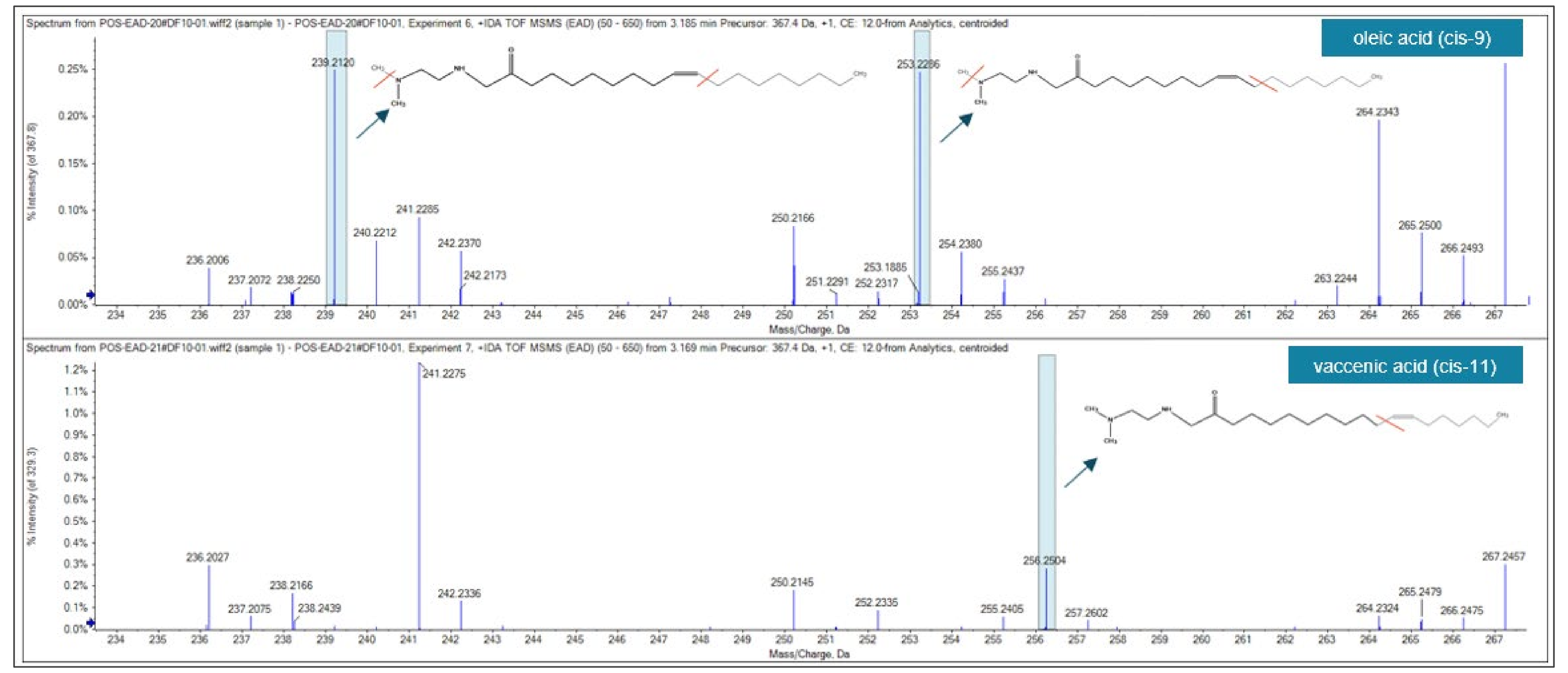

Figure 5. EAD of oleic acid and vaccenic acid. The diagnostic fragments for oleic acid and elaidic acid were both m/z 253.2274 Da and the diagnostic fragments for vaccenic acid and trans-vaccenic acid were both m/z 256.2509 Da.

