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# Identification and quantitation of C=C location isomers of unsaturated fatty acids by epoxidation reaction and tandem mass spectrometry

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## Abstract

Unsaturated fatty acids (FAs) serve as nutrients, energy sources and signaling molecules for organisms, which are the major components for a large variety of lipids. However, structural characterization and quantitation of unsaturated FAs by mass spectrometry remain an analytical challenge. Here we report the coupling of epoxidation reaction of the C=C in unsaturated FAs and tandem mass spectrometry (MS) for rapid and accurate identification and quantitation of C=C isomers of FAs in a shotgun lipidomics approach. Epoxidation of the C=C leads to the production of an epoxide which, upon collision induced dissociation (CID), produces abundant diagnostic ions indicative of the C=C location. The total intensity of the same set of diagnostic ions for one specific FA C=C isomer was also used for its relative and absolute quantitation. The simple experimental setup, rapid reaction kinetics (< 2 min), high reaction yield (>90% for monounsaturated FAs), and easy-to-interpret tandem MS spectra enable a promising methodology particularly for the analysis of unsaturated FAs in complex biological samples, such as human plasma and animal tissues.


## Introduction

As a key component of almost all lipids, including phospholipids and glycolipids, fatty acids (FAs) are essential for living organisms. Some FAs are important signaling molecules that are involved in a number of biological processes.[1] For instance, the derivatives of arachidonic acid, e.g. leukotrienes and prostaglandins, mediate inflammation and promote a variety of diseases, including allergy, obesity and cancer.[1] More recently, oxidized arachidonic acid was reported to capable to navigate cells to ferroptosis.[2]

The structures of unsaturated FAs are different in terms of the chain length and the number, location and geometry of the carbon-carbon double bond (C=C). A large number of studies on FAs have shown that the change in unsaturated FA composition is associated with certain diseases.[3,4] Determination of the number and locations of C=C bonds in unsaturated FAs will help understand

their biological functions. More recently, lipid desaturation has been identified as a metabolic marker and therapeutic target.[5] Mass spectrometry (MS) techniques have been widely used for FA analysis, among which gas-chromatography MS (GC-MS) is the most traditionally used.[6-8] Both the retention time and the corresponding mass spectrum are used to characterize FA structures. Currently, it is still a challenge to identify the location of a C=C in FAs using MS alone. FA C=C isomers cannot be distinguished by electron ionization (EI) as their mass spectra are almost the same.[9] Low-energy collisional induced dissociation (CID) is a powerful method to distinguish isomeric structures, unfortunately the fragment ions of FA C=C isomers cannot be used to locate the C=C bond, because no diagnostic ions specific to the C=C were produced due to the low acidity/basicity of the C=C relative to heteroatom positions.

In order to generate structurally informative fragment ions that allows one to accurately pinpoint the C=C location in a FA, a variety of strategies have been developed. With such strategies, MS can not only be used for mass measurement, but also allow for structural analysis. Charge-remote fragmentation (CRF), namely fragmentation of an ion in which the cleaved bond is isolated from the apparent charge site by a distance of several bonds, has been employed to locate C=C via high-energy CID of $[M-H]^-$.[10-12] Charge switch derivatization is another well-developed method for detection and characterization of FAs via conversion of the carboxylic group to a positive fixed-charge group, offering higher sensitivity of detection than the method reported in this work.[13-15] In intact FAs, by contrast, the charge site is at the distal end of the chain (carboxyl group) and the C=C is far away from the charge site by several carbon-carbon single bonds. However, abundant fragment ions arising from fragmentation channels other than that of the C=C are generated as well in a CRF experiment, especially for polyunsaturated FAs (PUFAs). To increase the relative abundances of fragment ions indicative of the C=C location, a variety of chemical reactions have been introduced to covalently modify the C=C,[16-20] making it to be more easily fragmented by CID. However, these methods were only compatible with FA analysis by gas-chromatography MS, following the conversion of unsaturated FAs to FA methyl esters (FAMEs). With the rapid development of shotgun FA analysis, new methodologies compatible with electrospray ionization-(ESI)- and nanoESI-MS are urgently needed. Blanksby and co-workers have successfully used ozone to directly cleave the C=C in a spectrum of lipids, directly offering diagnostic ions specific to the

C=C location without resorting to tandem MS.[21] They have integrated ozone reactions into an ESI source for the structural elucidation of unsaturated lipids, i.e. ozone electrospray ionization (OzESI). Alternatively, ozone can be introduced into the ion trap of a MS to react with a mass-isolated lipid, where the analysis of a complex lipid mixture was made possible (termed ozone-induced dissociation, OzID).[22-24] The inconvenience associated with OzID is the need of hardware modification to MS to allow ozone introduction into the trap. Recently, we have successfully demonstrated the identification and quantitation of lipid C=C isomers by using Paternò–Büchi (PB) reaction to convert a C=C to a four-membered oxetane ring, which is stable at room temperature but easily fragmented by collisional heating of CID to produce highly abundant diagnostic ions. [25-27] Since the C=C is not directly cleaved, PB reaction products can be mass-isolated to achieve rapid and accurate assignment of C=C location(s) in an unsaturated FA from a complex mixture, with a moderate PB reaction yield (~30%~60%). In comparison with other methods for FA analysis summarized here, PB reaction/tandem MS has the unique advantage that allows both identification and quantitation of C=C isomers of FAs, without any chromatographic separation.

In this study, we report a novel method to structurally characterize and quantify C=C isomers of FAs via epoxidation reaction and tandem mass spectrometry. Transformation of the C=C to a stable epoxide ring leads to complete fragmentation of the original C=C by CID. The epoxidation reaction between the C=C bond(s) in FAs and oxygen was facilitated by blowing a low-temperature plasma (LTP) into a solution of unsaturated FA(s) dissolved in acetone/water (50/50, v/v). A significant advantage is that monounsaturated FAs (MUFAs) can be almost quantitatively converted to their corresponding epoxides within a short reaction time of <1 min, a feature that is highly desirable for FA quantitation. Following CID, abundant diagnostic ions indicative of the C=C locations are generated from the epoxides, which not only offer structural information, but also enable rapid quantitation of unsaturated FAs. Oxygen in air served as the oxidizing reagent. As a practical application, the identification and quantitation of FA C=C isomers in human plasma were demonstrated using the developed method.

## Experimental Section

*Chemicals and reagents*. Oleic acid (FA 18:1 (9Z)), palmitoleic acid (FA 16:1 (9Z)), *trans*-10-heptadecenoic acid (FA 17:1 (10E)), linoleic acid (FA 18:2 (9Z, 12Z)), linolenic acid (FA 18:3 (6Z, 9Z, 12Z)), acetonitrile, and methanol were purchased from Sigma Aldrich (St. Louis, USA). Vaccenic acid (FA 18:1 (11Z)) and arachidonic acid (FA 20:4 (5Z, 8Z, 11Z, 14Z)) were purchased from Aladdin (Shanghai, China). Isooctane and ammonium hydroxide were purchased from J&K Scientific (Beijing, China). Acetone was purchased from Sinopharm Chemical Reagent (Beijing, China), and Dulbecco's phosphate buffered saline (dPBS) was purchased from Dingguo Changsheng Biotech. (Beijing, China). Tetradecenoic acid (FA 14:1 (9Z)) was purchased from Nu-Chek Prep, Inc. All solutions were prepared using deionized water with a resistivity of 18.2 MΩ cm, from a Milli-Q water purification system. Helium (purity: 99.999%) was used as the discharge gas for the low-temperature plasma (LTP) probe.

