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ARTICLE TYPE

Polyaromatic Label-Assisted External Matrix-free Mass Spectrometry: A New Analytical Technique for Selective Detection of Zinc Ion

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An external matrix-free LDI technique for selective detection of zinc ion is described. The method is free of cross-interference which as often faced in fluorescence based methods.

Selective and sensitive methods for the detection of metal ions have created a substantial impact in various research areas especially in molecular biology,¹ and environmental monitoring.² Metal ions, in particular the transition metal ions like Fe³⁺, Cu²⁺, Zn²⁺ play key roles in regulating important biological processes; for example, as cofactors for a host of enzymes (like Carbonic anhydrase,³ Cytochrome P450,⁴ amine oxidase,⁵ β-lactamase),⁶ maintenance of protein structural motif (Zn-finger),⁷ DNA-synthesis⁸ etc. Although these processes are essential for normal cellular function, presence of these ions in excess can have profound toxic effects on health and environment, even at extremely low concentrations. Thus it is important to be able to detect transition metals in low concentrations to assess health risks and for environmental monitoring. Development of such assay methods represents a significant challenge for scientists. Various analytical techniques reported in the literature for detection/estimation of transition metal ions, which include UV–vis spectroscopy,⁹ atomic absorption spectrometry,¹⁰ fluorescence spectroscopy,¹¹ Plasmon resonance energy transfer etc. However, these have their own limitations especially the interference by other contaminants (inorganic/organic). A method which can detect specific metal ions in actual biological or environmental samples will be extremely useful. Our attention was drawn to a recent paper¹³ where a large number of potential chemical reactions were screened by labelling one of the reactant with a polyaromatic tag and recording a matrix-free MALDI mass spectrometry (LA-LDI). We reasoned that such a technique can be used to design polyaromatic tagged ligands for detection of metal ions in solution. If the ligand is tailor made for a specific metal ion, it will be possible to detect the presence of that particular metal ion in a mixture of other metal ions as well as other organic contaminants. The principle is: in absence of matrix, only those peaks will be observed where the polyaromatic tagged ligand is present, free or bound to metal ions. The situation is depicted in **Figure 1**. In this communication, we describe a novel polyaromatic based oxinyloxy acetic acid **1** (oxine = 8-hydroxyquinoline) for detection of various transition

metal ions like Cu²⁺, Zn²⁺ and Ni²⁺ by LA-LDI mass spectrometry (Label Assisted Laser Desorption/Ionisation). Because of fluorophoric nature of the anthracene-based tag, the probe can also be useful for metal ion detection by exploiting any change in fluorescence behaviour.

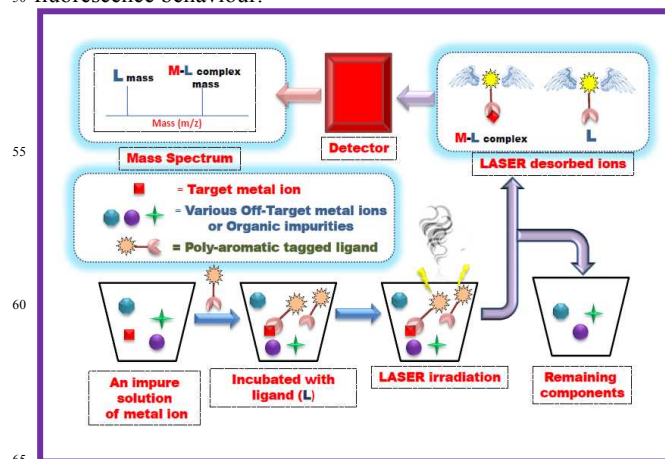
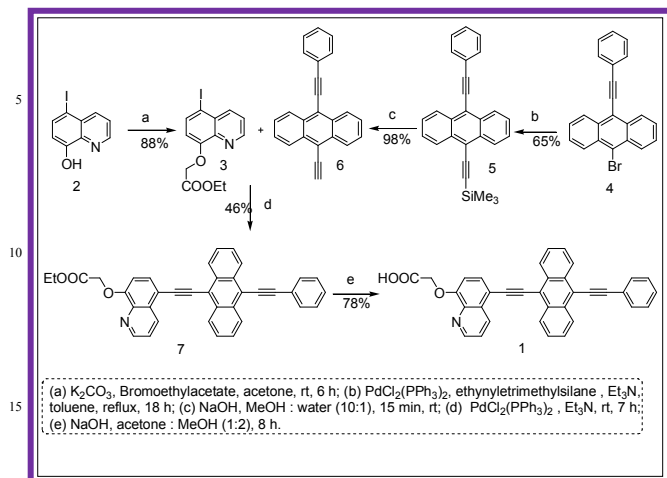


