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Electronic Supporting Information

Label-Assisted Laser Desorption/Ionization Mass Spectrometry (LA-LDI-MS): An Emerging Technique for Rapid Detection of Ubiquitous *Cis*-1,2-Diol Functionality

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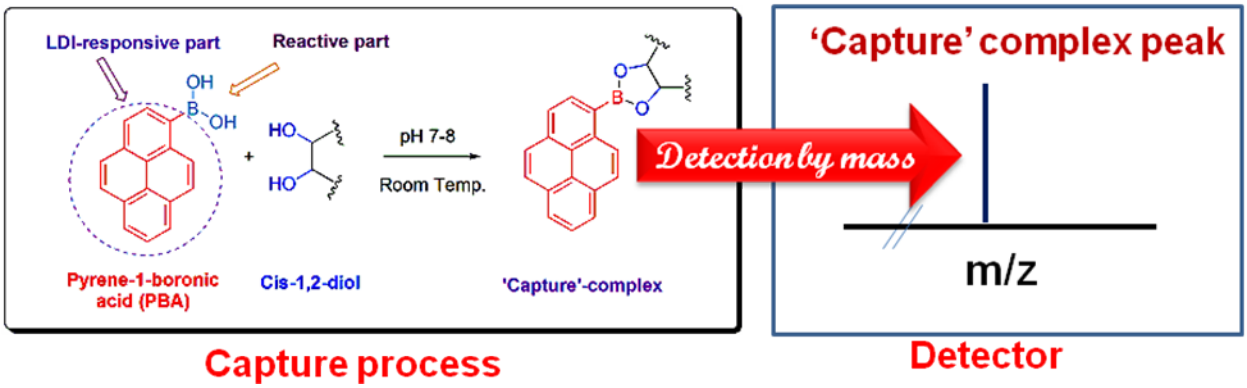

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S1: TOOLS AND TECHNIQUES

Chemicals: Pyrene-1-boronic Acid was purchased from Sigma-Aldrich®. Solvents and analyte compounds were also purchased from commercial sources and used directly without further purification. Dopamine was purchased as dopamine hydrochloride (1 mg/mL) in injectable form. Epinephrine was purchased as epinephrine bitartrate (1 mg/mL) in injectable form. Both dopamine and epinephrine were used directly without further purification. Phosphate Buffer (PB) of pH 7 and pH 8 were prepared in deionized water using commercially available sodium dihydrogen phosphate (NaH_2PO_4) and sodium hydrogen phosphate (Na_2HPO_4).

Instrumentation: All mass spectra were recorded on an Applied Biosystems Voyager-DE PRO Time-of-Flight Mass Spectrometer in either positive or negative ion mode. The instrument was calibrated for the mass range 50-2500 Da using a standard calibration kit (bought from AB SCIEX) and CHCA. The kit contains des-Arg1-Bradykinine, Angiotensin l, Glu1-Fibrinopeptide B, Neurotensin. By using this kit and CHCA, the whole mass range was calibrated in both positive and negative ion modes.UV laser: N_2 LASER, 337 nm wavelength; LASER Intensity 3200; LASER Rep Rate 20.0 Hz; Linear mode.

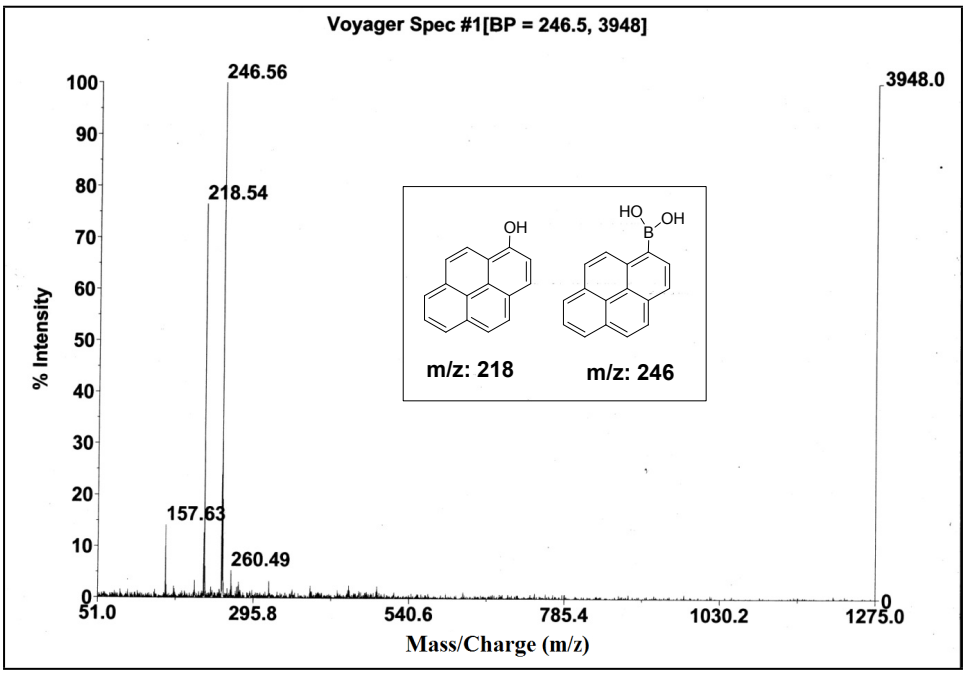
A general note on MS experiments: All incubation reactions were carried out at room temperature (~25^{o}C) in 1.5 mL microtubes without stirring or shaking. 2µL (unless stated otherwise) of each reaction mixture was aliquoted on a standard MALDI plate and air-dried before recording the mass spectra.

All the masses recorded by the mass spectrometer are quoted in integer value only due to technical reasons. Moreover, it is sufficient for the current work since any experiment has not been attempted where a resolution of less than 1 unit is required. The relative intensities for various peaks are quoted in percentage with respect to the base peak in that spectrum. The molecules where there are multiple sites of complexation, the exact structures of the complex are not drawn on their spectra to avoid ambiguity. Throughout, *small molecule* refers to molecules having mass less than 500 Da unless stated otherwise.

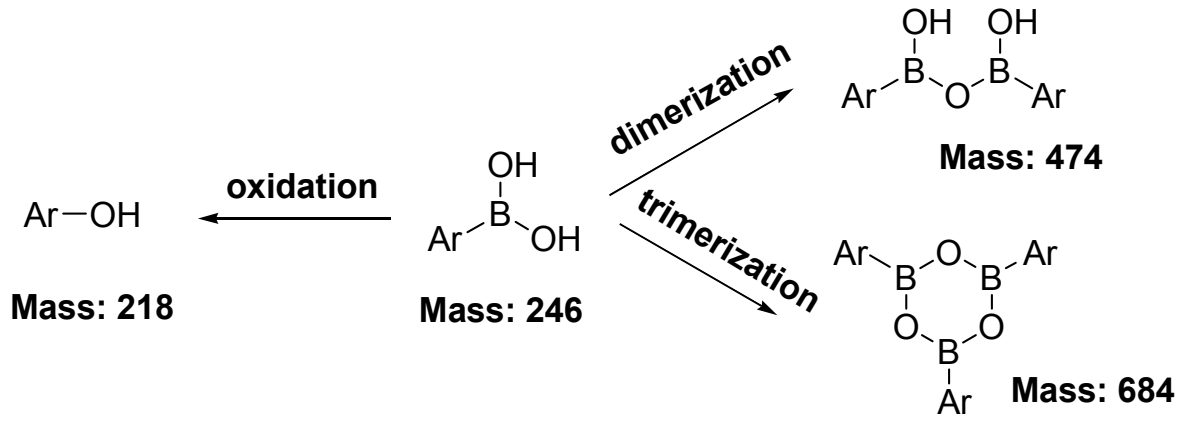
S2: MASS SPECTRA FOR SCREENED COMPOUNDS

Mass spectrum of Pyrene-1-Boronic acid and its characteristic features:

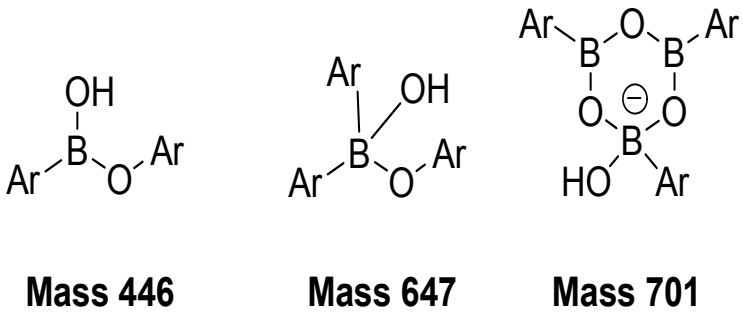
The mass spectrum of pyrene-1-boronic acid showed the intense molecular ion peak at m/z = 246 in +ve ion mode. In experiments performed in -ve ion mode, molecular ion peak was obtained at m/z = 245, owing to the loss of a proton. Under the given conditions, it also gives an intense fragmentation peak at m/z = 218 in +ve ion mode and m/z = 217 in –ve ion mode. This is probably due to the formation of pyrene-1-ol by oxidation of pyrene-1-boronic acid. Sometimes, peaks at m/z = 474 (m/z = 473 in –ve mode) and rarely at m/z = 684 were obtained, which can be attributed to formation of dimer and trimer of pyrene-1-boronic acid respectively due to self-condensation. All the peaks arising from boron-containing species gave a characteristic distribution of intensities about the central peak (quantitative data not given), reflecting the isotopic abundance of ^{10}B and ^{11}B – roughly 1:4:1 for (M-1):M:(M+1).