*Fabrication of the LTP probe.* Details on the fabrication of a LTP probe can be found elsewhere.[28] Briefly, the probe was fabricated using a quartz tube (o.d. 4 mm and i.d. 2mm) as the dielectric barrier, with two Cu electrodes surrounding the quartz tube surface, separated by ~2.5 cm. An alternating current (AC) power with a $V_{p-p}$ of 0.8-1.02 kV and a frequency of 0.18-0.27 kHz was applied to both electrodes to drive plasma generation. The flow rate of the helium supplied to the LTP probe was 5 L/min.

*Experimental setup for the epoxidation of unsaturated fatty acids.* The experimental setup used for the epoxidation of the unsaturated FAs is shown in Figures 1 and S1. Briefly, 0.1 mL unsaturated fatty acid solution dissolved in 1% $NH_4OH$ in acetone/water (50/50, v/v) was transferred to a 1.5 mL plastic vial. A low-temperature plasma was then blown into the solution to facilitate the epoxidation reaction. The resulting solution was then subjected to electrospray ionization-mass spectrometry (ESI-MS) and tandem MS analysis for identification and quantitation of unsaturated FAs.

*Mass spectrometry (MS) and nanoESI-MS*. All experiments were performed on a Thermo LTQ mass spectrometer (San Jose, CA, USA). The instrument parameters used were as follows: capillary temperature, 275 °C; capillary voltage, -10 V; tube lens voltage, -100 V; maximum injection time, 200 ms; microscans, 2. The commercial ESI source was removed and replaced with a nanoESI

source. Accurate mass measurement experiments were performed on a Thermo Q-Exactive Orbitrap mass spectrometer (Thermos Scientific, San Jose, CA, USA), operated in the full mass mode. The Q-Exactive Orbitrap MS instrumental parameters were as follows: capillary temperature: 320 °C, tube lens voltage: 50 V, mass resolution: 70,000, maximum injection time: 50 ms, and sum of microscans: 1. Epoxidized FAs were infused into a nanoESI tip of ~10 $\mu$m outer diameter pulled using borosilicate glass capillary tips (1.5 mm o.d. and 0.86 mm i.d.) on a P-1000 Flaming/Brown micropipette puller (Sutter Instrument, Novato, CA, USA). FA solution was loaded from the back opening of the borosilicate glass tip. A stainless steel (SS) wire was inserted to the nanoESI-tip to serve as the electric contact, with the tip aligned with the MS sampling orifice.

*Quantitation of fatty acids in human plasma*. For absolute quantitation, 10 $\mu$M tetradecenoic acid (FA 14:1 9Z) was used as the internal standard (IS). Taking palmitoleic acid as an example, a series of palmitoleic acid solutions of 5, 10, 20, 30, 40 $\mu$M were prepared respectively, each added with 10 $\mu$M tetradecenoic acid as the IS. After 60 s of epoxidation reaction, the intensity of the epoxidation products of palmitoleic acid (*m*/*z* 269, see Fig 2d) was compared to the intensity of the products of the IS (*m*/*z* 241) to calculate the intensity ratio ($I_{269}/I_{241}$). A good linear relationship (see Table S2) was obtained between $I_{269}/I_{241}$ and the concentration of palmitoleic acid. For the analysis of fatty acids in human plasma, 10 $\mu$M IS was also doped into the FA extract, which was reconstituted into a volume of solvent that is ten times that of the plasma. FAs were extracted by following the standard extraction protocol (see SI). Following epoxidation, the concentration of each fatty acid was calculated from the standard curve prepared for each FA. Other FAs, including palmitoleic acid (FA 16:1 (9Z)), linoleic acid (FA 18:2 (9Z, 12Z)) and linolenic acid (FA 18:3 (6Z, 9Z, 12Z)), were analyzed in the same way.

## Results and Discussion

### Epoxidation of unsaturated fatty acids facilitated by a low temperature plasma (LTP)

Oleic acid (one C=C between C9 and C10) was chosen as an example to demonstrate the methodology developed in this work. The whole analytical process consists of two steps, i.e.

epoxidation of the unsaturated FAs followed by MS analysis (Figure 1a). A centrifuge vial containing 0.1 mL of 70 $\mu$M oleic acid (1% $NH_4OH$ in 50/50 acetone/water, v/v) was used as the reactor placed under the LTP. Addition of $NH_4OH$ into the reaction system both accelerates the reaction and improves ESI-MS detection of FAs and their epoxidation products. After epoxidation, the FA solution was analyzed via (-)nanoESI-MS and (-)nanoESI-MS/MS. Before reaction, only oleic acid ($[M\text{-H}]^-$, $m/z$ 281.3) can be detected (Figure 1b) in the solution. By contrast, almost no oleic acid was detectable in the reaction system after a reaction time of 120 s. Instead, the only abundant peak detected on the mass spectrum was at $m/z$ 297.3, representing a 16 Da mass increase in comparison with intact deprotonated oleic acid (Figure 1c). Accurate mass measurement using a LTQ Q-Exactive Orbitrap mass spectrometer (Thermo, San Jose, CA) revealed the mass increase to be 15.99492 Da, which is exactly the mass of oxygen (15.994915 Da, error: 0.3 ppm, Figure S4). A significant advantage of this method is the high reaction yield of the epoxidation reaction for unsaturated FAs, without other major side reactions and is highly desirable for producing abundant C=C-specific diagnostic ions and quantitative analysis. CID of the epoxide for oleic acid ($m/z$ 297.3) produces four major fragments due to the shattering of the epoxide ring, including ions at $m/z$ 155.2, 171.2, 253.3 and 279.3 (Figure 1d). Products at $m/z$ 171.2 and 155.2, each carrying a negative charge at the carboxyl group, are generated via fragmentation of the three-membered epoxide ring in two different mechanisms (Scheme in Figure 1e). For one fragmentation channel, an aldehyde group was generated at the distal end in one fragment, and in the other complementary fragment, an ethylene group was formed at the site of cleavage at $\Delta 9$ C=C via intra-molecular hydrogen transfer. In each pair of fragments, only the fragment carrying the $COO^-$ group can be detected by MS. Therefore, two ions out of the four fragments can be detected, which are termed diagnostic ions as they can be used to accurately pinpoint the C=C location. Other product ions at $m/z$ 279.3 and 253.3 were formed following loss of $H_2O$ (18 Da) and $CO_2$ (44 Da) from parent ions. The scheme of the epoxidation reaction and subsequent decomposition of the epoxide of oleic acid can be found in Figure 1e.

