Verdel Instruments

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Lipid feature characterisation using Total Correlation Mass Spectrometry and ultraviolet photodissociation

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This is an application note and does not contain a detailed experimental section.

Abstract

In order to fully characterise lipids, it is important to determine the position, length and degree of unsaturation in their fatty acid moieties. Currently, determining all this information simultaneously is not possible. Typically, a combination of analysis (e.g., LC, GC, MS/MS and MS^3) is utilised to determine most of this information, however, no direct connection between the whole lipid specie and the fatty acid moieties' double bond position (e.g., ω-3, ω-6, etc.) can be made. Furthermore, it is very difficult and time consuming to determine the basic fatty acid composition of any appreciable number of lipids: since utilising MS/MS or MS^3 typically results in head group cleavage or drastically affects the sensitivity and number of lipids analysed due to the instrument limitations. Total correlation mass spectrometry (TOC-MS™) is a new data-independent analytical technique, which uses ultraviolet photodissociation in order to selectively fragment lipids at pi-bonds, such as carbon-carbon double bonds and/or ester or amide linkages. These fragments can then be intrinsically correlated to their precursor molecule without prior chromatographic separation or quadrupolar isolation. This application note shows how TOC-MS can be used to obtain a detailed characterisation of a complex lipid profile, gaining key characteristic information such as: the lipid species headgroup(s), the fatty acid composition(s) and the location of unsaturated bonds within those fatty acids, concurrently for all the detected lipid species within a given sample using a single direct-infusion analytical method without derivatisation.

Introduction

The importance of lipid characterisation in order to understand their function is well recognised but has only really been possible in the last 30 years (1) with the introduction of soft-ionisation mass spectrometry sources (e.g., electrospray) and tandem MS/MS approaches, which allowed for the isolation and targeted fragmentation of individual lipid species. Many of the reported approaches are reliant on lipid derivatisation, such as ozonolysis, in order to identify biologically important features such as the location of unsaturated bonds within the fatty acid moieties of whole lipids. Additionally, sub-optimal fragmentation is often required in order to identify the relative position of these fatty chains, which reduces the speed of analysis such that it is no longer compatible with online chromatographic separation methods, or is reliant on selective hydrolysis before analysis, reducing throughput (2).

Total correlation mass spectrometry (TOC-MS) is a new analytical technique that combines ultraviolet photodissociation (UVPD) with the ability to inherently link the fragments produced with their precursors (3). This correlation ability has been previously demonstrated on FT-ICR-MS instruments using a process known as 2D-MS (4). Where TOC-MS differs is in its adaptation to Q-ToF MS instruments, allowing for higher throughput due to the increased speed of analysis offered by the ToF compared to the ICR, whilst still giving low ppm level mass accuracy.

For lipid analysis, the use of UVPD allows for the potential to characterise lipids down to the structure defined level, as proposed by Liebisch et al. (5), which includes characterisation of the lipid head group (if applicable), double bond position, relative position of the tails (i.e. the sn-position) and the location of chain substitutions such as branching (6).

Methodology

Lipid standards and solvents of the highest purity possible were purchased from Sigma Aldrich. Two lipid standards were chosen for this work, a C2-C10 triglyceride mix contains equal amounts of triacetin (C2:0), tributyrin (C4:0), trihexanoin (C6:0), trioctanoin (C8:0) and tridecanoin (C10:0) and lyso-phosphatidylcholine (LPC). The triglyceride mixture was chosen as it allowed for an assessment of UVPD fragmentation efficiency and selectivity for the ester bond, as well as an assessment of how fragmentation efficiency changes with fatty acid chain length. LPC was selected because of its structural simplicity, containing one fatty acid chain, one site of unsaturation and one head group, which allowed for an assessment of UVPD fragmentation efficiency at each of these sites.

Solutions for analysis were prepared by diluting lipid standards with a 1:1:1 mixture of LC-MS grade water: acetonitrile: isopropyl alcohol with addition of 0.1 % (v/v) formic acid. C2-C10 triglyceride mix and LPC_(18:1-d_7) were diluted to a final concentration of 50 µg mL^{-1} and 25 µg mL^{-1} respectively. Samples were infused directly into the TOC-MS instrument at a rate of 0.25 µL min^{-1} using a borosilicate glass, 22 gauge, 500 µL Hamilton syringe and a NE-1000 programmable single syringe pump.