Figure 1: Principle behind metal ion detection

Regarding the basis of ligand design, the polyaromatic tag, namely the alkynyl anthracene moiety¹⁴ was chosen because of its expected ability to assist the ionization process in LDI measurement acting as an internal matrix. Secondly, it is strongly chromophoric with a wide absorption window. Also, the tag can act as a fluorophore where emission intensity or change in wavelength can be monitored during metal ion chelation thus adding an additional handle for detection. Oxinyloxy acetic acid moiety was chosen for its well-known¹⁵ behaviour as a tridentate ligand towards transition metal ions, especially towards zinc. The ligand was synthesized starting from 9, 10-dibromo anthracene as shown in **Scheme 1**. Three sequential Sonogashira coupling¹⁶ followed by hydrolysis completed the synthesis of **1**. The compound was fully characterized by NMR and mass spectral analysis. As expected, **1** showed a strong MH⁺ peak at 504 in the LDI spectrum, recorded without adding any matrix, thus confirming the ability of the alkynyl anthracene moiety to act as an internal matrix (**Figure 2**). The spectrum also showed peaks at m/z 548, 458, 445 assignable to structures A-C (**Figure**

3).



Scheme 1: Synthesis of compound 1

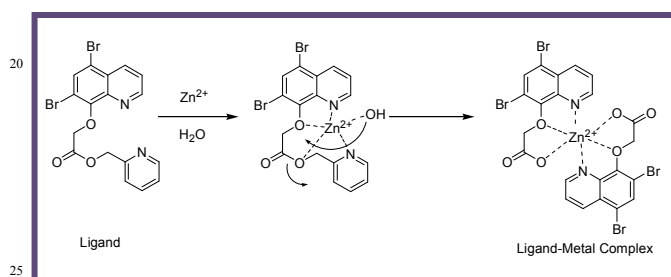
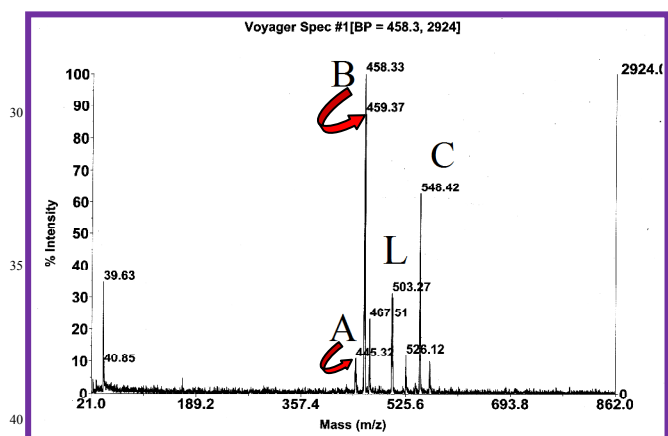
Scheme 2: Reported work by Chen *et al.*¹⁵

Figure 2: LA-LDI MS of Ligand

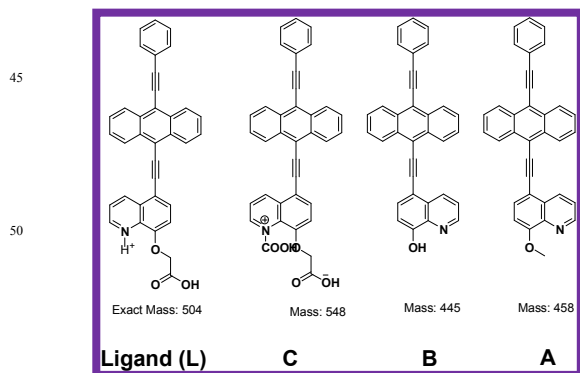


Figure 3: Ligand and its different fragments appeared in mass spectrum

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Because of the reported selective complexation of the *in situ* generated 8-hydroxyquinoline-based ligand **2** with zinc as shown in **Scheme 2**, the free acid **1** was first treated with $Zn(II)$ perchlorate, in acetonitrile/water (final concentration Ligand 100 μM , salt $\sim 60 \mu M$) for 1 h at room temperature to see its complexation behaviour. An aliquot (2 μL) from the reaction mixture was subjected to LDI MS (**Figure 4**) which showed the appearance of new peaks at m/z 543.5 and 607 generated due to 2:1 and 1:1-complex formation respectively (**X** and **Y** in **Figure 5**) between the tridentate ligand and with one molecule of solvent (H_2O or CH_3CN). In addition, the typical isotopic distribution expected for Zn^{2+} was also observed (see inset).