Formation of different species from pyrene-1-boronic acid during acquisition of mass spectra is shown as follows (***Ar**: pyrene*):



Seldom, few other peaks were also encountered in different spectra due to reaction among PBA molecules, especially during higher temperature or longer incubation time.



<u>Screening of substrates for pyrene-1-boronic acid</u>:

Here we have demonstrated how different cis-diols can be effectively captured using pyrene-1-boronic acid. Both aliphatic and aromatic cis-diols were chosen for screening. Solution of pyrene-1-boronic acid was prepared in 1:1 PB (0.1 M, pH 8) and THF and solutions of the substrates were prepared in either MeOH or PB. The concentration of the probe during screening was maintained at 2.5 mM and that of the substrates at 3.0 mM. The reaction mixtures were incubated overnight at room temperature. 2 μL aliquots from the reaction mixture were taken from each reaction mixture and analyzed by a Time of Flight Mass Spectrometer (TOF-MS) in both positive and negative ion mode.

Molecule	Expected mass of the captured compound	Mass obtained
Catechol	320	320
2, 3-dihydroxy naphthalene	370	370
Ethylene glycol	272	272
Dopamine	363	363
Epinephrine	393	376 (M^+-$OH^•$)
Mannitol	392, 602, 812	602, 812

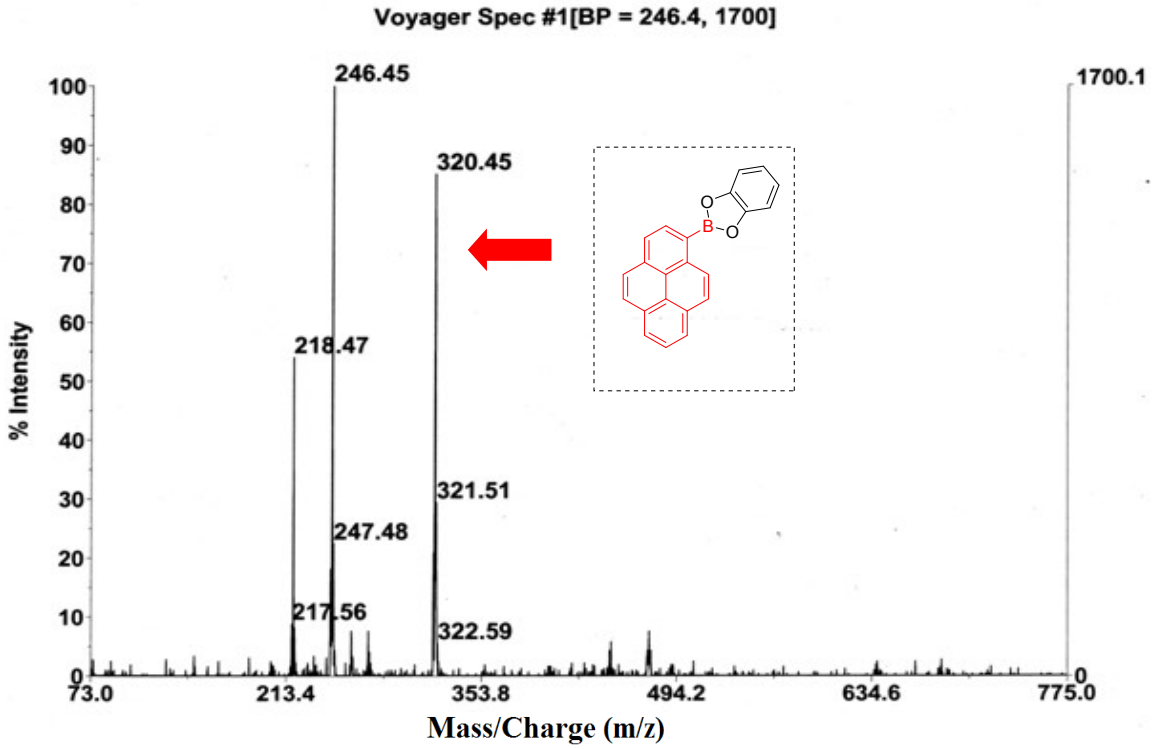
Table 1: Molecules detected in positive ion mode

Molecule	Expected mass of the captured compound	Mass obtained (minus H^+)
Tartaric acid	360	359
Ascorbic acid	386	385
Citric acid	402	401
L-DOPA	407	406

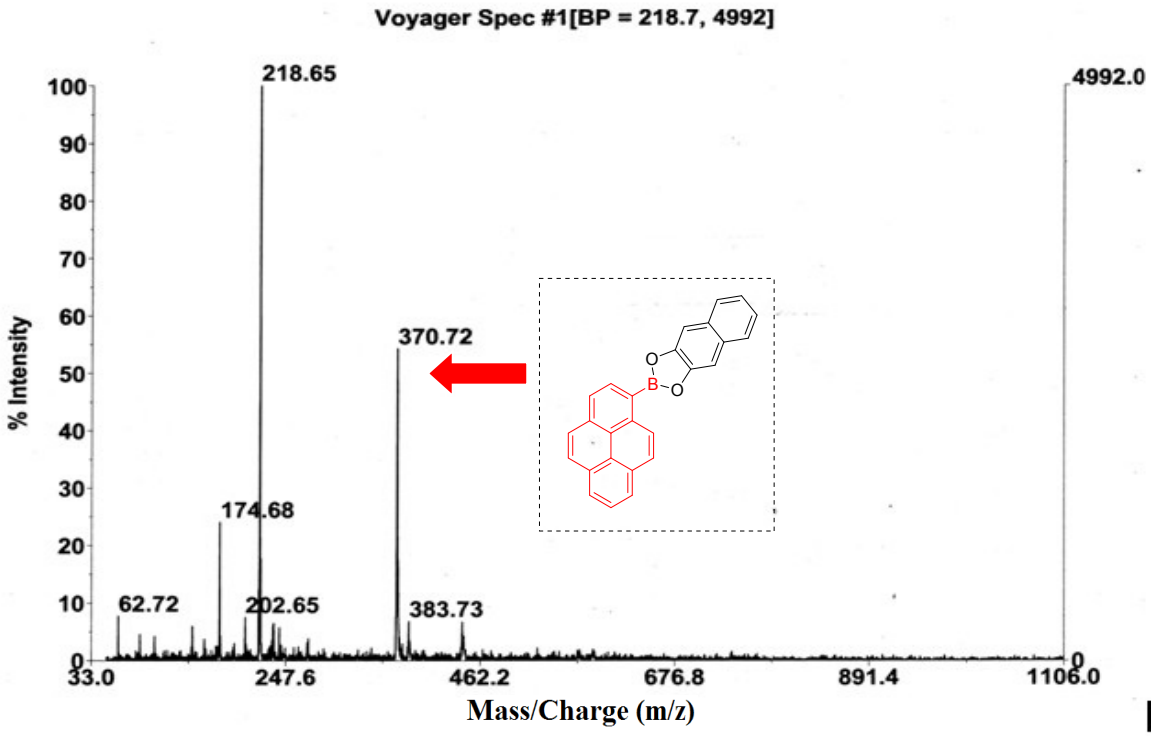
Table 2: Molecules detected in the negative ion mode

The mass spectra for capture of different screened compounds are given as follows:

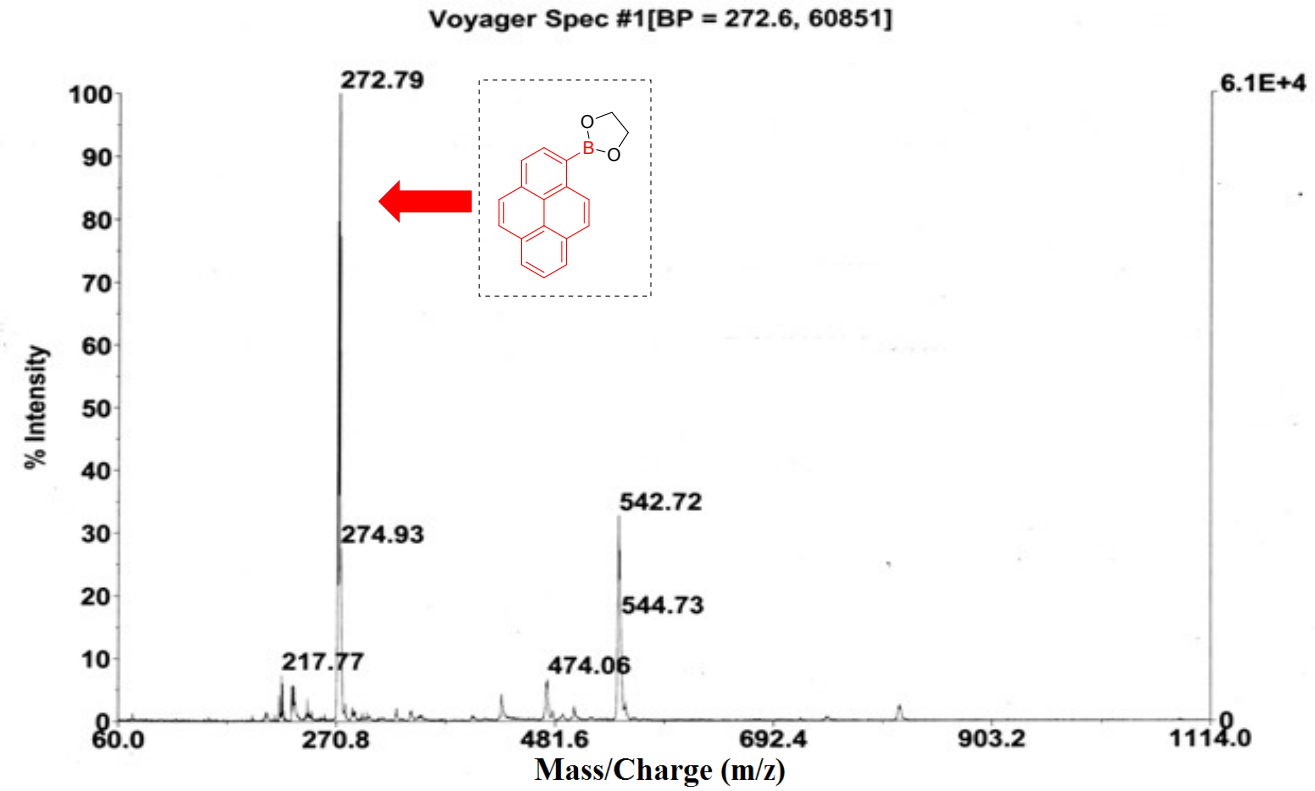
Catechol: +ve mode



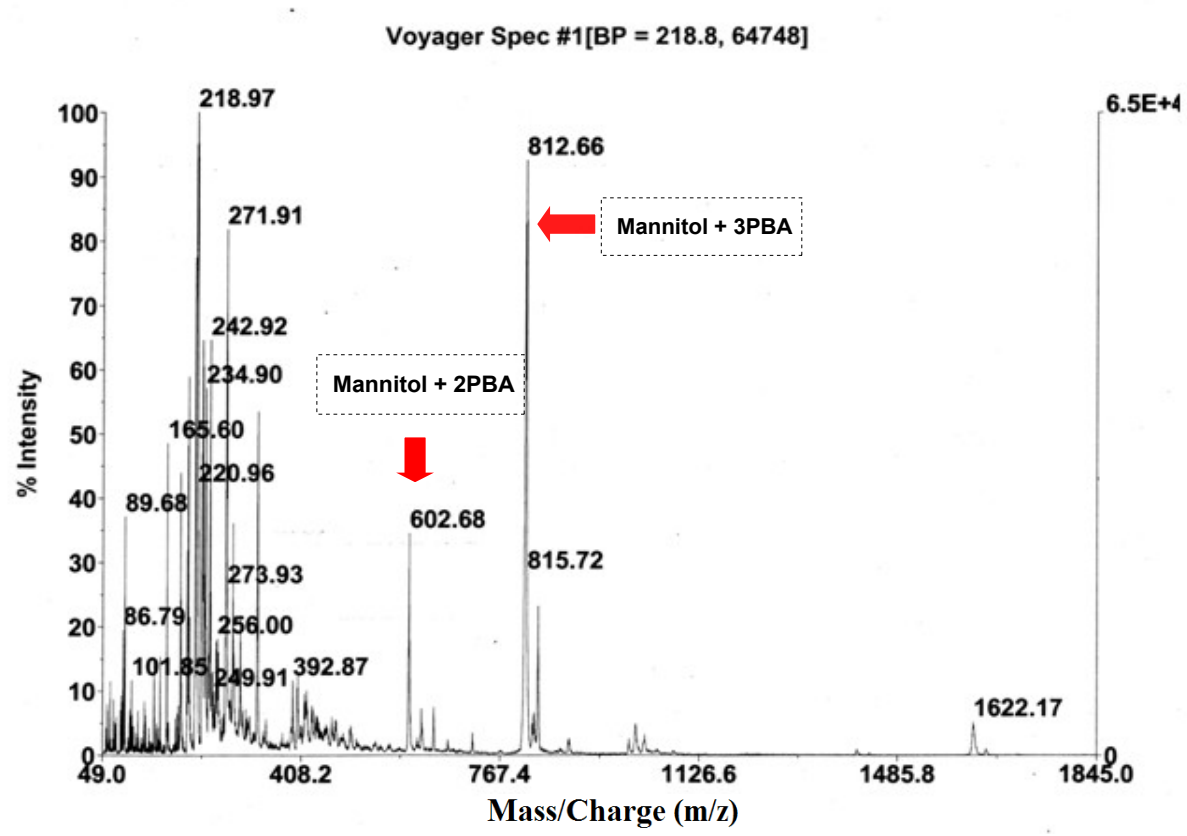
2,3-Dihydroxynapthalene: +ve mode



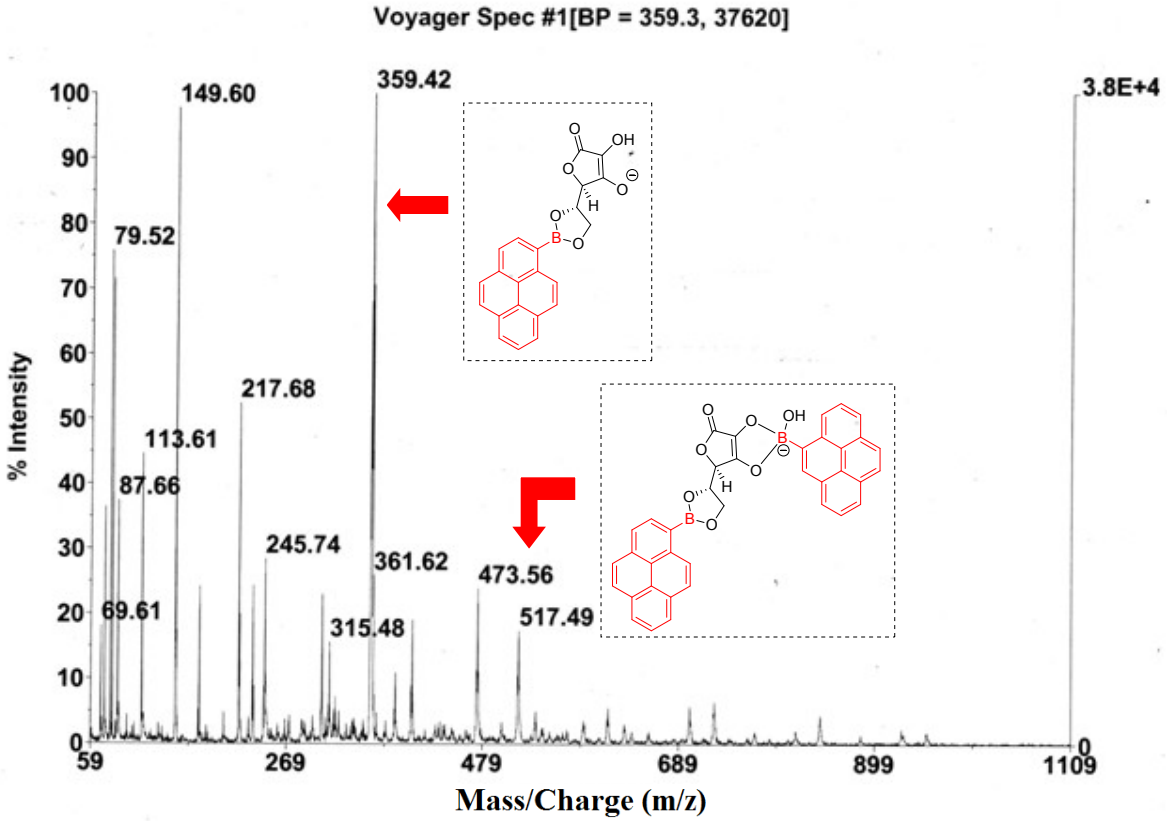
Ethylene glycol: +ve mode



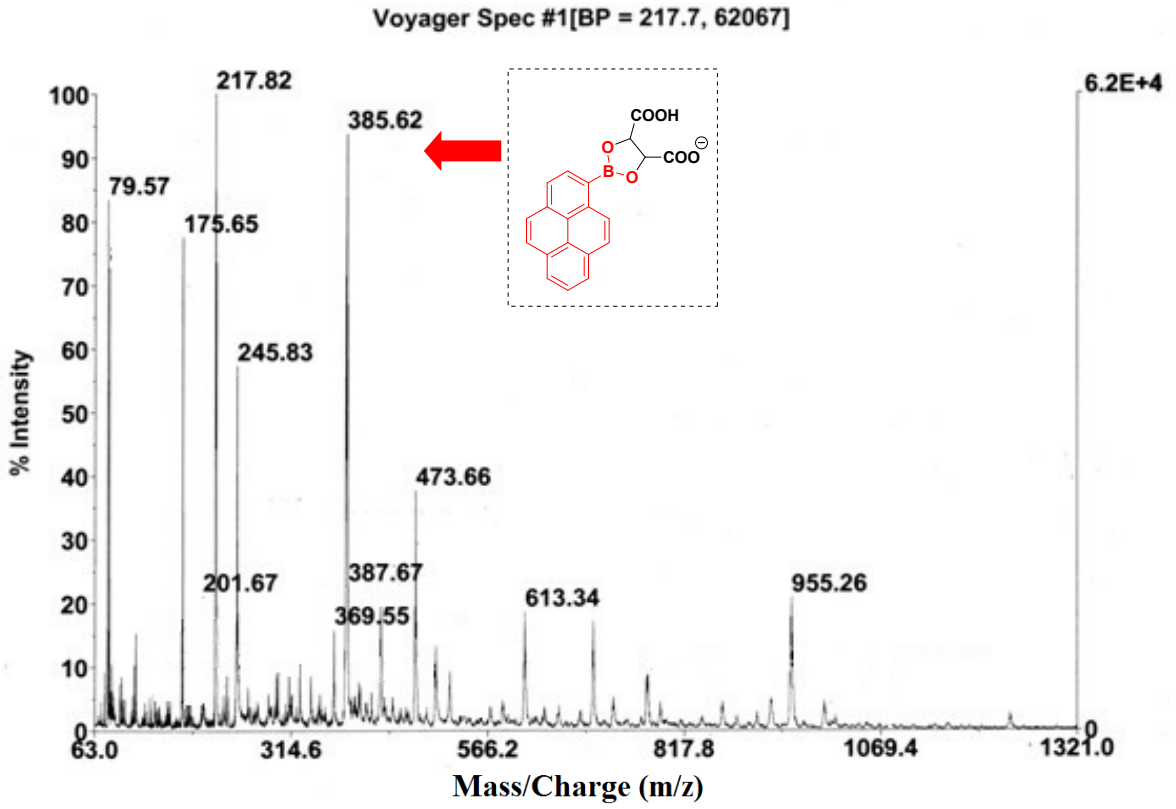
Mannitol: -ve mode



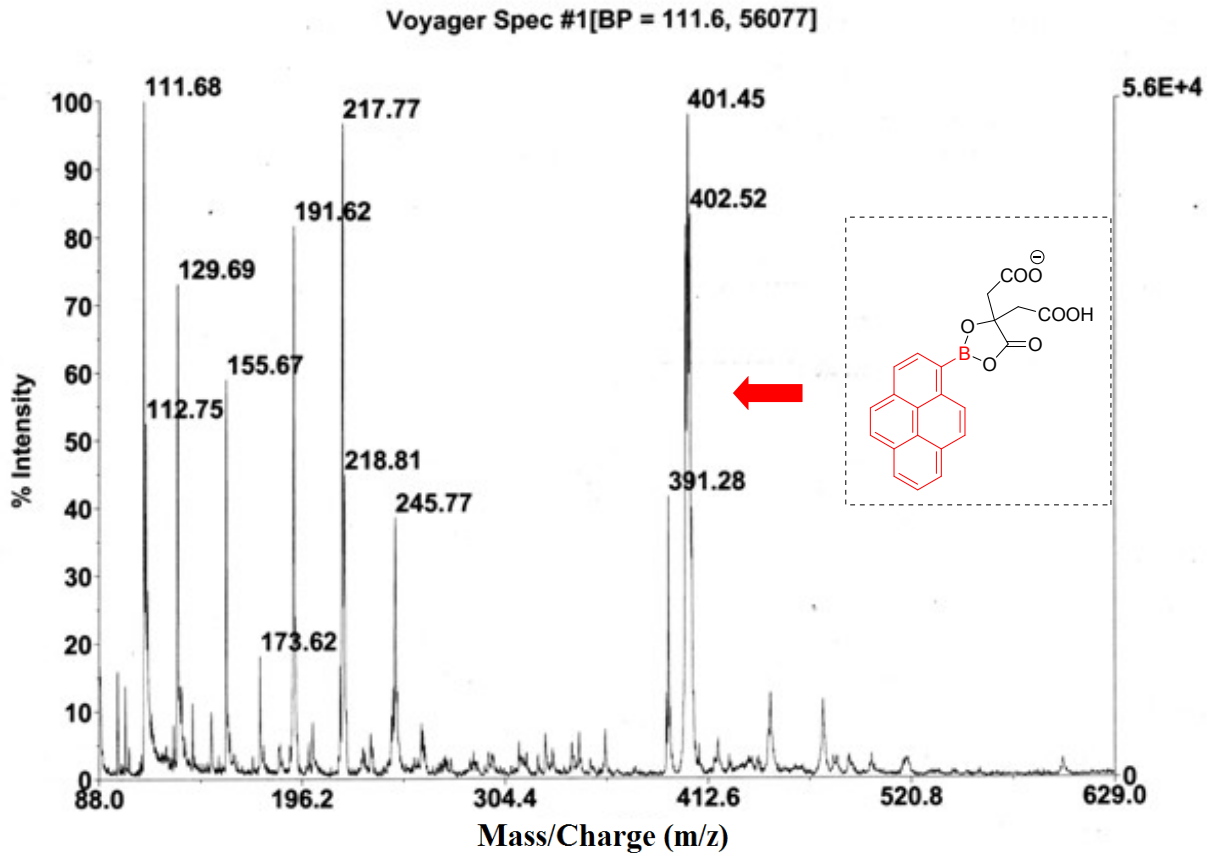
Tartaric acid: -ve mode



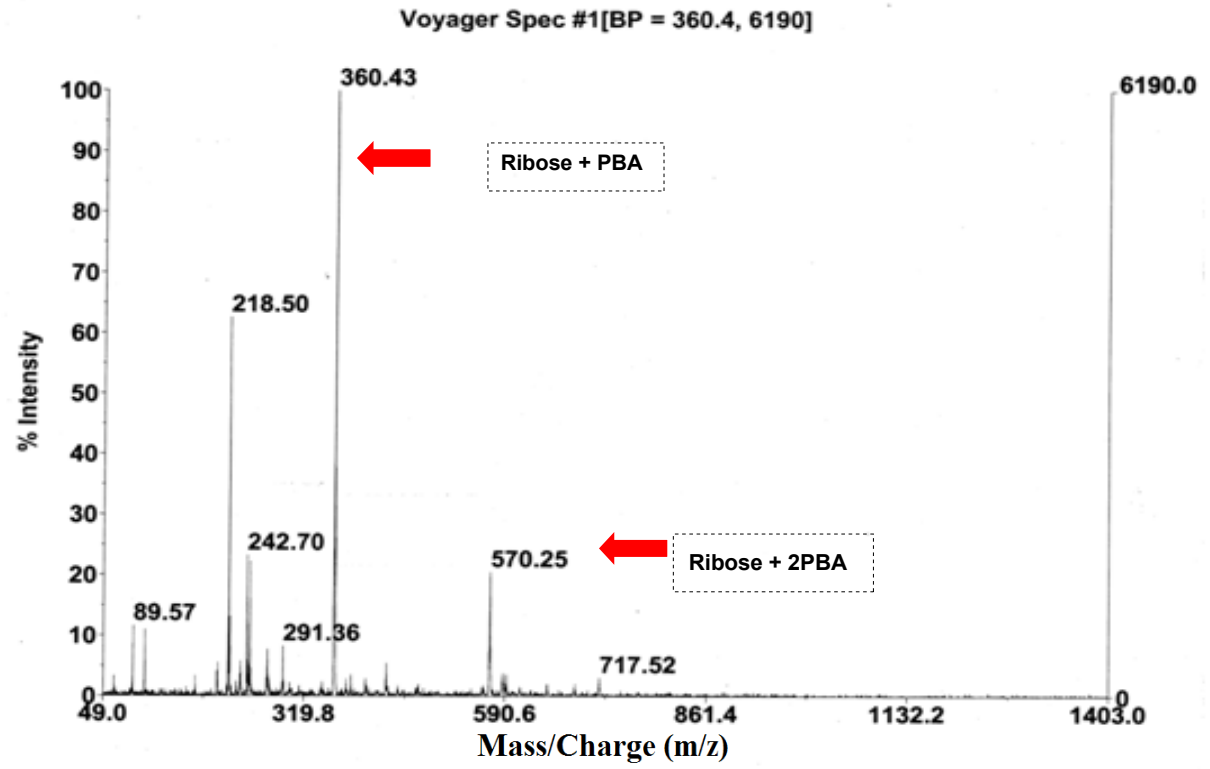
Ascorbic acid: -ve mode



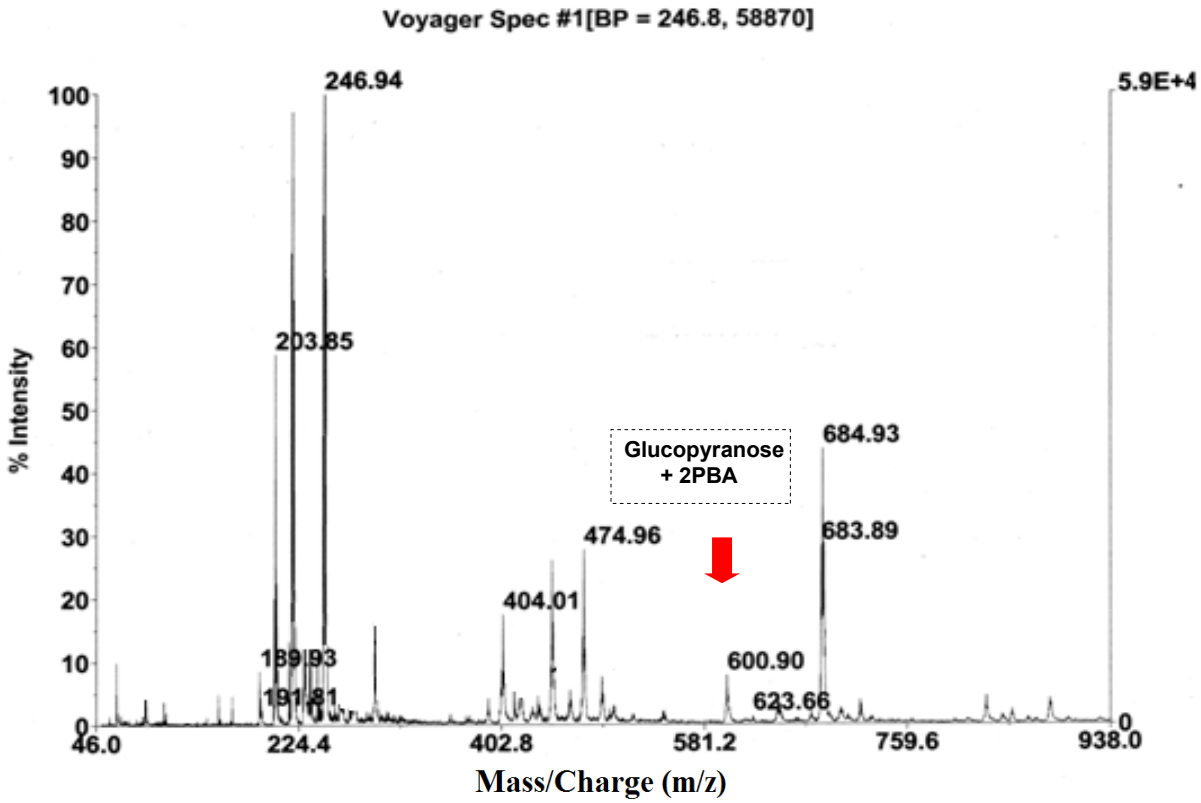
Citric acid: -ve mode



