Electronic Supplementary Material (ESI) for RSC Advances.

Electronic Supporting Information

Label-Assisted Laser Desorption/Ionization Mass Spectrometry (LA-LDI-MS): Use of Pyrene Aldehyde for Detection of Biogenic Amines, Amino Acids and Peptides

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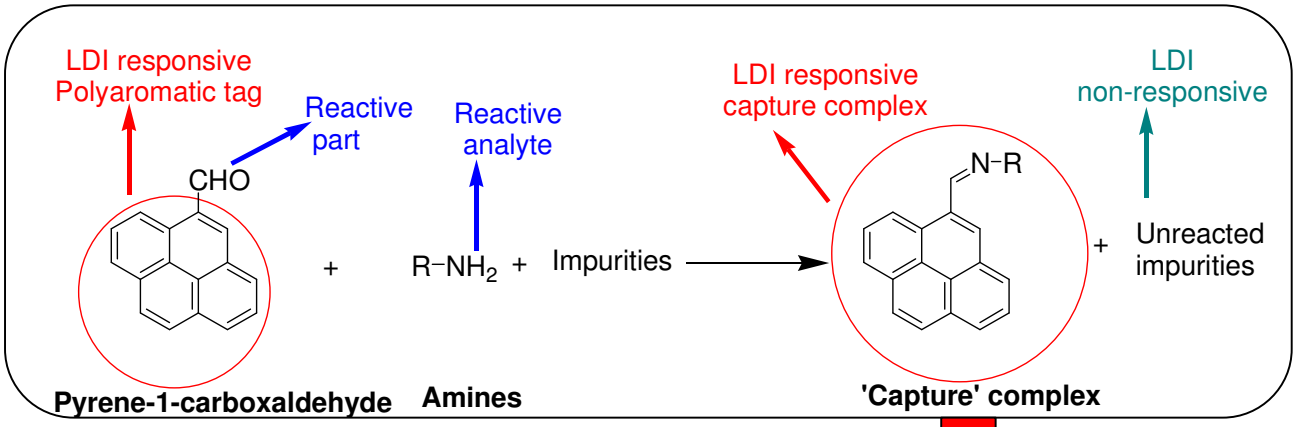
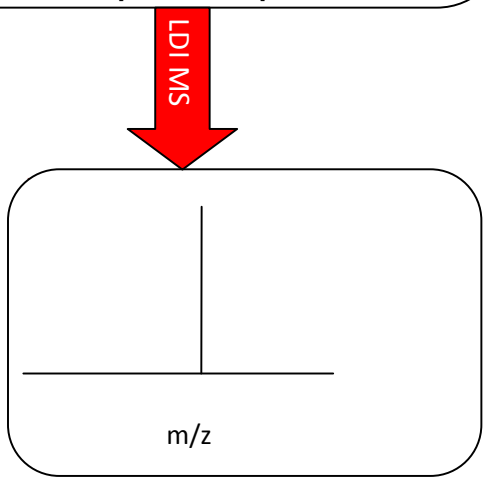


Capture process



Detector

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Tools and Techniques

Chemicals: - 1-Pyrenecarboxaldehyde was purchased from Sigma Aldrich. Solvents and analyte compounds were purchased from commercial sources and used directly without further purification. Dopamine was purchased as dopamine hydrochloride in injectable form (1mg/ml).Dopamine was used without further purification.

Instrumentation: - All mass spectra were recorded on ultraFlextrene MALDI Time-of-Flight Mass Spectrophotometer from Bruker in positive ion mode. The instrument was calibrated for the mass range 50-2500 Da using a standard calibration kit (Bruker). The kit contains Bradykinin, Angiotensin II, Angiotensin I, Substance_P, Bombesin, Renin_substrate, ACTH_clip (1-17), ACTH_clip (18-39), Somatostatin. By using this kit whole mass range was calibrated in positive ion mode. UV laser: Smartbeam II (N_2, NdYag), 355 nm wavelength, Laser rep rate 2000 Hz, Reflector mode.

MS experiment: - All incubation reactions were done at room temperature in 1.5 ml microtubes. 1μL aliquot from each reaction mixture was aliquoted on MALDI plate and air dried before recording mass spectra and analyzed by a Time of Flight Mass Spectrometer (TOF-MS) in positive ion mode.

All the masses recorded by mass spectrometer are quoted in integer value only due to technical reason. 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.

Capture Experimental Process and Mass Spectra of Screened Compounds

Mass spectra of 1-pyrenecarboxaldehyde:-

1-Pyrenecarboxaldehyde was dissolved in Methanol and LDI-MS was recorded at positive ion mode. It showed a strong molecular ion peak at m/z = 230.