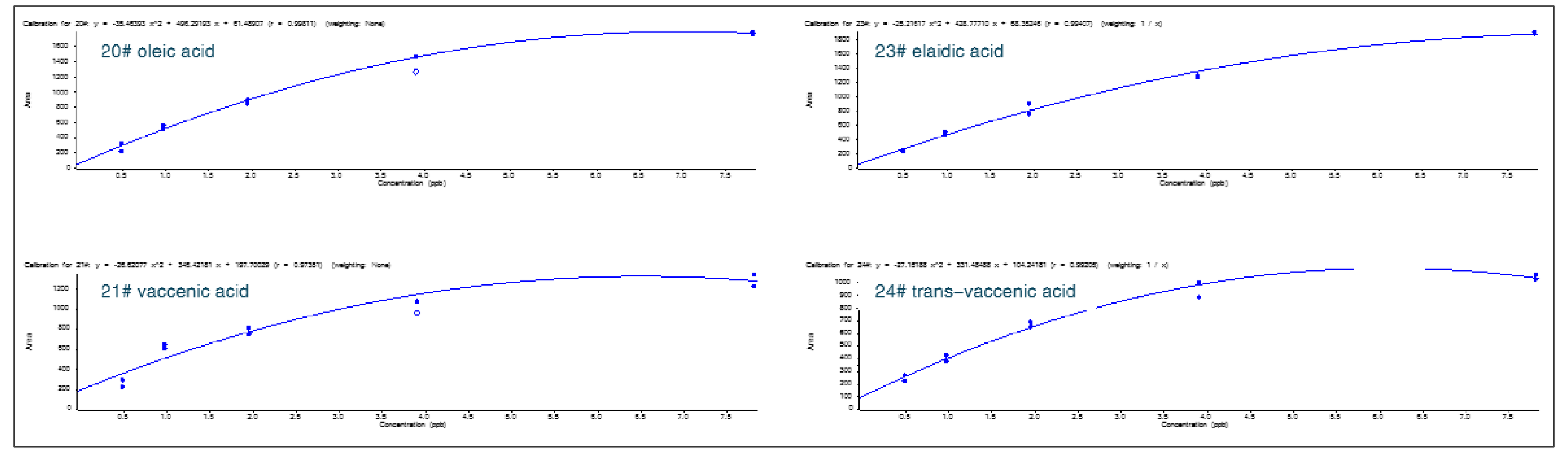

Figure 6. Concentration curves with a linear dynamic range spanning from 0.48to7.81 ng/mL were generated for 5 different concentrations of each standard. The r values of the four curves were all ＞ 0.990.

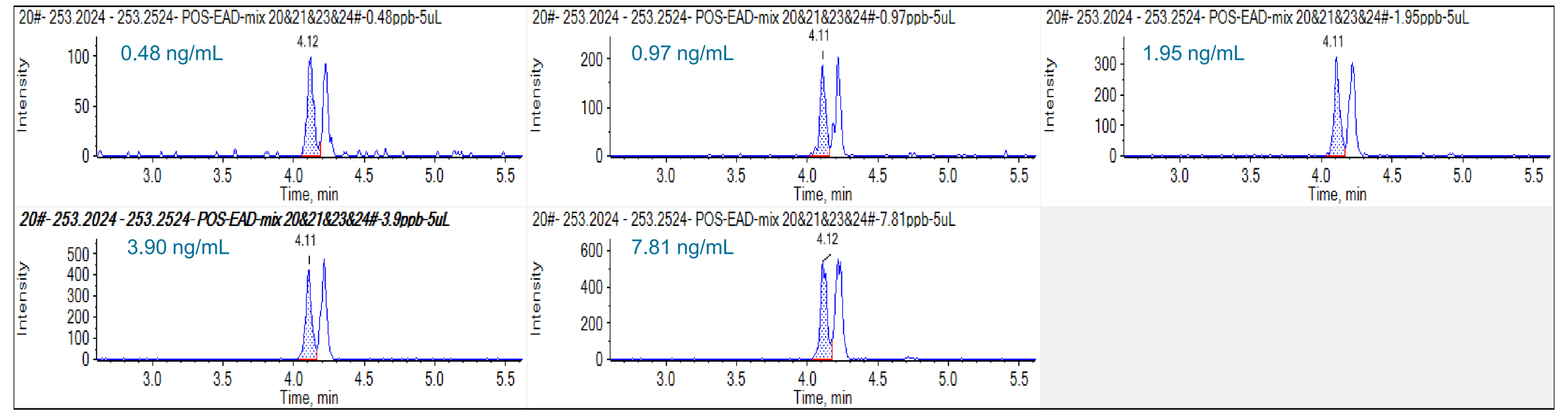

Figure 7. Extracted ion chromatograms(XICs) of standard curve concentration points for oleic acid, with another peak noted as elaidic acid.

Figure 8. The quantitative accuracy and cross-channel interference. These parameters were tested in reference to a single standard. The results show that derivatized fatty acids can be detected with a high degree of sensitivity. Additionally, EAD generated diagnostic fragments to distinguish the 4 octadecenoic acid isomers.

CONCLUSIONS

This poster uses the SCIEX ZenoTOF™ 7600 system to establish an LC-MS/MS method that can effectively identify and distinguish octadecanoic acids at different double bond positions. Further, it establishes a quantitative method for EAD characteristic fragments. This method used a simple process, enabled effective identification, and can be used to more accurately explain biological significance.

REFERENCES

1. Identification and quantification of unsaturated fatty acids using electron-activated dissociation (EAD) fragmentation on the ZenoTOF™ 7600 system. RUO-MKT-02-15564-ZH-A

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