MS source conditions: End plate offset voltage: 500 V, Capillary voltage: 4.0 kV, Nebulising gas pressure: 0.3 Bar, Dry gas flow rate: 4.0 mL min^{-1}, Dry gas temp: 200°C

UVPD parameters: Laser wavelength: 213 nm.

Results and discussion

The determination of glycerolipids structures

The C2-C10 triglyceride mix TOC-MS spectra showed four clear precursor ion peaks on the diagonal auto-correlation line, representing the sodiated parent ions for C4:0-C10:0 (figure 1). The auto-correlation line represents where the mass detected by the instrument (x-axis) matches the mass of the precursor (y-axis), and as such should only contain molecules present in the instrument before fragmentation, i.e., only precursors. In the top-down TOC-MS plot below, features with the same y-axis coordinates are related to each other as they have the same molecular precursor and as such have been linked by the TOC-MS process.

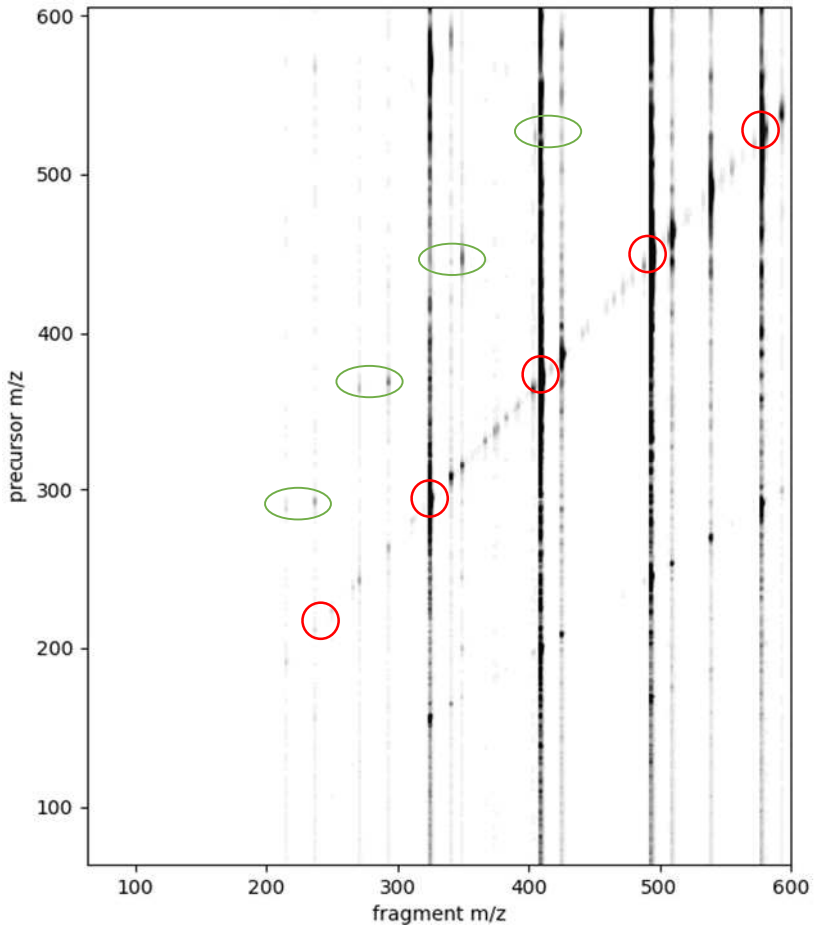

Figure 1. TOC-MS spectra of C2-C10 triglyceride mix showing precursor ions (red) and fragments (green).

From this plot it is possible to see the fragments visible associated with their respective precursor ions, as shown in figure 1, where the *m/z* of these fragment ions can be determined from the x-axis. For the triacylglyceride standards two fragment ions were identified from each precursor ion: the loss of a fatty acid $[M+Na-C_4H_7O_2]^+$ and loss of a fatty aldehyde $[M+Na-C_4H_7O]^+$. Of these two fragments the loss of the whole fatty acid was the most abundant of the two fragments. A horizontal extract can then be taken out of the plot in order to identify fragments associated with each precursor ion, including triacetin (figure 2). For tributyrin, trihexanoin, trioctanoin and tridecanoin there no other fragments identified. However, for triacetin the fragment $[C_7H_{11}O_4+Na]^+$, associated with loss of the whole fatty acid tail, was identified despite not being visible on the initial TOC-MS spectra (figure 1).