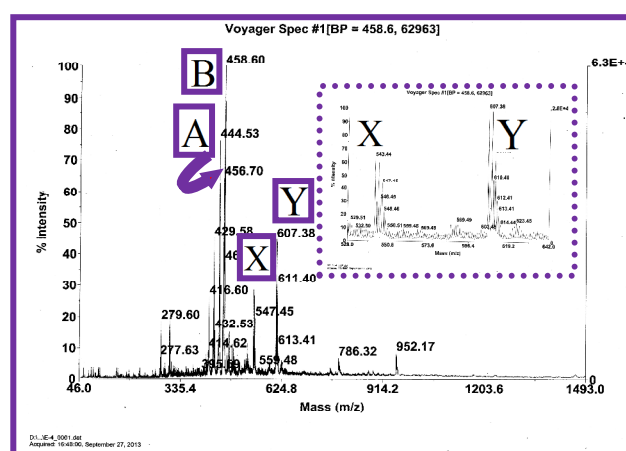
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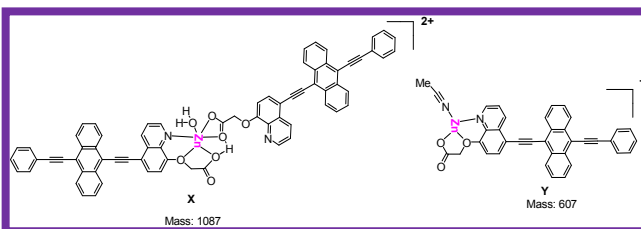
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Figure 4: LA-LDI MS of Ligand + $Zn(II)$ perchlorate

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Figure 5: Proposed structures of complexes between Ligand and Zn^{2+}

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The complexation experiment was then repeated with several other metal ions (Cr^{3+} , Fe^{3+} , Co^{3+} , Ni^{2+} , Cu^{2+} , Cd^{2+} and Hg^{2+}) as perchlorates keeping the same concentrations of ligand and salt. Except for Ni^{2+} which showed a small peak (<10% intensity) at m/z 602 corresponding to a 1:1 complex (**Figure 6**), for all other metal ions, no peak corresponding to metal ion-ligand complex appeared. Thus except for a small interference from Ni^{2+} , our ligand is capable of selectively forming complex with Zn^{2+} as revealed by the LA-LDI MS.

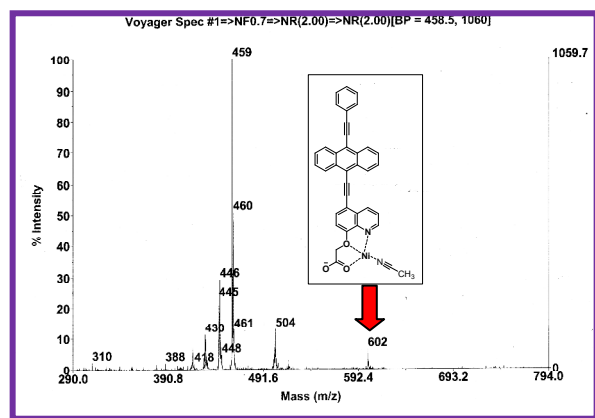


Figure 6: LA-LDI MS of Ligand + Ni(II) perchlorate
 One important attribute of this LA-LDI based detection is that the peak for the complex with Zn²⁺ is not affected by the presence of other metal ions or organic molecules. Thus, the peak for the ligand-Zn²⁺ complex at m/z 607 could be seen in the presence of metal ions like Hg²⁺/Fe³⁺/Cd²⁺/Cu²⁺ (only the spectrum for Zn²⁺/Hg²⁺ is shown in **Figure 7**, for others see ESI) and organic molecules like amino acids (**Figure 8**). A spectrum taken in presence of an external matrix sinapinic acid with ligand, Zn²⁺ and amino acids are also shown for comparison. The spectrum without matrix is much cleaner and mostly showed the peaks of species containing the ligand.