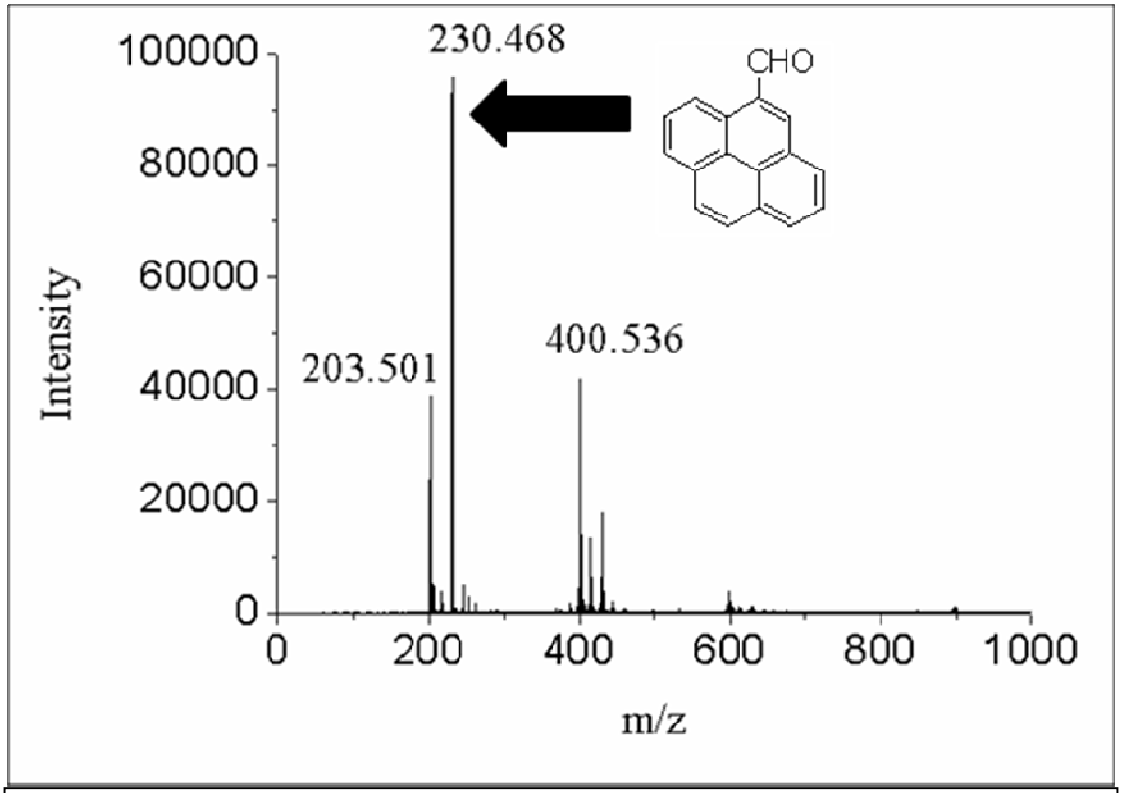

Fig 1: LDI-TOF Mass Spectrum of 1-Pyrenecarboxaldehyde (m/z= 230) in positive ion mode

Screening of Primary amines (mono amines and bis amines) for reaction with 1-pyrenecarboxaldehyde:-

Experimental procedure: - Solution of 1-pyrenecarboxaldehyde was prepared in Methanol and substrates were prepared in water. All amines were treated with excess with excess amount of triethyl amine to liberate the free amine. The concentration of probe during screening was maintained at 5mM and the substrate at 5mM (for monoamines) and 2.5mM (for diamines). The reaction mixtures were incubated for 30 minutes at room temperature. 1μL aliquot from the reaction mixture were taken from each reaction mixture and analyzed by a Time of Flight Mass Spectrometer (TOF-MS) in positive ion mode. The results of the screening are summarized as follows.