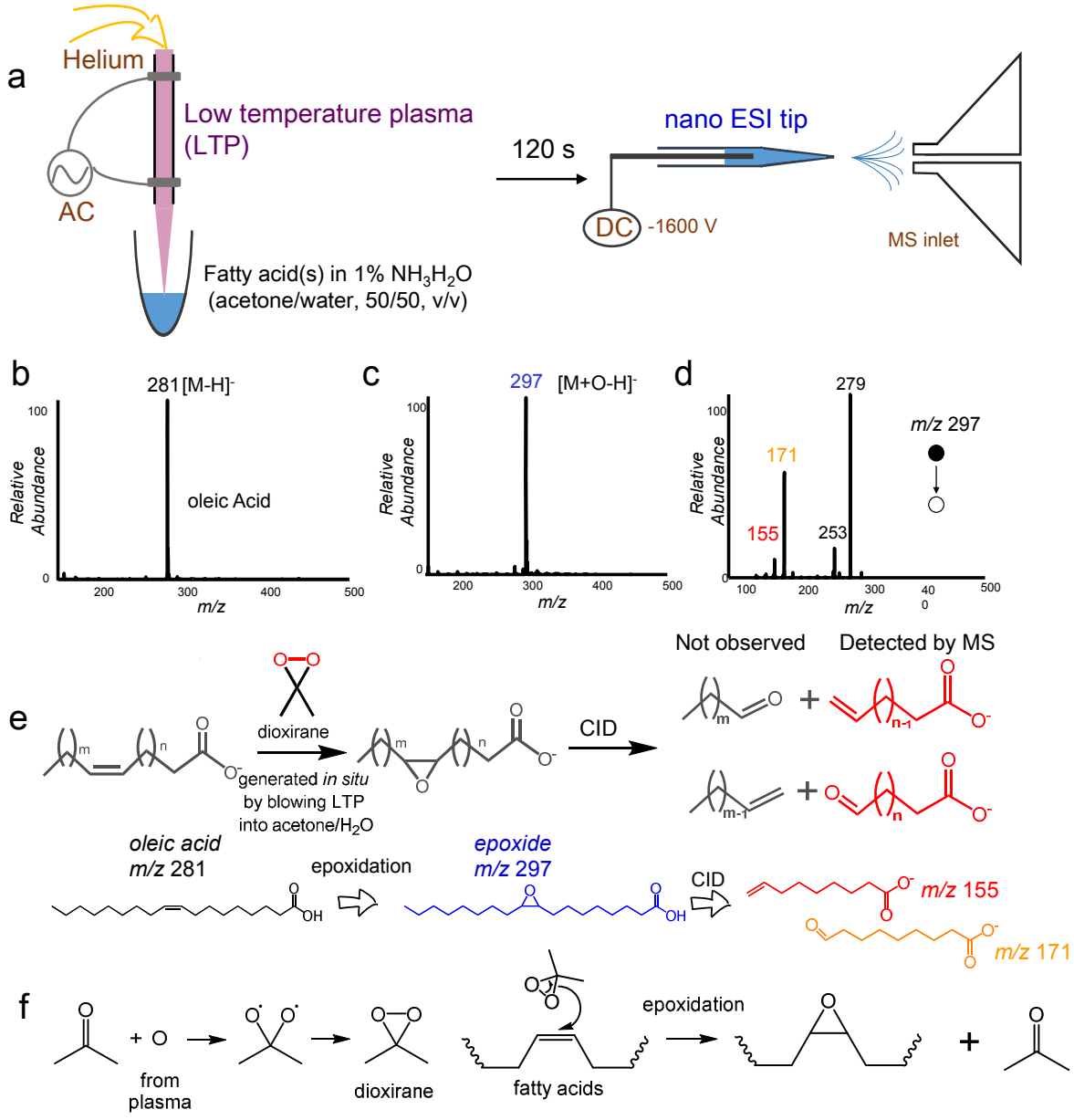


**Figure 1.** (a) Experimental setup for the epoxidation of unsaturated FAs catalyzed by a low-temperature plasma, followed by MS and tandem MS analysis. (b-d) Epoxidation of oleic acid followed by tandem MS analysis of its epoxidation product. NanoESI mass spectra of 70 $\mu$M oleic acid in the negative ion mode: before (b) and after (c) epoxidation, indicating an almost full conversion of oleic acid without other reaction channels. (d) MS/MS spectrum of oleic acid epoxide at *m/z* 297. (e) A general mechanism for the epoxidation of an unsaturated FA and the CID mechanism of its epoxide to release diagnostic ions. (f) Scheme for the *in situ* generation of dioxirane in acetone/water (50/50, v/v) by LTP, and the epoxidation of unsaturated fatty acids by dioxirane.[29,30]

To evaluate the versatility of the epoxidation strategy towards other unsaturated FAs,

palmitoleic acid (FA 16:1 (9Z)), *trans*-10-heptadecenoic acid (FA 17:1 (10E)) and *trans*-vaccenic acid (FA 18:1 (11E)) were selected and diagnostic ions similar to those observed for oleic acid were detected via MS/MS analysis of the epoxides. Regardless of the C=C location and *E/Z* geometry, all unsaturated FAs tested were quantitatively converted to their corresponding epoxides (Figure 2a-c). For FA 16:1 (9Z) which contains two less carbons than FA 18:1 (9Z), the mass-to-charge ratios of its diagnostics ions are identical to those of the latter because the C=C locations are identical in both FAs (Figure 1d). *Trans*-vaccenic acid has the same chain length as oleic acid, and both contain one C=C, albeit at different C=C locations. However, as the C=C in FA 18:1 (11E) is two more carbons away from the $COO^-$ than FA 18:1 (9Z) (oleic acid), the diagnostic ions of FA 18:1 (11E) were 28 Da higher in mass (*m/z* 183/199 vs. *m/z* 155/171) than those of oleic acid (Figure 2f). C=C Locations in other unsaturated FAs, such as FA 17:1 (10E), can be identified efficiently and accurately in a similar way (Figure 2e). The fragmentation schemes of the epoxides, which lead to the generation of diagnostic ions observed in Figure 2d-f, were shown in Figure 2g.