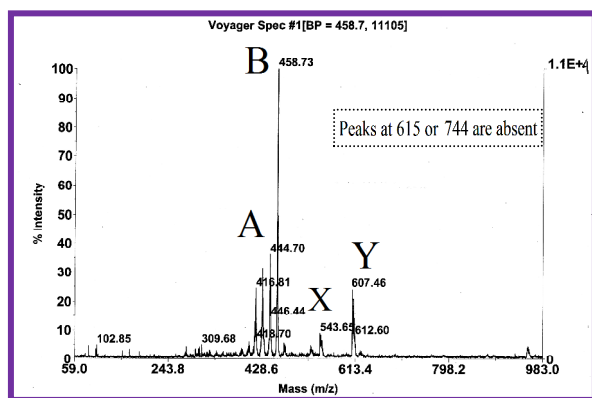


Figure 7: LA-LDI MS of Ligand + Ni(II) and Hg(II) perchlorate

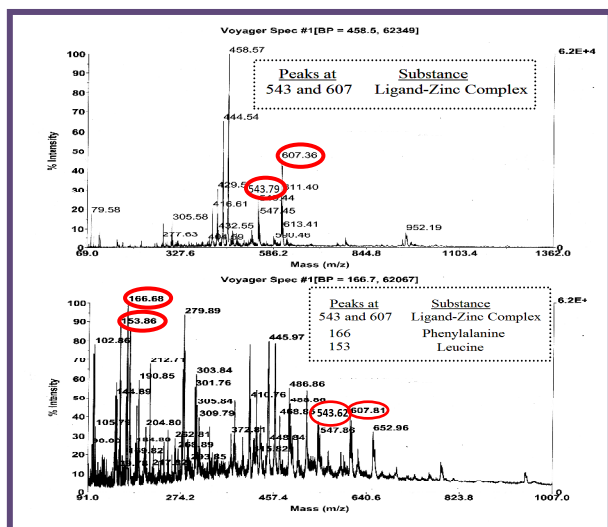


Figure 8: LA-LDI MS of (a) Ligand + Leucine + Phenylalanine + Zn(II) perchlorate without sinapinic acid matrix. (b) Ligand + Leucine + Phenylalanine + Zn(II) perchlorate + sinapinic acid matrix

Having been successful in using the LA-LDI MS technique for detection of Zn²⁺ with our ligand 1, the fluorescence behaviour of the latter in isolation and in presence of metal ions was then studied. For the ligand, excitation at 380 nm showed two emission maxima at 490 and 518 nm. (Fluorescence quantum yield, $\Phi = 0.32$). While metal ions like Cu²⁺, Co³⁺, Ni²⁺ and Mn²⁺ showed only quenching of fluorescence, other ions like Fe³⁺, Cr³⁺ and Hg²⁺ did not cause any appreciable change. The situation was interesting with Zn²⁺. In this case, a new broad hump centering at 580 nm causing an orange fluorescence (Fluorescence quantum yield, $\Phi = 0.09$) started to appear along with quenching of the original emission peak (**Figure 9A**). Thus the ligand acted as a fluorescence sensor for Zn²⁺ (for Fluorescence spectra see ESI). However, the sensitivity of sensing is seriously affected as the orange fluorescence observed for Zn²⁺ is quenched in presence of those ions (the situation is shown for Ni²⁺). However, the LA-LDI MS of a mixture of Zn²⁺ and Ni²⁺ clearly showed the presence of Zn²⁺ (**Figure 9B**) This clearly demonstrated the matrix-free LA-LDI MS method may be a suitable alternative to the fluorescence based techniques for sensing of metal ions.