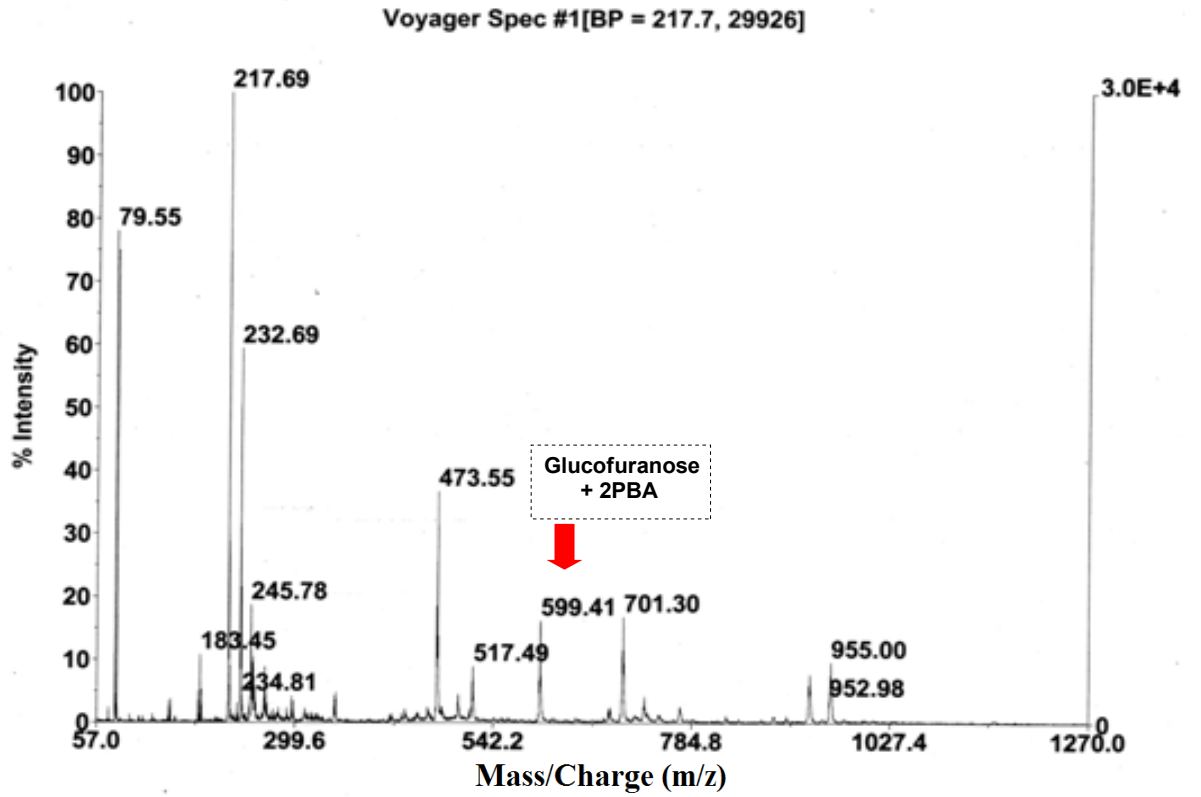
Ribose: -ve mode



Glucose (+ve mode)



Glucose (-ve mode)



S3: DETECTION OF CATECHOLAMINE NEUROTRANSMITTERS AND RELATED MOLECULES

Dopamine and epinephrine were procured in injectable form as dopamine hydrochloride and epinephrine bitartrate respectively. L-DOPA was commercially purchased in solid form. Solutions containing 0.1 mM of dopamine and epinephrine were incubated with 1 mM of pyrene-1-boronic acid (in 1:1 PB (0.1 M, pH 8) and THF) at room temperature