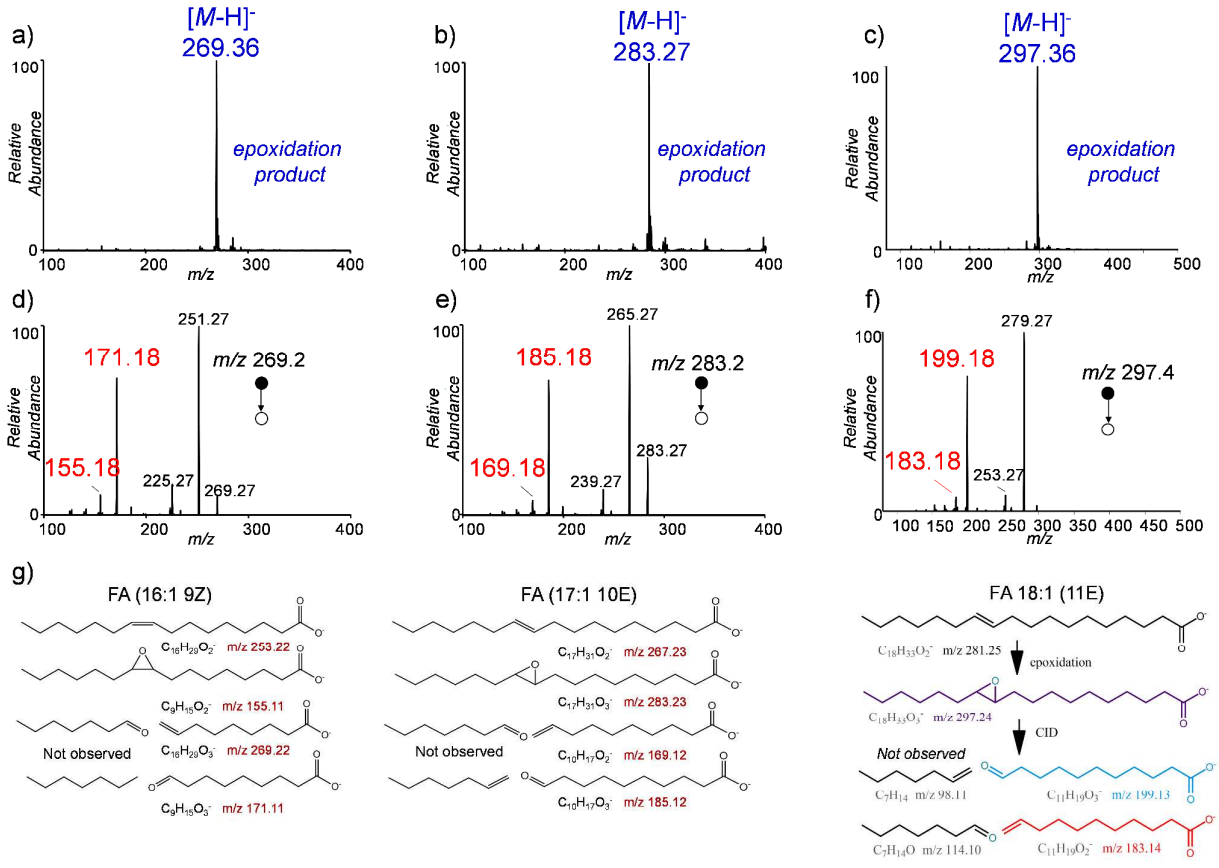


**Figure 2.** Structural analysis of palmitoleic acid (FA 16:1 (9Z)), *trans*-10-heptadecenoic acid (FA 17:1 (10E)) and *trans*-vaccenic acid (FA 18:1 (11E)) by epoxidation and MS/MS analysis. (a,b,c) Mass spectra

of FA 16:1 (9Z), FA 17:1 (10E) and FA 18:1 (11E) after epoxidation. (d,e,f) Tandem mass spectra of the epoxides of FA 16:1 (9Z), FA 17:1 (10E) and FA 18:1 (11E) (collision energy: 30 eV). (g) Proposed mechanisms of epoxide fragmentation for the release of diagnostic ions upon CID.

**Analysis of polyunsaturated FAs (PUFAs) by epoxidation and tandem MS**

When an unsaturated FA contains more than one C=Cs, the epoxidation reaction occurs non-selectively at each C=C location. This fact was verified through the analysis of linoleic acid (FA 18:2 (9Z, 12Z)) and linolenic acid (FA 18:3 (6Z, 9Z, 12Z)). As linoleic acid and linolenic acid contain two and three C=Cs, their epoxidation products can contain up to two and three epoxide rings, respectively (Figure 3a-d). Taking linoleic acid as an example, ions at *m*/*z* 295.36 correspond to epoxides generated after addition of one oxygen to either C=C, while ions at *m*/*z* 311.36 correspond to the epoxide after addition of two oxygens to both C=Cs. When ions at *m*/*z* 295.36 were mass-isolated and subjected to CID, two pairs of diagnostic ions were detected, at *m/z* 155.18/171.18 and *m/z* 195.18/211.18 (Figure 3b). Identification of each pair of diagnostic ions was straightforward due to characteristic 16 Da mass difference, as has been discussed previously. Diagnostic ions at *m/z* 155.18/171.18 were generated after C9-C10 bond cleavage and those at *m/z* 195.18/211.18 were generated after C12-C13 bond cleavage (Figure 3c). Together, this set of experimental data showed that when one oxygen was attached to a PUFA to form an epoxide, it can be attached at any C=C location. This phenomenon is very useful in identifying the locations of multiple C=Cs in a PUFA as first-step epoxidation products containing one oxygen are enough to reveal all C=C locations in a PUFA. In fact, the MS/MS spectrum of the final reaction product (added with two oxygens, *m/z* 311.36) is much more difficult to interpret than the products containing only one oxygen (SI, Figure S2). Similar results were observed for linolenic acid (Figure 3d,e), except that its epoxides contained up to three oxygens and its epoxides containing one oxygen released three pairs of diagnostic ions after CID (*m/z* 113.18/129.18, *m/z* 153.18/169.18, *m/z* 193.18/209.18), corresponding exactly to its three C=Cs at different locations (6Z, 9Z, 12Z). Therefore, for all unsaturated FAs we only use the epoxides containing one oxygen for MS and tandem MS analysis.