The identification of the fatty acid composition of these triglycerides was important as it pointed to the ability for TOC-MS to be able to identify the fatty acid in lipids where there are tails of different lengths.

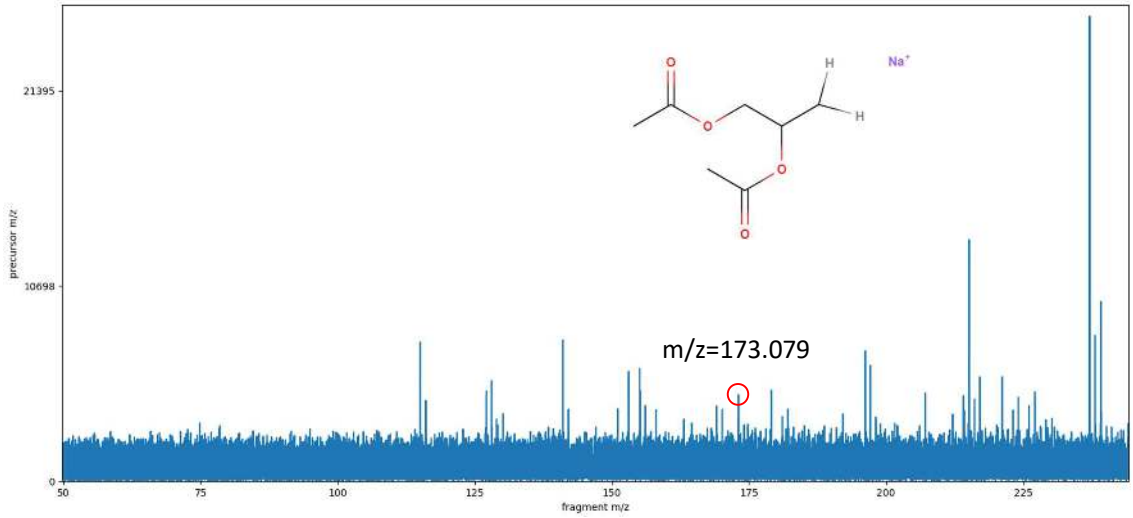

Figure 2. Triacetin spectra showing fragment (red) associated with fatty acid loss.

The determination of phospholipid structures

The structure of LPC_(18:1-d_7) is shown below (figure 3) and in contrast to the triglycerides, the protonated parent ion was more abundant than the sodiated (figure 4). From the initial TOC-MS plot a horizontal extract was taken to produce an MS spectra showing the precursor ion and associated fragments (figures 5 and 6). From this, three fragments were identified (figures 7-9) showing fragmentation at the C=C double bond at the n-9 position $[M+H\text{-}C_{10}D_7H_{12}]^+$, loss of the phosphatidylcholine headgroup $[M+H\text{-}C_5H_{14}NPO_4]^+$ and its corresponding fragment where charge is retained on the headgroup $[M+H\text{-}C_{18}H_{32}D_7O_3]^+$.

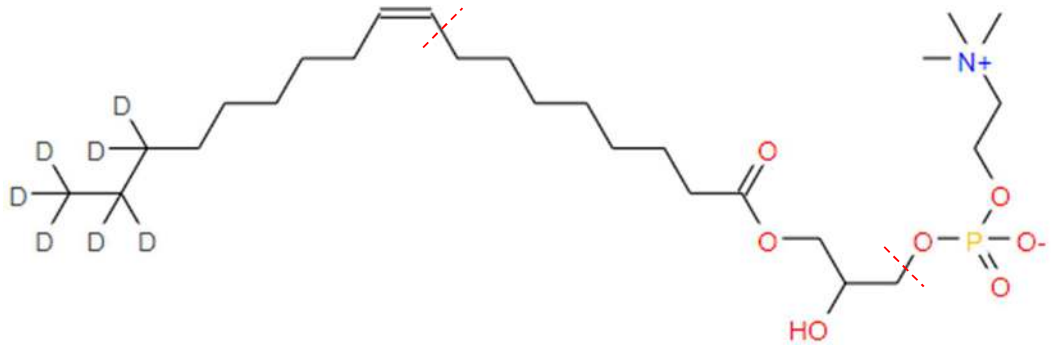

Figure 3. Structure of LPC_(18:1-d_7) and proposed locations of bond fragmentation (red) to form the deuterated C_{10} and PC headgroup and loss of PC headgroup fragments respectively.