for 1 hr. Because of its low aqueous solubility, L-DOPA was incubated at pH 10, maintained by aq. NaOH.

Dopamine:

Mass spectrum was recorded in both +ve and –ve ion mode. Peaks were found only in +ve ion mode.

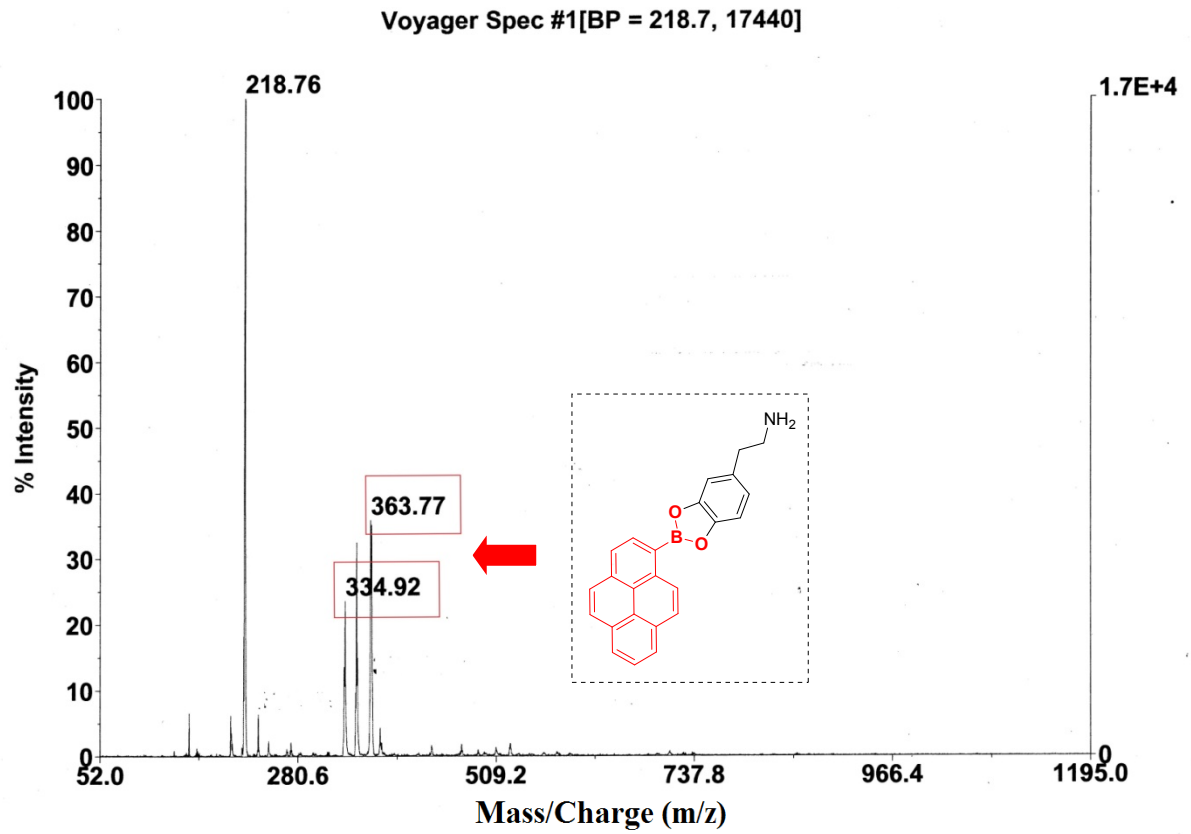


Fig: LA-LDI-TOF Mass Spectrum of dopamine (+ve ion mode)

Besides the molecular ion peak at m/z = 363, dopamine showed prominent fragmentation peaks at m/z = 347 and m/z = 333, possibly due to loss of NH_2· and ·CH_2-NH_2 respectively.