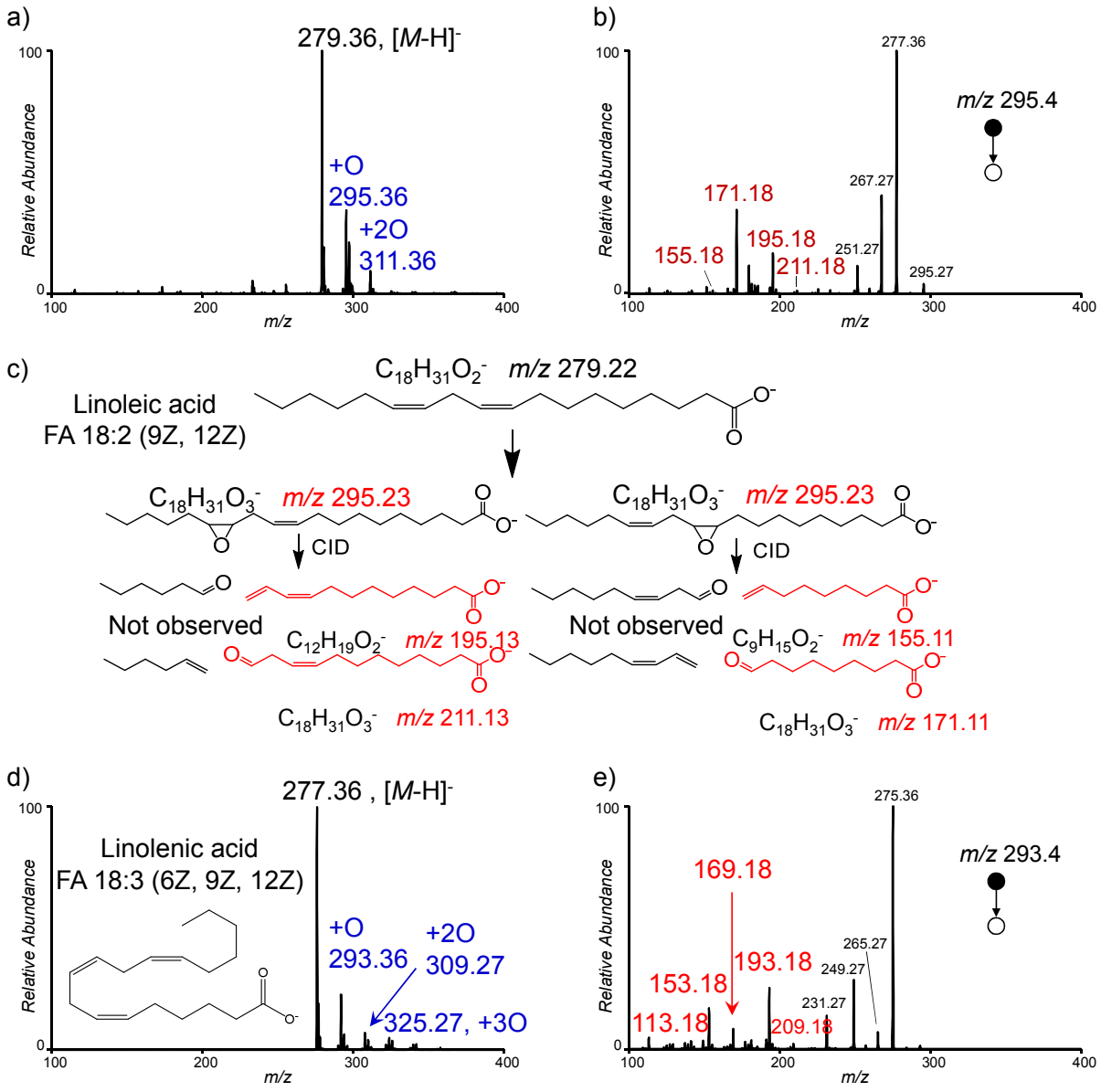


**Figure 3**. Structural analysis of linoleic acid (FA 18:2 (9Z, 12Z)) by epoxidation and MS/MS analysis. (a) MS spectrum of linoleic acid after epoxidation reaction. (b) MS/MS spectrum of the epoxide (+16 Da) at *m/z* 295. (c) Fragmentation schemes of the +16 Da epoxidation reaction products of linoleic acid. Note that the oxygen is non-selective for the C=C location when linoleic acid was epoxidized. (d) MS spectrum of linolenic acid (FA 18:3 (6Z, 9Z, 12Z)) after epoxidation reaction. (e) MS/MS spectrum of epoxide (+16 Da) at *m/z* 293.

**Mechanistic study on the source of oxygen that leads to the production of epoxides**

To study the source of oxygen that leads to the production of epoxides, we repeated the epoxidation of oleic acid by replacing $H_2O$ with $H_2{}^{18}O$. If the oxygen in water is responsible for epoxidation, then the epoxide of oleic acid should have an *m/z* of 299 rather than 297. However, after

reaction the *m/z* of the epoxide of oleic acid was still 297 (SI, Figure S3), strongly suggesting that oxygen in water was not involved in the epoxidation process. Moreover, since the oxygens in acetone and water can exchange with each other,[31] acetone is not involved in reaction either. We thus hypothesize that the oxygen gas in air may be the O source for epoxidation. To verify this hypothesis, different amounts of pure oxygen ($O_2$) were mixed with the discharge gas of helium for generating the LTP. As the amount of oxygen was gradually increased from 0% to 1.5% (by volume), the relative intensity of the epoxide (*m/z* 297) increased significantly after the same 20s of reaction (Figure 4a-e). When no oxygen was present in helium, most of the oleic acid was not reacted and the percentage of oleic acid intensity in the total ion intensity ($I_{281}/(I_{281} + I_{297} + I_{313})$) was >80% (Figure 4a). By contrast, the introduction of only 0.05% oxygen into helium resulted in ~50% of oleic acid converted to its epoxide (Figure 4b). We propose that under the influence of LTP, acetone was converted *in situ* to 3,3-dimethyldioxirane (DMDO) (see Figure 1e), which was capable to epoxidize the C=C in FAs.[29,30] Conventionally, DMDO is generated *in situ* by adding peroxymonosulfate ($HO_2SO_3^-$) to acetone in buffered aqueous solution.[32] In the helium-oxygen plasma, O and HO• are the primary species formed via well documented pathways.[33-35] We suspect that the reaction between O and acetone leads to the *in situ* generation of DMDO for the epoxidation of unsaturated FAs (Figure 1f). Such a mechanism explains why acetone has to be present in the FA solution, while other solvents (e.g. methanol, acetonitrile and $H_2O$) did not promote the epoxidation (Figure S7). Peroxidation, the major side-reaction channel resulting in a net addition of oxygen atom(s) to unsaturated FAs, competes more favorably for PUFAs than MUFAs. This is likely due to the fact that the rate constant of hydrogen atom abstraction, the first step for peroxidation, for the bis-allylic hydrogens in PUFAs is about 35 times higher than of the allylic hydrogens in MUFAs. [36] Besides, the direct cleavage of C=C by ozone ($O_3$) generated in LTP are also possible side reaction products, although they can be barely detected in our experiments.

With the introduction of pure oxygen unwanted over-oxidation products of oleic acid emerged at *m/z* 313 (addition of two oxygens) and even 329 (addition of three oxygens). Oleic acid was further consumed as the amount of oxygen was elevated (0.1%~1.5%), producing more epoxides and side-reaction products (Figure 4c-e). Together, these experimental results present strong and direct evidence that pure oxygen was the oxygen source for epoxidation of unsaturated FAs. Even if no oxygen is introduced into the discharge gas, $O_2$ in air will inevitably diffuse into the plasma and the

reaction system. However, with the significantly reduced oxygen concentration in helium, reaction selectivity was greatly improved. Therefore, throughout this work pure helium was used although $O_2$ can drastically accelerate the epoxidation reaction. With an optimal reaction time of 120 s (SI, Figure S5), the reaction yield can reach ~90% ($I_{281}/(I_{281} + I_{297})$). Other experimental parameters, such as helium flow rate (SI, Figure S6) and solvent composition (acetone/water, 50/50, v/v, see SI, Figure S7 and S9), were also studied for optimal reaction selectivity. Addition of 1% $NH_3$•$H_2O$ greatly enhanced the MS detection sensitivity for FAs and their epoxides in negative ion mode (SI, Figure S8).