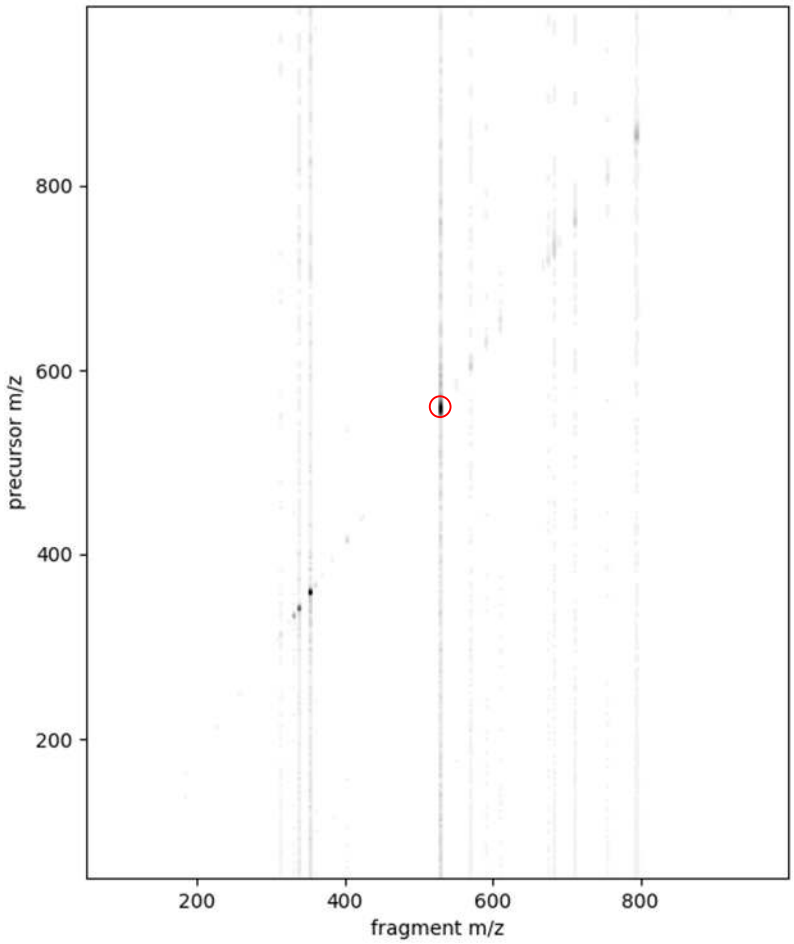

Figure 4. TOC-MS spectra showing LPC_(18:1-d_7) precursor ion (red).