The pathways leading to the generation of different fragment ions is shown in the following scheme:

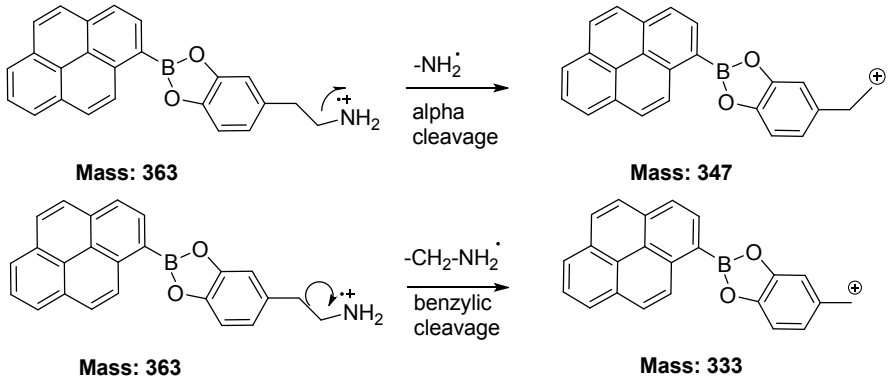


Fig: Schematic diagram showing the generation of different ion fragments from PBA-DA complex

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Epinephrine:

Pyrene boronate complex of epinephrine did not give rise to the molecular ion peak at m/z=393. Instead, a peak at m/z=376 was obtained only. This may be explained by the loss of OH radical from the highly reactive benzylic position even under soft ionization conditions.

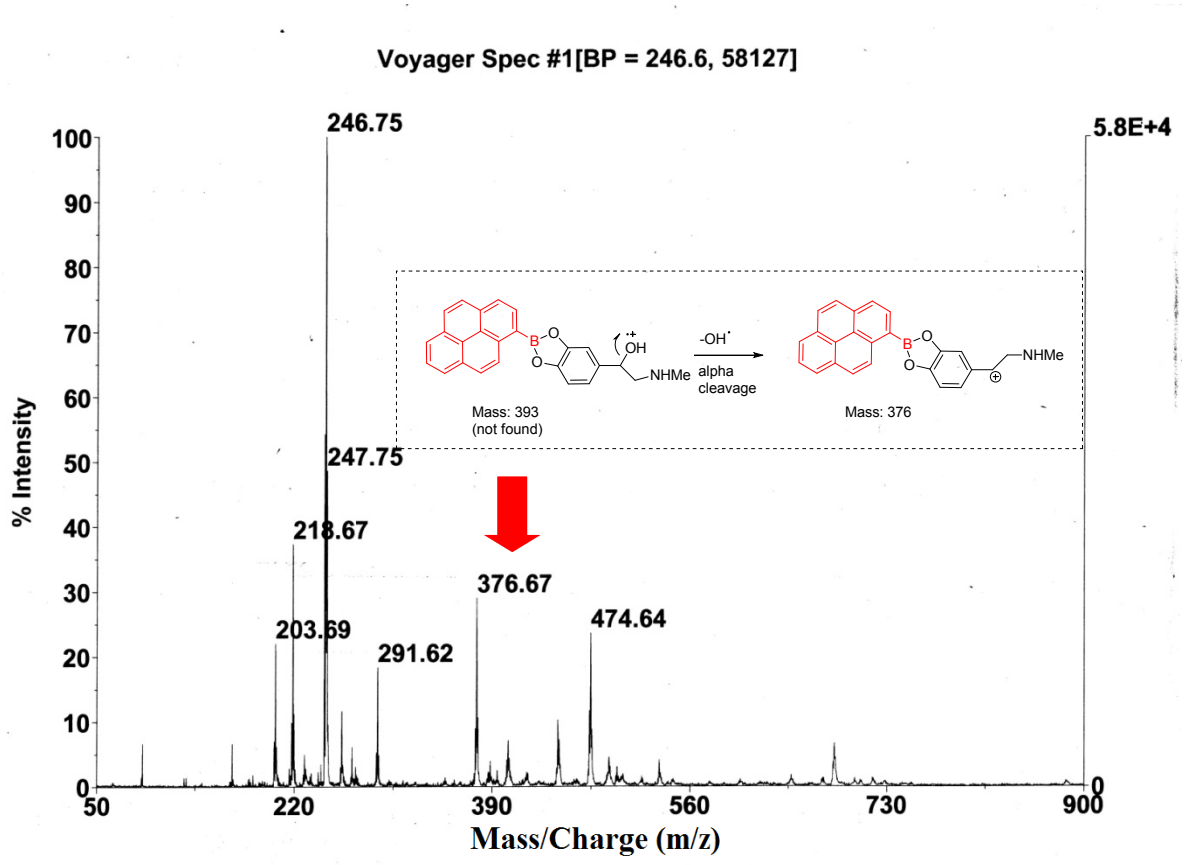

Fig: LA-LDI-TOF Mass Spectrum of epinephrine (+ve ion mode)

L-DOPA:

Molecular ion peak for L-DOPA was detected in the –ve ion mode at m/z 406. However, fragment peak at m/z 333 (on benzylic cleavage) was also found in +ve ion mode (spectrum not shown). Due to very low solubility of L-DOPA in the pH range 7-8, it was dissolved in aq. NaOH (pH ~10) and then incubated with PBA.

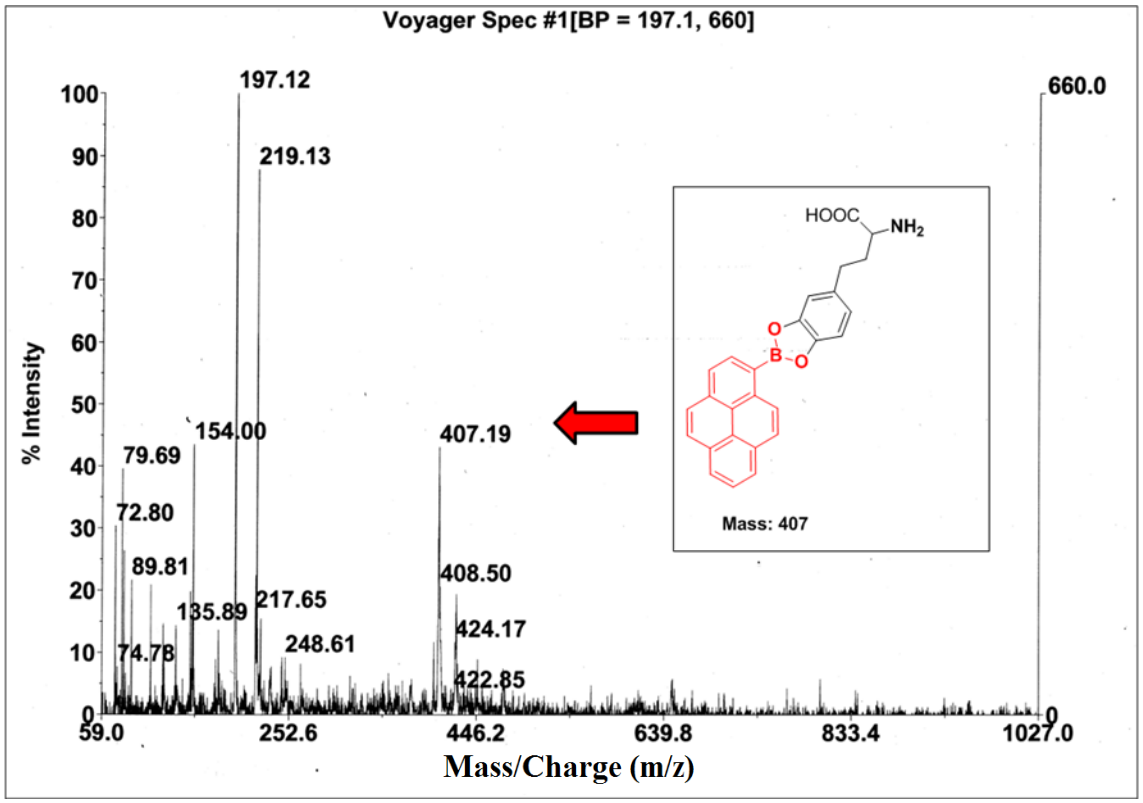

Fig: LA-LDI-TOF Mass Spectrum of L-DOPA (+ve mode)

Detection of Dopamine in presence of glucose:

It was also checked whether presence of glucose interferes with the detection of dopamine. Solutions of dopamine of various strengths (100 µM, 50 µM and 10 µM) were kept incubated with PBA (2.5 mM) in presence of glucose (10 mM). It was found that, glucose has no interference with detection of dopamine in positive ion mode even when the concentration of dopamine is 10 µM, that is, glucose is present in 1000-fold excess by molar ratio. However, glucose gives a peak at m/z = 599 in negative ion mode as described in the previous section. However, in positive mode, only the peak at m/z = 363, which is due to capture of dopamine, is detected – thus demonstrating that dopamine could be captured with high selectivity. This can be explained by considering the higher reactivity of the rigid 1, 2-diol moiety present in dopamine and low abundance of the glucofuranose form.