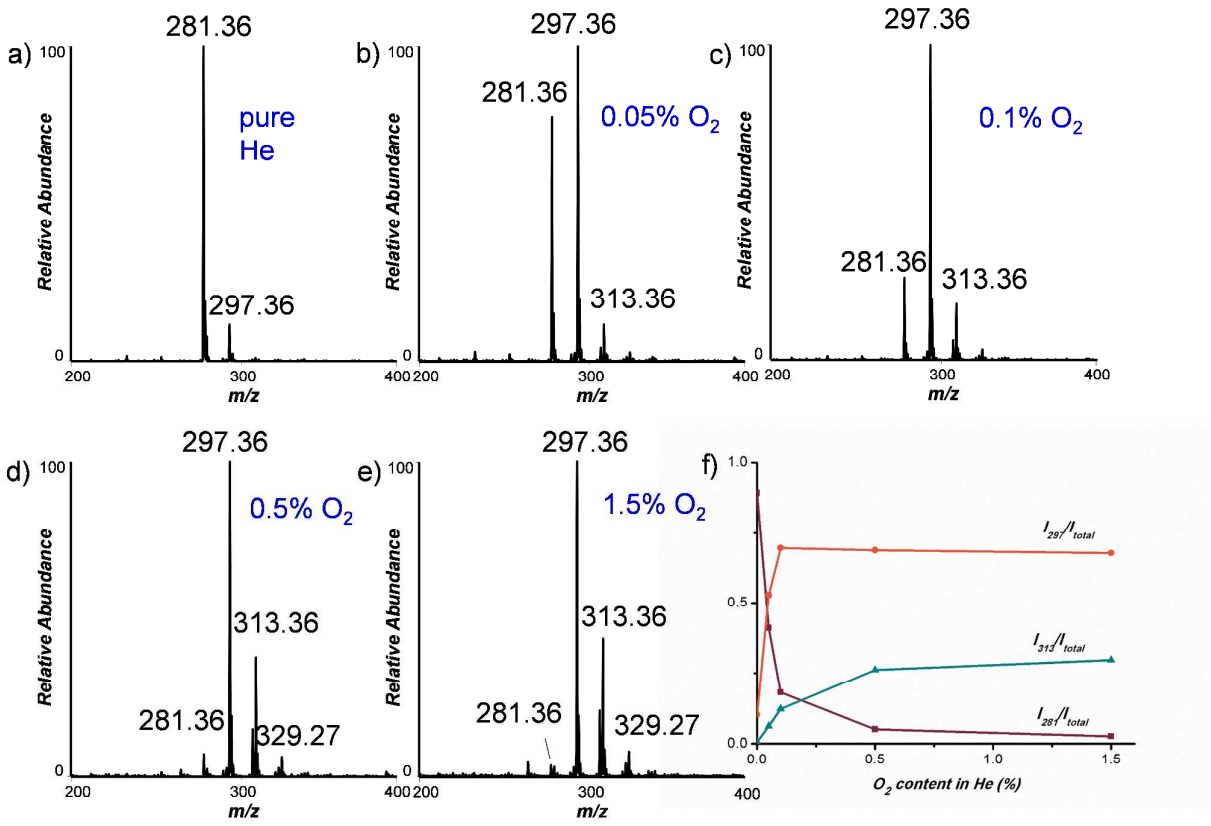


**Figure 4.** Discharge gas (helium) doped with varied amounts of oxygen for the mechanistic study of epoxidation. Reaction mass spectra collected using (a) pure helium, (b) 0.05% $O_2$ in helium, (c) 0.1% $O_2$ in helium, (d) 0.5% $O_2$ in helium, or (e) 1.5% $O_2$ in helium as the discharge gas for a reaction time of 20 s. (f) The ratios of the intensity of oleic acid (*m/z* 281), epoxidation product (*m/z* 297) and side reaction products (*m/z* 313) to the total intensity ($I_{281} + I_{297} + I_{313}$) as the amounts of oxygen introduced into helium were varied.

## Analytical performance

To evaluate the analytical performance of the developed method, the limit of detection (LOD) was evaluated using oleic acid as the example. When the concentration of oleic acid was reduced to 0.07 $\mu$M, the peak of the epoxide (*m/z* 297) can still be clearly observed (SI, Figure S10). More importantly, MS/MS CID mass spectrum of *m/z* 297 clearly shows the peaks of diagnostic ions indicative of the C=C location (Figure S11). As the absolute amount of oleic acid decreases, the time needed for its derivatization also decreases. For instance, 10 s of reaction time was enough for the full conversion of 0.07 $\mu$M oleic acid (0.1 mL). The relationship between the reaction yield and reaction time for 7 $\mu$M oleic acid was shown in SI, Figure S12.

## Identification and quantitation of unsaturated FAs in human plasma

A variety of FAs exist in the human plasma and a substantial proportion of them are unsaturated. In this study, as a real application we aim to use the developed method to identify and quantify all FA C=C isomers in human plasma. For absolute quantitation, tetradecenoic acid (FA 14:1 9Z) was used as the internal standard (IS). The spectrum of the FA extract from human plasma doped with the IS before epoxidation was shown in Figure 5a. The mass of unsaturated FAs increased by 16 Da after 60 s of reaction while saturated FAs remained intact (Figure 5b). Tandem MS analysis were performed to epoxides of unsaturated FAs to determine the C=C locations. It has been found that FA 16:1, FA 18:2 and FA 20:4 were all in pure forms of FA 16:1 (9), FA 18:2 (9, 12) and FA 20:4 (5, 8, 11, 14), respectively. By contrast, FA 18:1 was a mixture of two C=C location ($\Delta$9 and $\Delta$11) isomers. A table that summarizes the diagnostic ions for these FAs is listed in Table S1 in SI. For relative quantitation, a calibration curve ($y$=1.4466$x$+0.031) was prepared using a mixture of FA 18:1 Δ9 and Δ11 isomers mixed at different molar ratios, at a total concentration of 20 $\mu$M. The diagnostic ions intensity ratio ($I_{11Z}/I_{9Z}$, $y$) was plotted against the concentration ratio ($x$) for preparing the curve. $I$ represents the total intensity of diagnostic ions. For instance, $I_{11Z}$ is the sum of $I_{199}$ and $I_{183}$. A good linear relationship ($R^2$ = 0.9967) was obtained for the molar ratio (11Z/9Z) ranging from 1:15 to 3:1. In human plasma, FA 18:1 was a mixture of 92.15% $\Delta$9 and 7.85% $\Delta$11 isomers, as evidenced from the two sets of diagnostic ions at *m/z* 199/183 and *m/z* 171/155 (Figure 5c). FA 16:1 and FA 18:2 were identified to be FA 16:1 (9) and FA 18:2 (9, 12), respectively. The concentrations of FA 16:1,

FA 18:1 (*Δ*9 and *Δ*11 isomers) and FA 18:2 in the human plasma sample were quantified to be 104±14 $\mu$M, 214±23 $\mu$M, and 181±35 $\mu$M. The amounts of unsaturated FAs obtained by the current method are in line with those reported in the literature,[37] with the concentration of FA 16:1 (9Z) in the range of 0.9-93.2 $\mu$M, the concentration of FA 18:1 in the range of 36.1-676 $\mu$M and the concentration of FA 18:2 in the range of 78.7-586.4 $\mu$M (Table S3, SI).

