Identifying Lipids and Other Small Molecules from Imaging Mass Spectrometry Experiments Using Tandem Mass Spectrometry and Exact Mass

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Introduction

Imaging mass spectrometry (IMS) provides for compound localization in thin tissue sections, often producing very intriguing images. Critical to IMS studies and disease characterization is identification of these compounds detected from a tissue section. Besides the m/z value of an ion, which is typically not sufficient in the small molecule regime, identification of ion signals is accomplished by tandem MS using characteristic structural fragments. Using an instrument capable of tandem MS for IMS enables the collection of fragmentation spectra directly from the tissue to assist in compound identification. Exact mass is an additional tool that can be utilized for identification of unknown signals and is often critical in correct assignment. The use of tandem MS directly from tissue sections and tissue extracts using LC/MS and LC/MS/MS and exact mass is employed for these studies. The evaluation of post-acquisition software for exact mass measurements on a triple quadrupole is compared to data acquired on a high mass accuracy TOF system.

Experimental

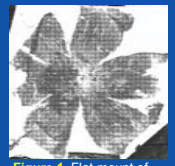

Figure 1. Flat-mount of eye section.

Flat-mounted eye sections were first analyzed by imaging mass spectrometry on a Thermo LTQ fitted with a vMALDI ion source operating at intermediate vacuum pressure. Full scan MS and MS/MS data were collected for several ions of interest in the phospholipid mass region (m/z 600-900) and in the lower m/z region (<600) for structural characterization. For further structural identification and exact mass calculations, extracts from the same eye tissues were prepared using the Folch method. LC/MS experiments were performed on a TSQ Quantum Ultra with Accela HPLC system (50 mm x 2.1mm, 5µm phenyl column) and an Agilent ESI-TOF with Agilent 1200 HPLC system. Exact mass measurements were performed using MassWorks from Cerno Biosciences (TSQ data) and Agilent's MassHunter (ESI-TOF data).

Results and Discussion

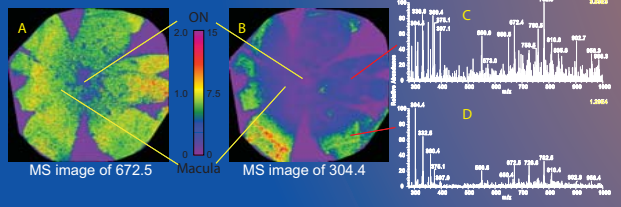

Figure 2A shows an MS image of m/z 672.5 acquired from a flat-mounted eye section (human donor age 56) and Figure 2B shows an MS image of m/z 304.4. The spectra at the right show the ions detected in two areas of the flat-mount: nearer to the optic nerve, ON (C) and in the periphery (D). The ion at m/z 304.4 shows a greater distribution in the peripheral regions of the flat-mount and very little distribution nearer to the ON, while the ion at m/z 672.5 shows a more uniform distribution. Unique images such as these are often created using imaging MS. In the small molecule range, additional tools are necessary to identify the ion. The first step towards identification of these compounds involved tandem mass spectrometry. Scale bars are in the thousands.

Results and Discussion

Tandem MS of the ion at m/z 672.4 (Figure 3) showed fragmentation similar to a phosphatidylcholine (PC) with a loss of 59 after MS/MS and a loss of 124 after MS³. Additional products at m/z 441.4 and m/z 357.2 identify the fatty acyl chains as palmitic acid (16:0) and capric acid (10:0). The location of the glycerol backbone was difficult to determine because the intensity of the two fragments is nearly equal. Knowledge of PC fragmentation was necessary for postive ID and thus exact mass was not needed here.

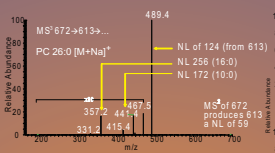

Figure 3. MS³ of m/z 672.4. This ion is identified as PC 26:0, $[M+Na]^+$.

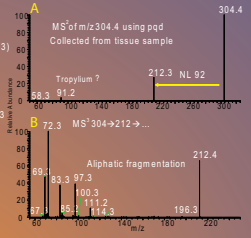

Figure 4. MS² of m/z 304.4 (A) and MS³ of m/z 304.4 to 212.4 (B).

Tandem MS of the ion at m/z 304.4 was performed. After MS², using pqd (Figure 4A), a neutral loss of 92 was the most abundant product and an ion at m/z 91 is produced; therefore, the compound likely contains a phenyl substituent. MS³ of m/z 212 (Figure 4B) indicates the presence of a long carbon chain and with an even m/z value, the compound contains an odd number nitrogens. The next step towards identification is finding related species, which may help to determine a class of compounds.

Finding related species with IMS

Images showing similar distributions in the tissue may help to identify compounds that are related. Figure 5 shows three ions with a similar distribution in the tissue as m/z 304.4. All these ions are separated by 28 mass units, including m/z 304. The images seem to suggest the ions are related and the MS² data in Figure 4B confirms their similarity to m/z 304. The differences of 28 seemed to indicated that a long carbon chain is present with varying lengths. The intensity decreases as the mass increases.

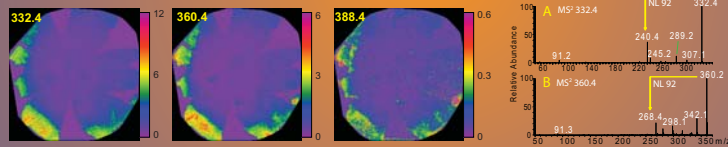

Figure 5. MS images of 3 ions that appear to be related to m/z 304. All the ions are separated by 28, indicating a varying carbon chain. MS² spectra of m/z 332.4 and 360.4 are shown in A and B, respectively. Similar fragmentation as m/z 304 is seen. Scale bars are in the thousands.