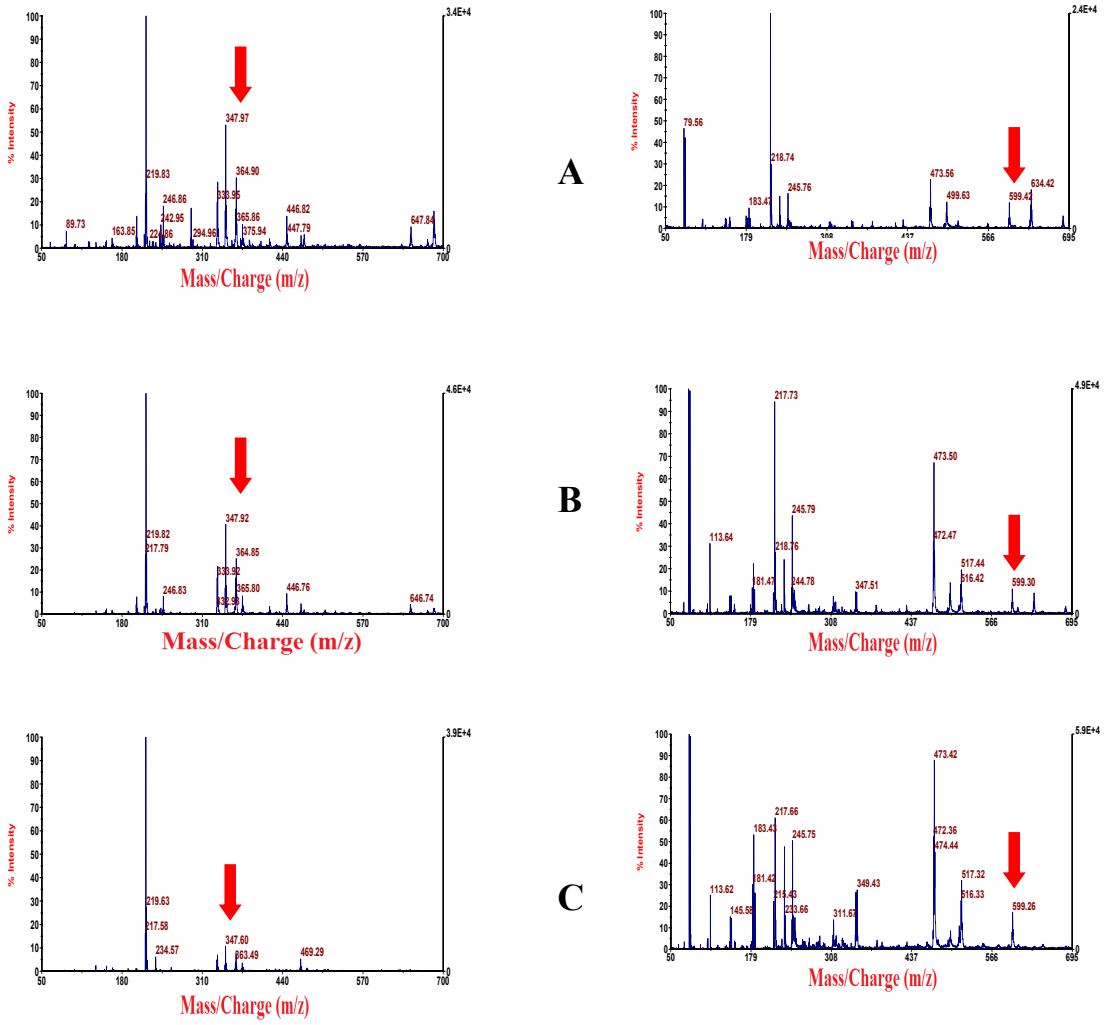

Fig: Incubation of dopamine of different strengths in presence of glucose (10 mM) A) 100 µM B) 50 µM C) 10 µM

Sensitivity of detection: Sensitivity of detection of dopamine was determined by incubating solutions of dopamine of different strengths with PBA. A series of solutions (solvent composition as described before) 2.5 mM in PBA and varying concentrations of dopamine (25 µM, 20 µM, 15 µM, 10 µM, 5 µM and 2.5 µM) were incubated at RT for 2 hr. From each solution, 1 µL of aliquot was spotted onto MALDI plate. It was found that; pyrene boronate complex of dopamine [m/z 363 (C) and fragment peaks at 347 (M) and 333 (N)] was reliably detectable upto 10 µM; although some of the fragment ions were detected even at lower concentrations. In terms of amount, this translates to 10 picomoles of dopamine.

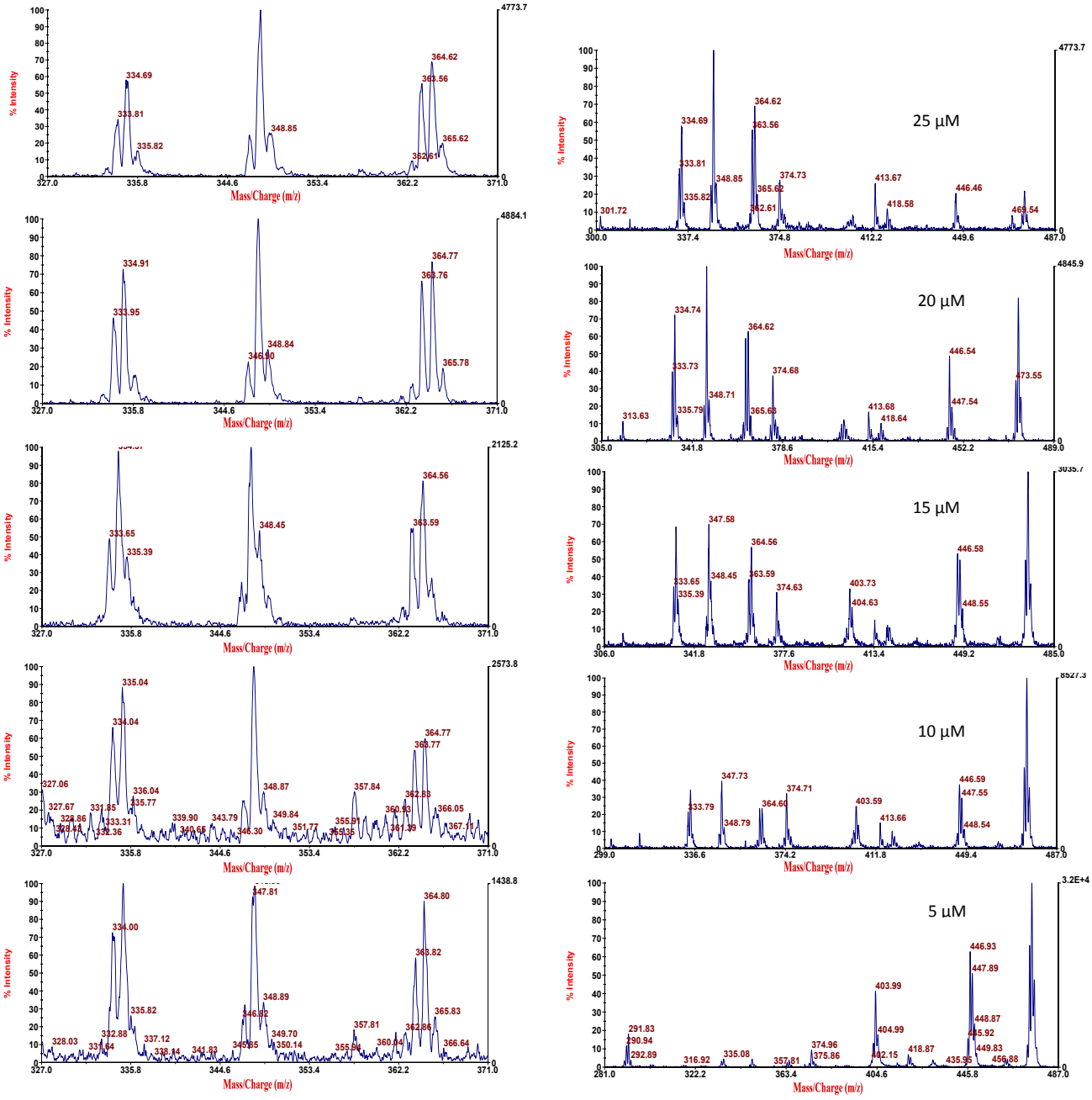

Fig: Concentration dependent variation of intensities of peaks for dopamine at m/z 363, m/z 347 and m/z 334 (Left panel is showing variation of dopamine complex. 2.Right panel is showing relative variation of dopamine complex with respect to boronic acid derivative).