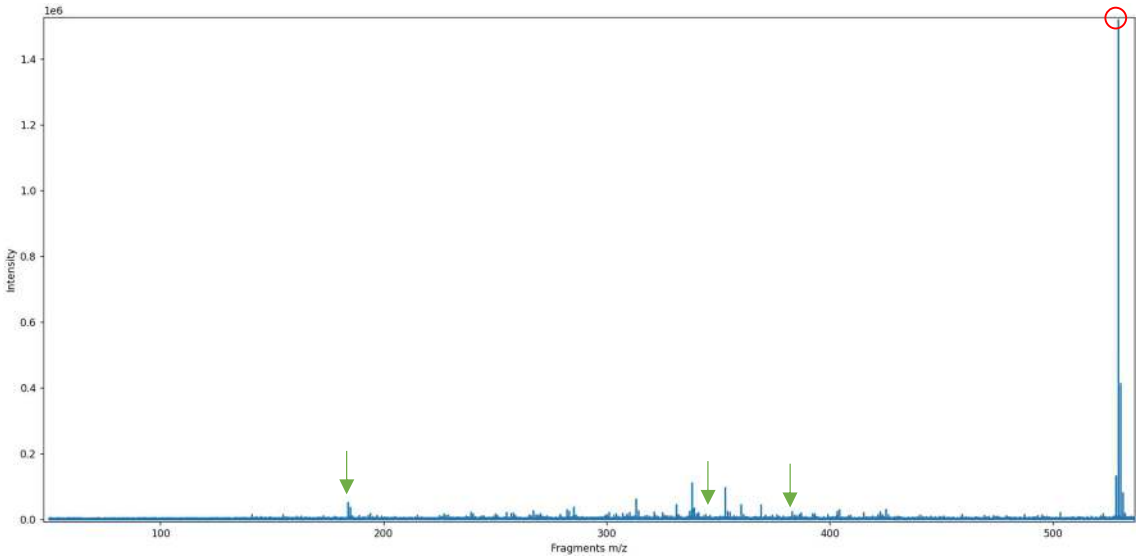

Figure 5. Extracted TOC-MS spectra showing LPC_(18:1-d_7) precursor ion (red) and fragments (green).

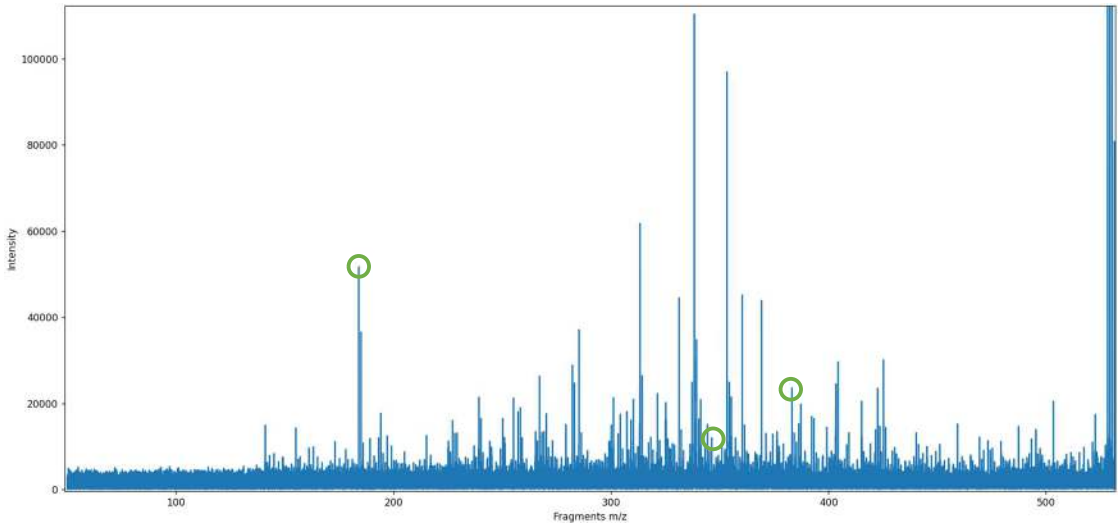

Figure 6. Extracted TOC-MS spectra showing LPC_(18:1-d_7) fragments (green).