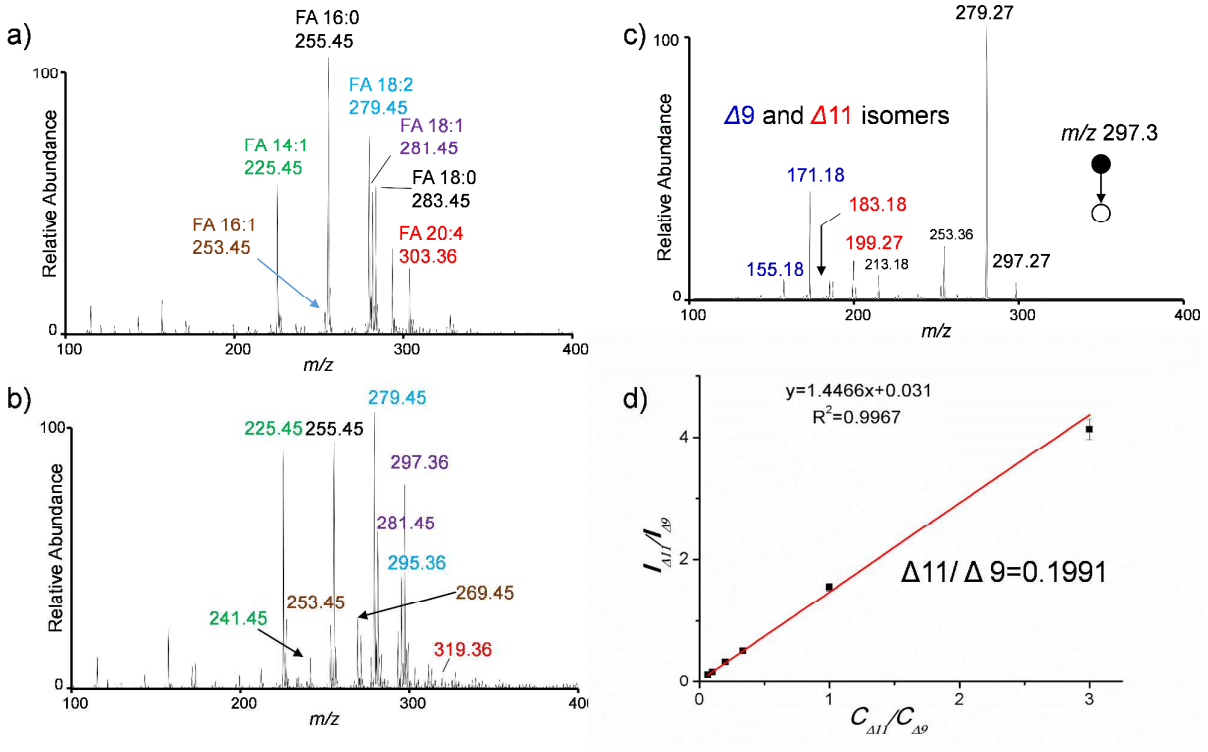


**Figure 5.** Identification and quantitation of C=C location isomers of FAs from human plasma. (-)NanoESI mass spectra of the FA extract from human plasma: (a) before and (b) after 60 s of epoxidation reaction. (c) MS/MS CID of the epoxide (*m/z* 297) of oleic acid. (d) The calibration curve for the relative quantitation of FA 18:1 *Δ*9 and *Δ*11 isomers.

## Conclusion

In conclusion, we have developed a novel method for the structural characterization and quantitation of FA C=C isomers via epoxidation reaction and tandem MS analysis. The epoxidation reaction between unsaturated FAs and oxygen was realized by blowing a low-temperature plasma into a solution of FAs dissolved in acetone/water. The almost quantitative transformation of MUFAs

makes the developed method especially attractive for the accurate quantitation of simple FA mixtures by using epoxide intensities. For determination of the C=C location and quantitation of FA C=C isomers, CID can be performed to FA epoxides of interests to release abundant diagnostic ions. The total relative intensities of diagnostic ions for a specific C=C isomer allow accurate quantitation. This analytical capability was successfully demonstrated via the analysis of unsaturated FAs extracted from human plasma. We are currently extending the developed method to identify and quantify many other types of unsaturated lipids, with the ultimate goal of using the composition of lipid C=C isomers for disease diagnosis and pathological studies.

## Associated Content

Supporting Information. The Supporting Information is available free of charge on the ACS Publications website.

Details for fabrication of the LTP probe, mechanistic studies, optimization of experimental parameters, and calibration curves for quantification (Figures S1−S12 and Table S1-S2) (PDF)