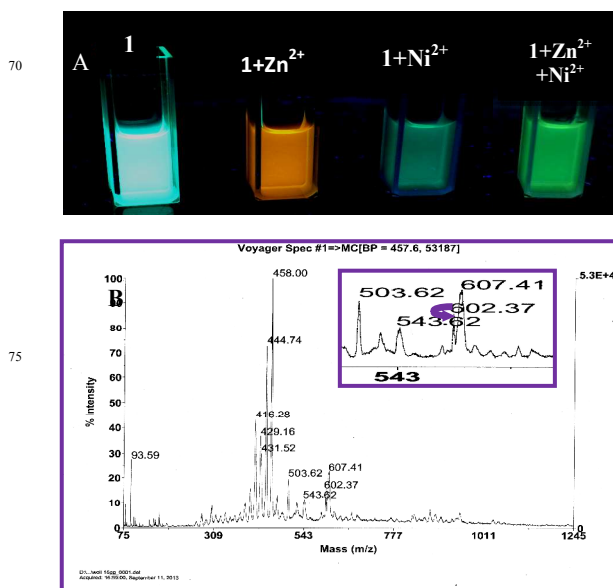


Figure 9: A) Fluorescence Spectrum of 1+ Zn²⁺; B) LA-LDI MS of 1+ Zn²⁺+Ni²⁺;

As already pointed out that Chen *et al.* has reported on the basis of ESI MS and NMR the formation of a 2:1 (ligand: zinc) complex formation with *in situ* generated oxinyloxy acetic acid derivative. However, in our case, both 2:1 and 1:1 complex were seen in the LDI MS. To explore the possibility that the stoichiometry may be concentration dependent, we carried out the complex formation at different concentrations of ligand and zinc and recorded the LDI MS which is shown in **Figure 10**. The spectra clearly supports the existence of equilibrium between 2:1

and 1:1 complex (ligand:metal). At a concentration of 10 μM , the peak at 543.5 is the only prominent peak indicating the formation of mostly the 2:1 complex. With increment of metal ion concentration, the equilibrium shifts from 2:1 complex to 1:1 complex which is clearly reflected in the spectra. The study showed that upto a concentration of 30 μM of Zn^{2+} (with ligand concentration kept at 100 μM), 2:1 complex exists in major amount; concentration beyond 50 μM shifts the equilibrium in favour of the 1:1 complex. JOB's plot (based on UV titrations) also suggests the coexistence of these two complex.

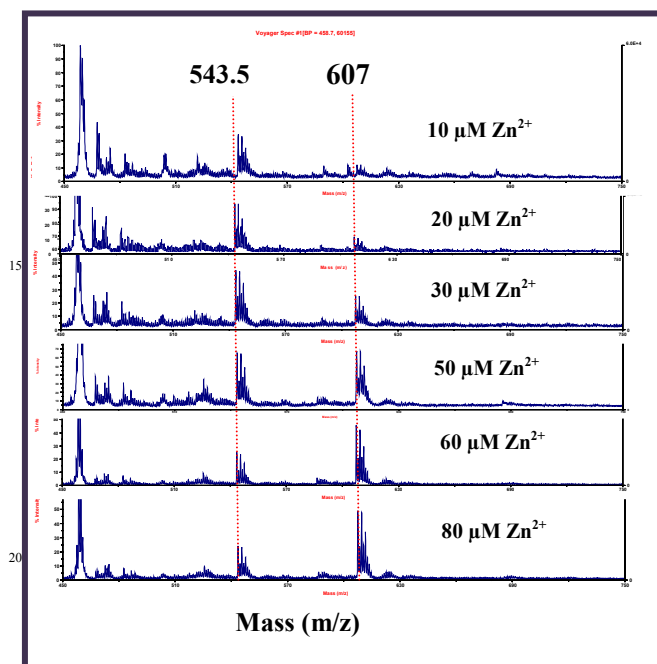


Figure 10: LA-LDI MS of Ligand + Zn(II) perchlorate at different concentrations (ligand concentration kept at 100 μM).

In conclusion, we have shown the application of label assisted LDI technique in detecting metal ions. The method has been validated by the ability of the alkynyl anthracene-oxinyloxy acetic acid hybrid **1** to capture metal ions like Zn and Ni, as confirmed from their LDI MS. Although the current detection limit is ~ 10 μM with the present label-ligand hybrid **1**, we believe the sensitivity may be improved by perturbing the label or the ligand. Current research activities are aimed towards that aspect.

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†Electronic Supplementary Information (ESI) available: Experimental procedure, compound characterization, copies of NMR, various MS spectra and fluorescence pictures.