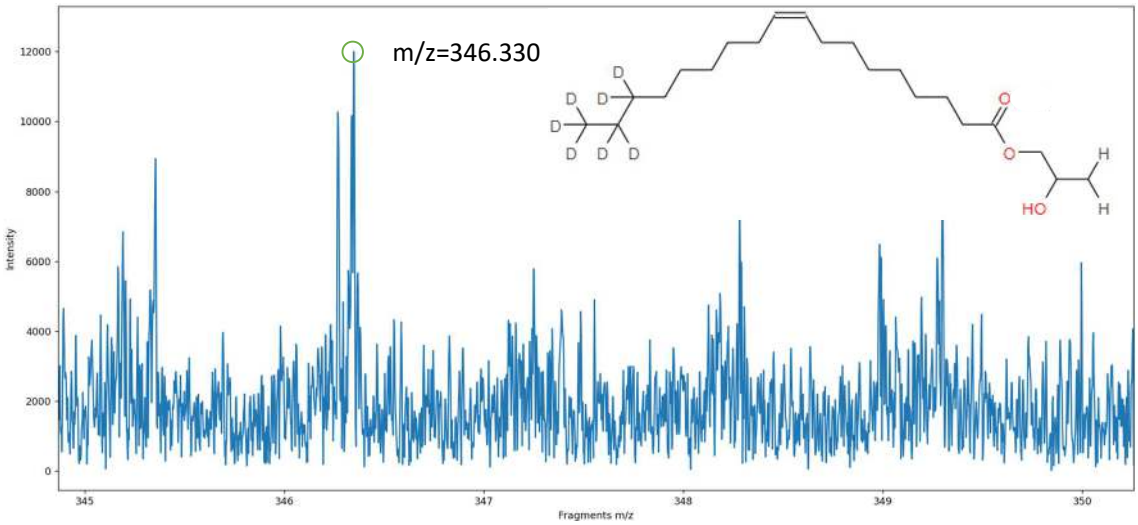

Figure 7. Extracted TOC-MS spectra showing a fragment resulting from loss of the PC head group.

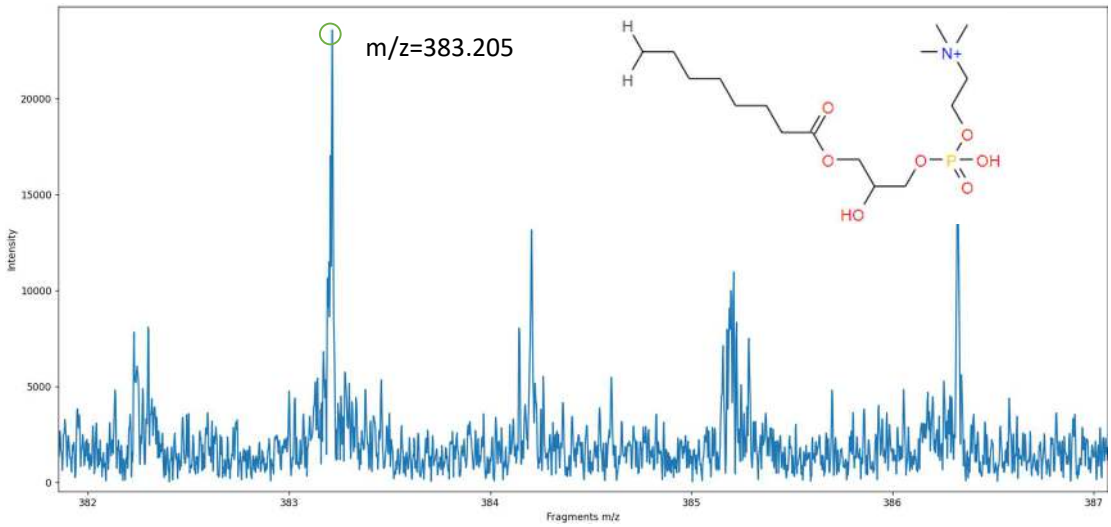

Figure 8. Extracted TOC-MS spectra showing a fragment at the C=C double bond position (n-9).