Exact mass studies

An eye flat-mount was extracted following the Folch method (Chloroform:methanol). A lipid extraction method was selected because the compound at m/z 304 was thought to be fatty acid related. Exact mass measurements were performed in two ways:

1. LC/MS Analysis of the extract was performed on an ESI-TOF (Agilent Technologies), Agilent 1200 HPLC and autosampler. MassHunter was used for exact mass calculations.

2. LC/MS and LC/MS/MS Analysis of the extract was performed on a ESI-Triple Quadrupole (TSQ Quantum Ultra, ThermoFisher), Accela HPLC and autosampler. Exact mass measurements were performed using MassWorks (Cerno Biosciences). Full-scan MS data was collected at an FWHM of 0.2 in Q1 (resolution at m/z 300 was 1500). PEG standards were infused post acquisition for peak shape verification and mass accuracy calucations. Exact mass of product ions was performed by setting Q1 to an FWHM of 4 (pass all isotopes) and Q3 at unit mass resolution (FWHM of 0.7) acquiring a 10 amu wide window in Q3. External calibration for MS/MS involved infusing compounds which produced products of similar m/z values as the unknown.

Results and Discussion

An example of the separation achieved of the unknown compounds by HPLC on the ESI-TOF system is shown in Figure 6. In addition to these compounds, multiple phospholipid species were detected, as expected using the Folch method of extraction. An example of the MS data obtained on the TSQ quantum at FWHM of 0.2 is shown in Figure 7. Acquiring full-scan MS data at a higher resolution on the TSQ was necessary for exact mass calculations using an external program (MassWorks). The downside of acquiring at a higher resolution is the decrease in scan speed. Table 1 compares the exact mass calulations on the two systems. For m/z 304, both systems provide the same chemical formula, but the TOF has less error. MS/MS data on the TSQ was acquired for exact mass calculations using MassWorks Table 2 lists the results for the two major products m/z 91 and 212. The product ion at m/z 91 is confirmed as tropylium and the product ion at 212 appears to be an unsaturated carbon chain. We know the following: 1) A phenyl ring is present and must be able to produce the tropylium ion upon CID, 2) one nitrogen, 3) long carbon chain, and 4) no oxygen, (not a fatty acid). How are they all connected? The key to finding out came from a literature search , finding a paper that used field desorption to analyze complex mixtures, one being quaternary ammonium salts (benzylalkyldimethylammonium salts-BAC).[1] MS/MS spectra matched closely to the ones generated here.

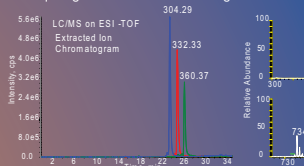

Figure 6. RIC for m/z 304, 332, and 360 from LC/MS (ESI-TOF) of the eye extract

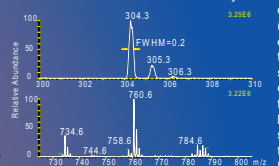

Figure 7. Mass spectra from LC/MS on the TSQ Quantum with a FWHM of 0.2.

Instrument	Formula	Mono isotope	Mass error (mDa)	Mass error (PPM)
TSQ	$C_{21}H_{38}N$	304.3004	11.4748	37.7073
TOF	$C_{21}H_{38}N$	304.2998	2.023	6.6481
TOF	$C_{23}H_{42}N$	332.3311	-2.6771	-2.0374
TOF	$C_{25}H_{46}N$	360.3624	0.8227	2.283

Table 1. Exact mass calculations from LC/MS analysis on a TOF and Triple quadrupole.

Product ion	Formula	Mono isotope	Mass error (mDa)	Mass error (PPM)
91	$C_7H_7^+$	91.0548	0.3752	3.0225
212	$C_{14}H_{30}N^+$	212.2378	-4.826	-21.7619

Table 2. Exact mass calculations from MS/MS data acquired on the triple quadrupole for m/z 304 for the two major product ions 212 and 91.

Conclusions

Why is a BAC in the eye? BACs are used in common eye products, such as Visine®, as anitmicrobials. They typically have chain lengths of 12, 14, 16, and 18 carbons (Figure 8). The eye tissue used was from a human donor, and that donor may have been a user of this product. The MS image shows what could be expected from a product designed to work in the periphery of the eye. An SRM experiment was designed to compare Visine to the eye extract. Figure 8 shows the result of this SRM experiment. The retention times are shifted likely due to the presence of co-eluting lipids in the eye extract. Using Imaging MS, tandem MS, exact mass and previous studies, we were able to determine an exogenous substance found in the eye tissue of a donor.

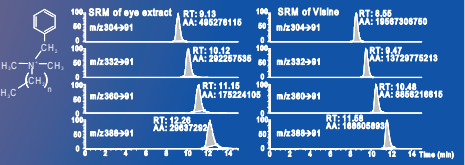

Figure 8. Basic structure of BACs is shown (left) and an SRM experiment comparing they eye extract to diluted in MeOH Visine®.

Acknowledgements

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References

1. Jackson, A.T; Jennings, R.C.K.; Scrivens, J.H.; Green, M.R.; Bateman, R.H. Rapid Commun. Mass Spectrom., 1998, 12, 1914-1924.