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## References


(1) Wymann, M. P.; Schneiter, R. *Nat. Rev. Mol. Cell Bio* **2008**, *9*, 162-176.
(2) Kagan, V. E.; Mao, G.; Qu, F.; Angeli, J. P.; Doll, S.; Croix, C. S.; Dar, H. H.; Liu, B.; Tyurin, V. A.; Ritov, V. B.; Kapralov, A. A.; Amoscato, A. A.; Jiang, J.; Anthonymuthu, T.; Mohammadyani, D.; Yang, Q.; Proneth, B.; Klein-Seetharaman, J.; Watkins, S.; Bahar, I.; Greenberger, J.; Mallampalli, R. K.; Stockwell, B. R.; Tyurina, Y. Y.; Conrad, M.; Bayir, H. *Nat. Chem. Biol.* **2017**, *13*, 81-90.
(3) Chavarro, J.; Stampfer, M.; Loda, M.; Campos, H.; Sesso, H.; Ma, J. *Cancer Res.* **2009**, *69*.
(4) Zhao, Y.; Wei, Z.; Zhao, H.; Jia, J.; Chen, Z.; Zhang, S.; Ouyang, Z.; Ma, X.; Zhang, X. *Anal. Chem.* **2016**, *88*, 10805-10810.
(5) Li, J.; Condello, S.; Thomes-Pepin, J.; Ma, X.; Xia, Y.; Hurley, T. D.; Matei, D.; Cheng, J.-X. *Cell Stem Cell* **2017**, *20*, 303-314.e305.
(6) Brenna, J. T. *Current Opinion in Clinical Nutrition and Metabolic Care* **2013**, *16*, 548-554.
(7) Christie, W. W. *Lipids* **1998**, *33*, 343-353.
(8) Seppanen-Laakso, T.; Laakso, I.; Hiltunen, R. *Anal. Chim. Acta* **2002**, *465*, 39-62.
(9) Vrkoslav, V.; Cvačka, J. *J. Chromatogr. A* **2012**, *1259*, 244-250.
(10) Davoli, E.; Gross, M. L. *J. Am. Soc. Mass Spectrom.* **1990**, *1*, 320-324.
(11) Trimpin, S.; Clemmer, D. E.; McEwen, C. N. *J. Am. Soc. Mass Spectrom.* **2007**, *18*, 1967-1972.
(12) Voinov, V. G.; Claeys, M. *Int. J. Mass. Spectrom.* **2001**, *205*, 57-64.
(13) Bollinger, J. G.; Thompson, W.; Lai, Y.; Oslund, R. C.; Hallstrand, T. S.; Sadilek, M.; Turecek, F.; Gelb, M. H. *Anal Chem* **2010**, *82*, 6790-6796.
(14) Wang, M.; Han, R. H.; Han, X. *Anal. Chem.* **2013**, *85*, 9312-9320.
(15) Yang, K.; Dilthey, B. G.; Gross, R. W. *Anal. Chem.* **2013**, *85*, 9742-9750.
(16) Kuklev, D. V.; Smith, W. L. *J. Lipid. Res.* **2003**, *44*, 1060-1066.
(17) Michaud, A. L.; Yurawecz, M. P.; Delmonte, P.; Corl, B. A.; Bauman, D. E.; Brenna, J. T. *Anal. Chem.* **2003**, *75*, 4925-4930.
(18) Moe, M. K.; Anderssen, T.; Strom, M. B.; Jensen, E. *J. Am. Soc. Mass Spectrom.* **2005**, *16*, 46-59.
(19) Moe, M. K.; Jensen, E. *Eur. J. Mass. Spectrom.* **2004**, *10*, 47-55.
(20) Van Pelt, C. K.; Brenna, J. T. *Anal. Chem.* **1999**, *71*, 1981-1989.
(21) Thomas, M. C.; Mitchell, T. W.; Harman, D. G.; Deeley, J. M.; Murphy, R. C.; Blanksby, S. J. *Anal. Chem.* **2007**, *79*, 5013-5022.
(22) Pham, H. T.; Maccarone, A. T.; Campbell, J. L.; Mitchell, T. W.; Blanksby, S. J. *J. Am. Soc. Mass Spectrom.* **2013**, *24*, 286-296.
(23) Poad, B. L. J.; Pham, H. T.; Thomas, M. C.; Nealon, J. R.; Campbell, J. L.; Mitchell, T. W.; Blanksby, S. J. *J. Am. Soc. Mass Spectrom.* **2010**, *21*, 1989-1999.
(24) Thomas, M. C.; Mitchell, T. W.; Harman, D. G.; Deeley, J. M.; Nealon, J. R.; Blanksby, S. J. *Anal. Chem.* **2008**, *80*, 303-311.
(25) Ma, X.; Chong, L.; Tian, R.; Shi, R.; Hu, T. Y.; Ouyang, Z.; Xia, Y. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113*, 2573-2578.
(26) Ma, X.; Xia, Y. *Angew. Chem. Int. Ed.* **2014**, *53*, 2592-2596.
(27) Ma, X.; Ouyang, Z. *Trac-Trend. Anal. Chem.* **2016**, *85*, 10-19.

(28) Harper, J. D.; Charipar, N. A.; Mulligan, C. C.; Zhang, X.; Cooks, R. G.; Ouyang, Z. *Anal. Chem.* **2008**, *80*, 9097-9104.
(29) Wong, O. A.; Shi, Y. *Chem. Rev.* **2008**, *108*, 3958-3987.
(30) Murray, R. W. *Chem. Rev.* **1989**, *89*, 1187-1201.
(31) Roberts, I. *Ann. Ny. Acad. Sci.* **1939**, *39*, 375-394.
(32) Murray, R. W.; Jeyaraman, R. *J. Org. Chem.* **1985**, *50*, 2847-2853.
(33) Sankaranarayanan, R.; Pashaie, B.; Dhali, S. K. *Appl Phys Lett* **2000**, *77*, 2970-2972.
(34) Fehsenfeld, F. C.; Mosesman, M.; Ferguson, E. E. *J. Chem. Res.* **1971**, *55*, 2115-2120.
(35) Laux, C. O.; Spence, T. G.; Kruger, C. H.; Zare, R. N. *Plasma Sources Sci. Technol.* **2003**, *12*, 125.
(36) Xu, L.; Porter, N. A. *Free Radic. Res.* **2015**, *49*, 835-849.
(37) Ukolov, A. I.; Orlova, T. I.; Savel'eva, E. I.; Radilov, A. S. *J. Anal. Chem.* **2015**, *70*, 1123-1130.

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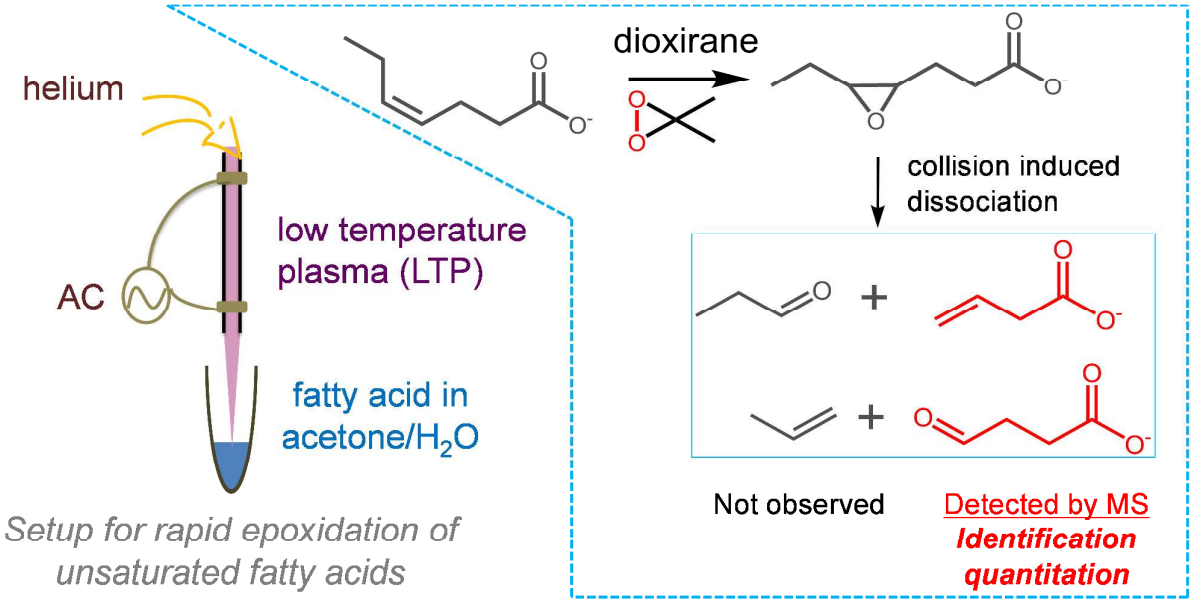


*Setup for rapid epoxidation of unsaturated fatty acids*