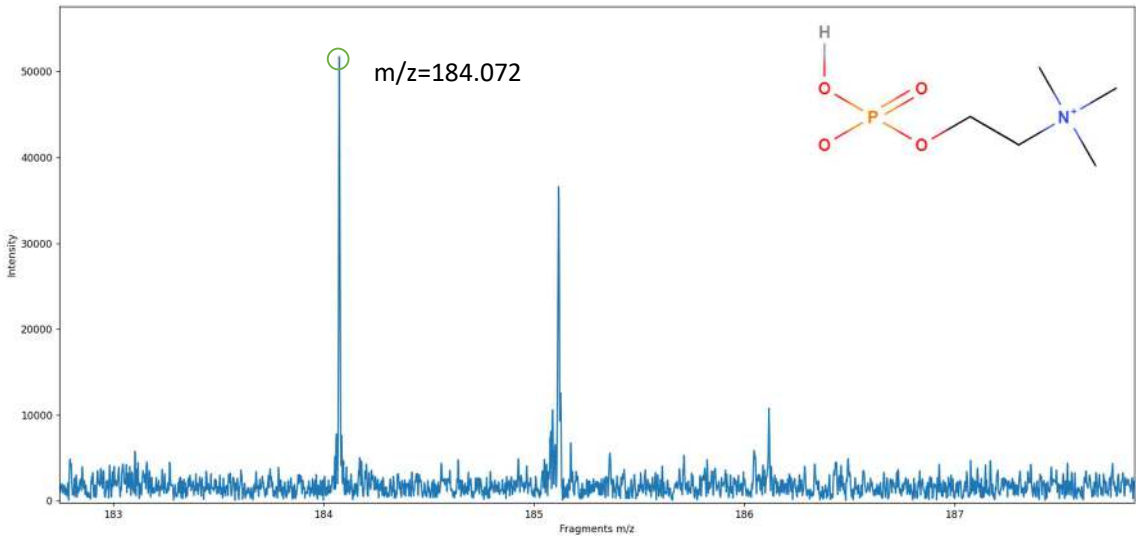

Figure 9. Extracted TOC-MS spectra showing the phosphatidylcholine headgroup

The combination of these two fragments allowed for TOC-MS to be able to confirm key structural features of the lipid including double bond position, head group composition and the confirmation of the location of deuteration (figure 10).

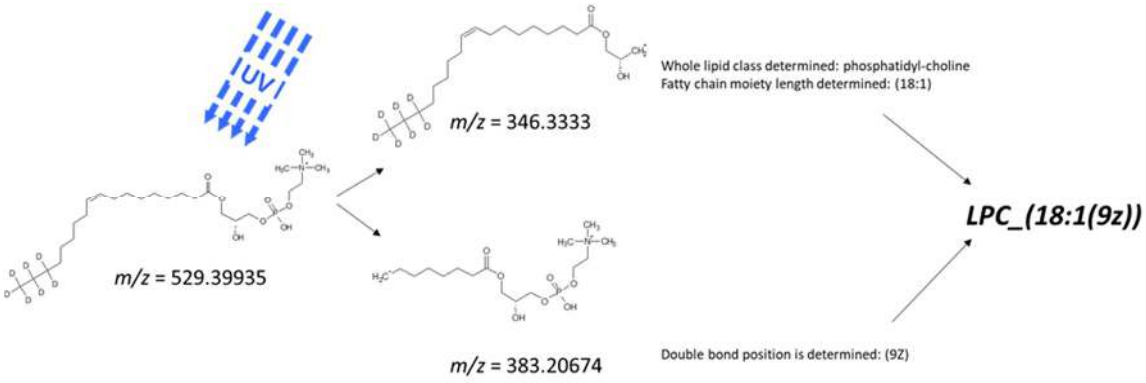

Figure 10. Fragmentation pathway for LPC_(18:1)d_7 via UVPD

Loss of the intact fatty acid was not observed, perhaps due to the presence of "stronger" chromophores, such as the C=C double bond. The presence of deuterium was not expected to influence the extinction coefficient of the C=C chromophore due to the length of the alkyl carbon chain between them. This is, perhaps, beneficial as it is often harder to locate the double bond location than it is to determine the fatty acid composition, which could be deduced in figure 10 from the 346 m/z fragment.

When analysing an unknown lipid, the combination of fragments would allow for full characterisation of the lipid including double bond position, whilst the loss of intact fatty acids demonstrated for the triglycerides would potentially allow for the characterisation of the sn-position, as defined by Leibsich *et al*. (5). This was achieved without the need for derivatisation, analyte separation or complex method development in order to produce fragmentation that was both sufficient for characterisation and could be linked to the parent molecule.

Conclusion

The analytical method outlined in this publication was able to sufficiently characterise lipids down to the double bond position level using a simple direct infusion TOC-MS method. The benefits of UVPD for selectively fragmenting lipids at regions of interest, such as carbon-carbon double bonds and esters, combined with the ability of TOC-MS to inherently link molecular precursors with fragments allowed for the successful characterisation of a series of triglycerides and a lyso-phosphatidylcholine. This ability was demonstrated even where the precursor was not detected in the final TOC-MS spectra. This approach can be applied to lipids of any class or degree of unsaturation in order to provide lipid characterisation using a single analytical platform.

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