Another synthesized probe:

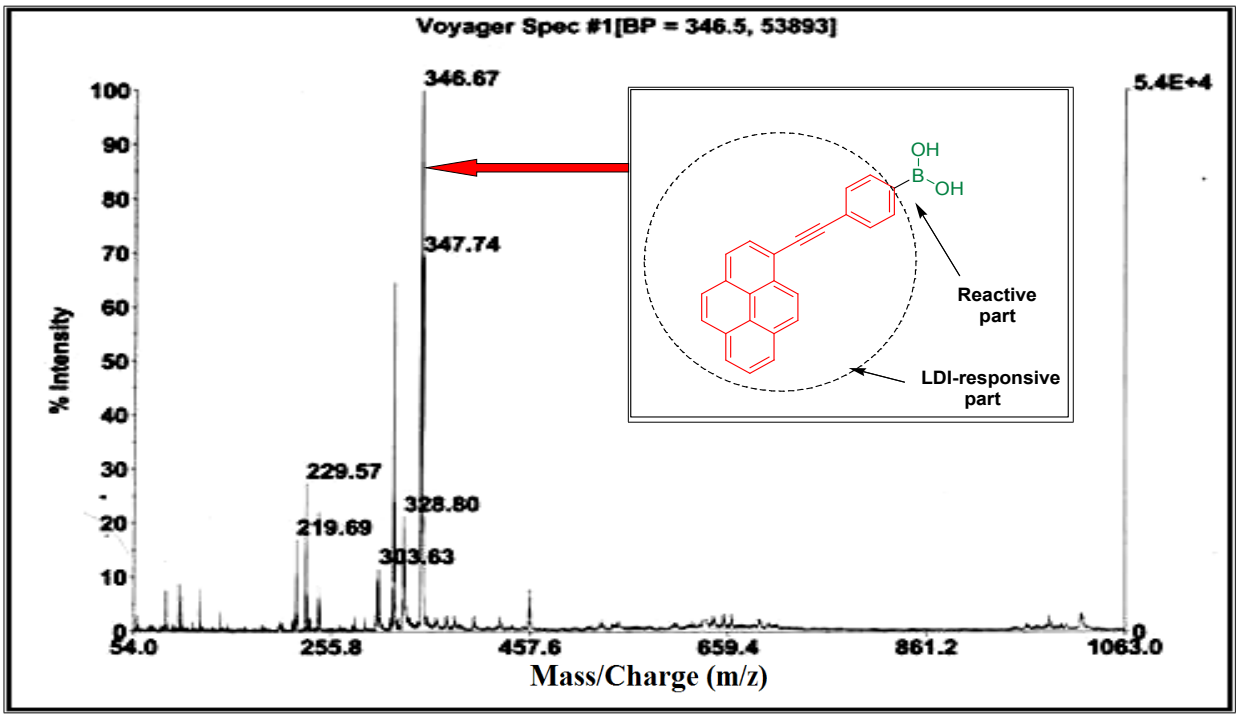

Fig: LA-LDI spectrum of the synthesised probe (+ve ion mode).

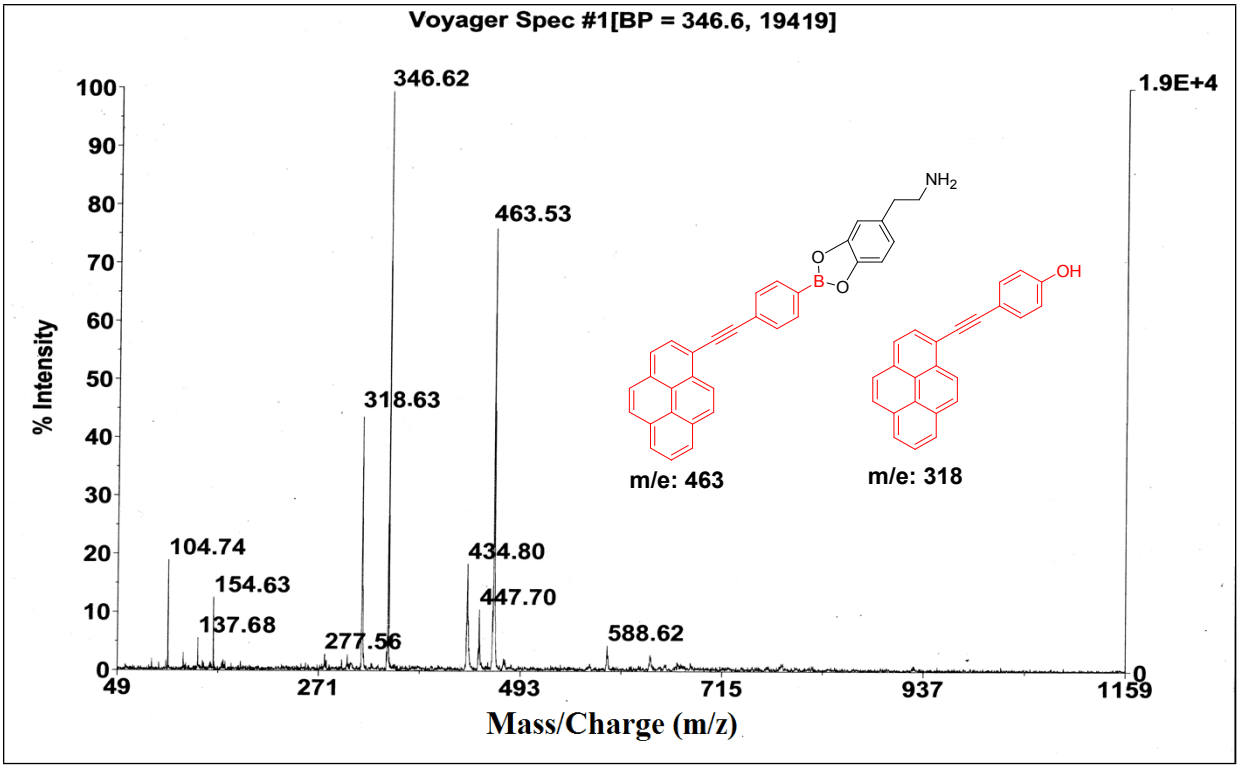

Fig: LA-LDI spectrum of the synthesised probe and Dopamine complex (+ve ion mode, Intensity is much higher).

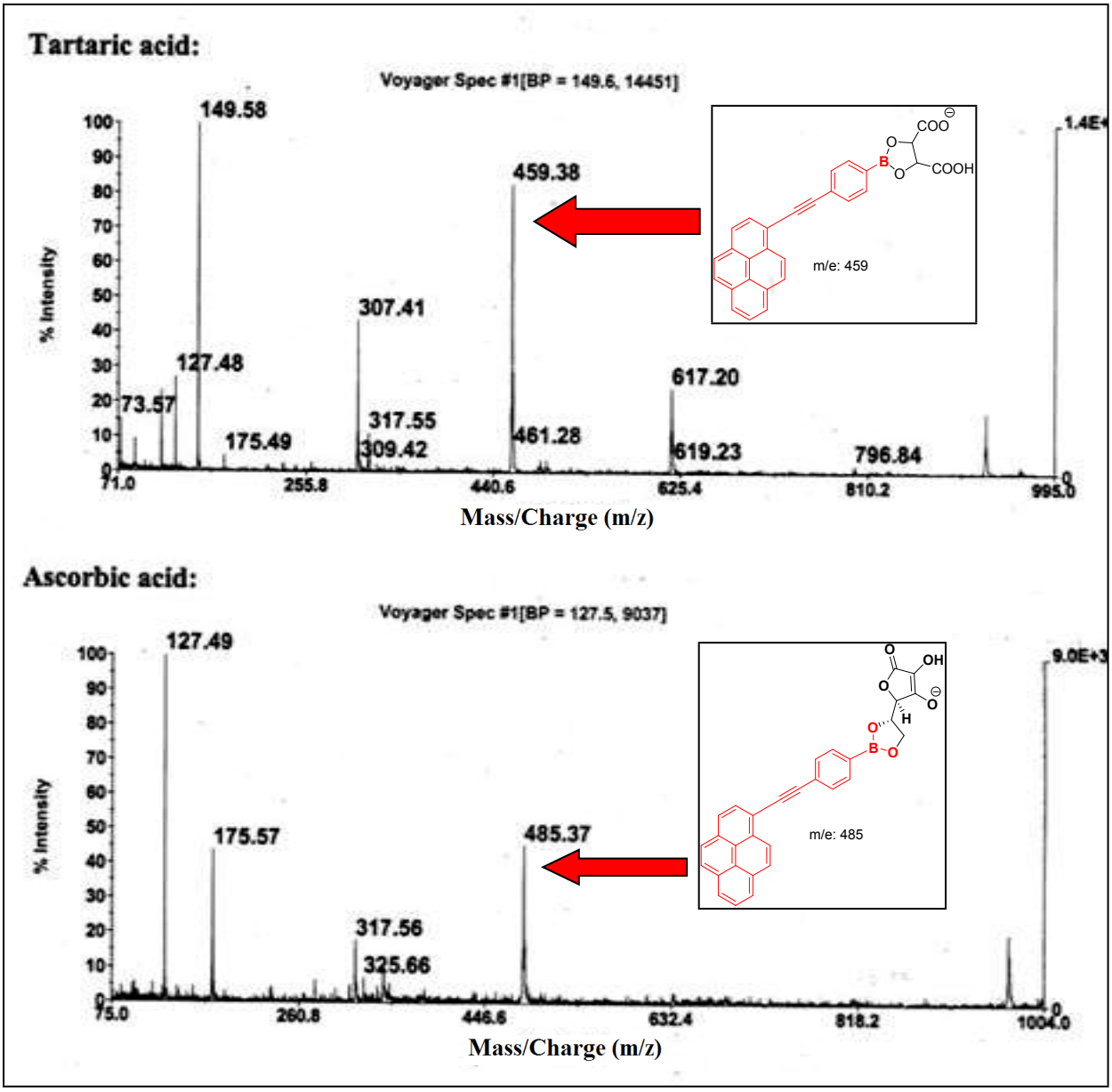

Fig: LA-LDI spectrum of the synthesised probe with Tartaric and ascorbic acid (Both the spectra were recorded in **negative ion mode**. The spectra are much clean compare to PBA complexes).

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