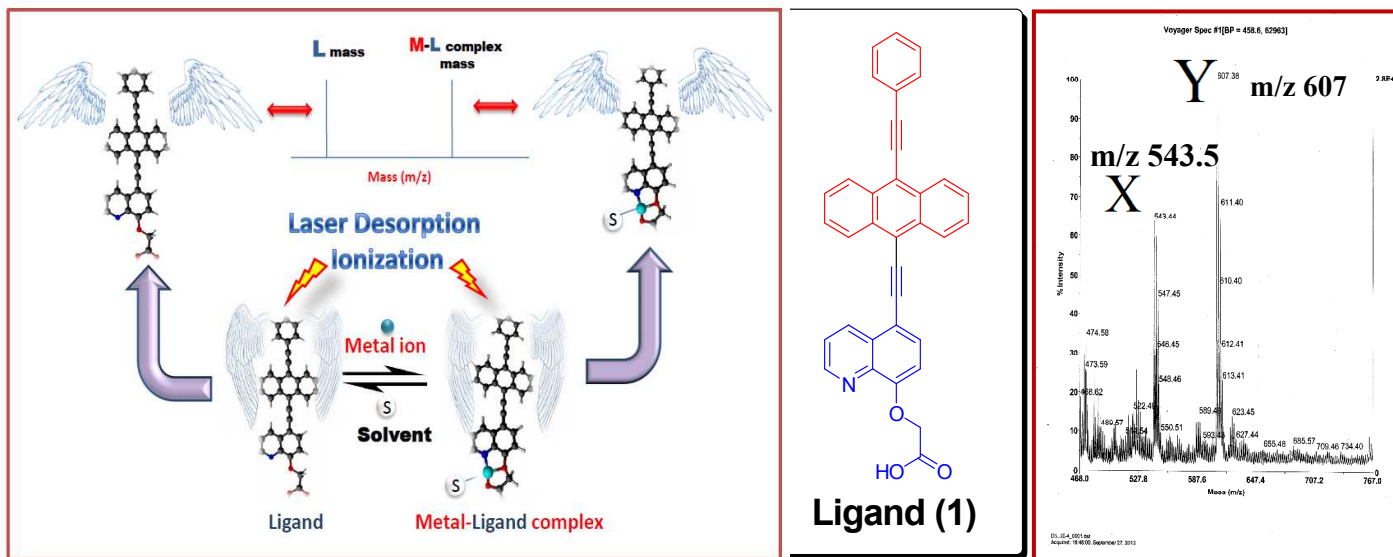
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- [17] Spectroscopic data: For **1**: ¹H-NMR (400 Mz, d₆-DMSO): 9.09 (1H, d, J = 4 Hz), 9.01 (1H, d, J = 8Hz), 8.76-8.69 (4H, m), 8.24 (1H, d, J = 8.1 Hz), 7.95 (1H, bs), 7.89 - 7.82 (5H, m), 7.53 (3H, s), 7.39 (1H, d, J = 8.1 Hz), 5.1 (2H, s). ¹³C-NMR (100 MHz, d₆-DMSO): 170.2, 154.4, 149.7, 133.5, 132.2, 132.0, 131.8, 129.9, 129.5, 129.3, 129.2, 128.5, 128.3, 127.4, 125.4, 124.1, 123.6, 123.2, 122.7, 118.2, 112.8, 111.3, 103.4, 100.0, 90.6, 86.3, 66.0. HRMS: Calcd for C₃₅H₂₂NO₃ + H⁺ 504.1594 found 504.1608.
- [18] **Metal capture protocol**: Compound **1** (2.5 μL , 4 mM in DMSO) was added to an acetonitrile solution of metal perchlorates (100 μL , 9.6 μM - 80 μM). The final concentration of the ligand was thus maintained at 100 μM . The solution was incubated for 1 h at 30 $^{\circ}\text{C}$. 2 μL from this solution was spotted into the well of the plate for LA-LDI (Label Assisted Laser desorption Ionization) and the MS spectrum was recorded.

Graphical Abstract

Polyaromatic Label-Assisted Laser Desorption Ionization Mass Spectrometry (LA-LDI MS): A New Analytical Technique for Selective Detection of Zinc Ion

Partha Sarathi Addy, Sohini Basu Roy, Santi Mohan Mandal and Amit Basak



Principle of Label-Assisted MS detection of metal ions; X, Y are 2:1 and 1:1 complexes consecutively