Table 1 (Monoimines):-

Monoamine Compound	**Expected imine peak (m/z)**	**Obtained Peaks (m/z)**
n-butyl amine	285	286
Benzyl amine	319	320
Ampicillin	561	562, 318 (benzylic fragmentation), 242
Dopamine	365	366, 215,
Tyramine	349	350, 242, 215
Histamine	323	324, 242, 215
Ethanol amine	273	274,242, 215

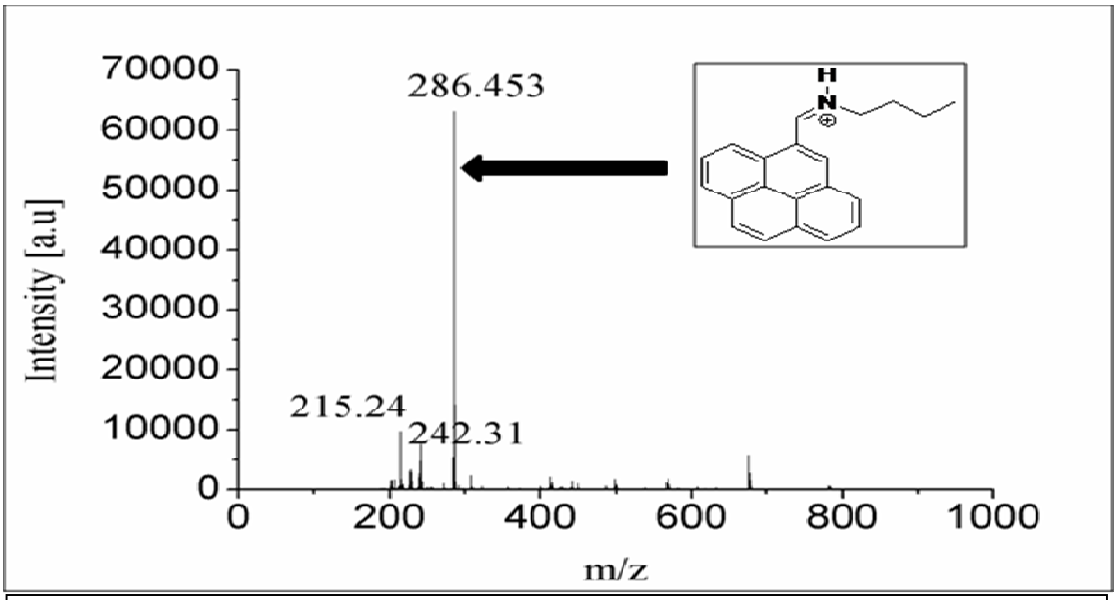

Fig 2: LDI-TOF Mass Spectrum of capture complex of n-Butylamine (m/z= 286) in positive ion mode

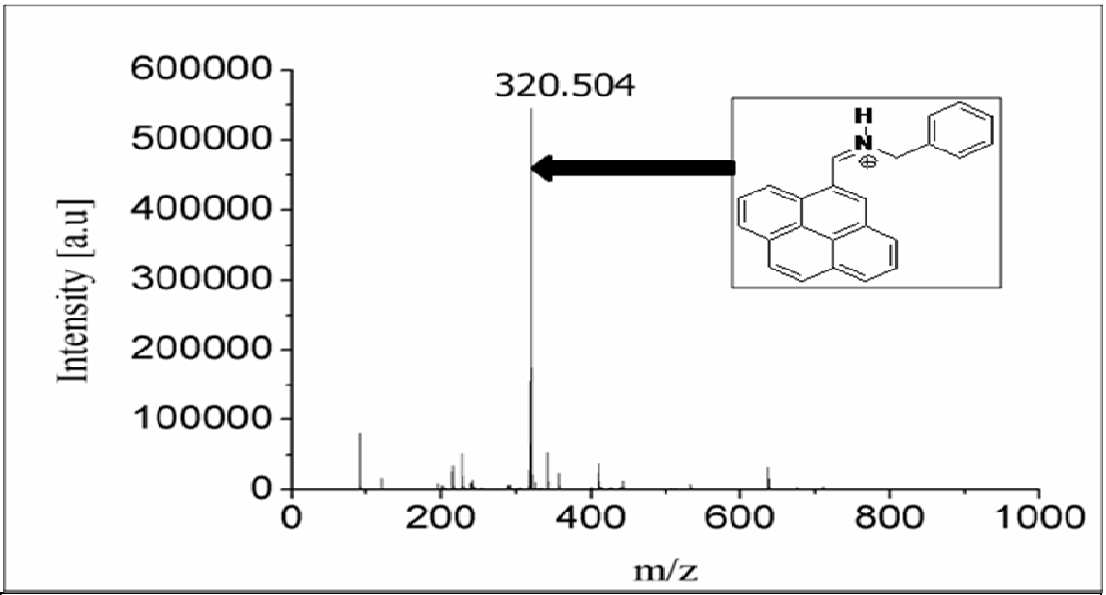

Fig 3: LDI-TOF Mass Spectrum of capture complex of Benzylamine (m/z= 320) in positive ion mode

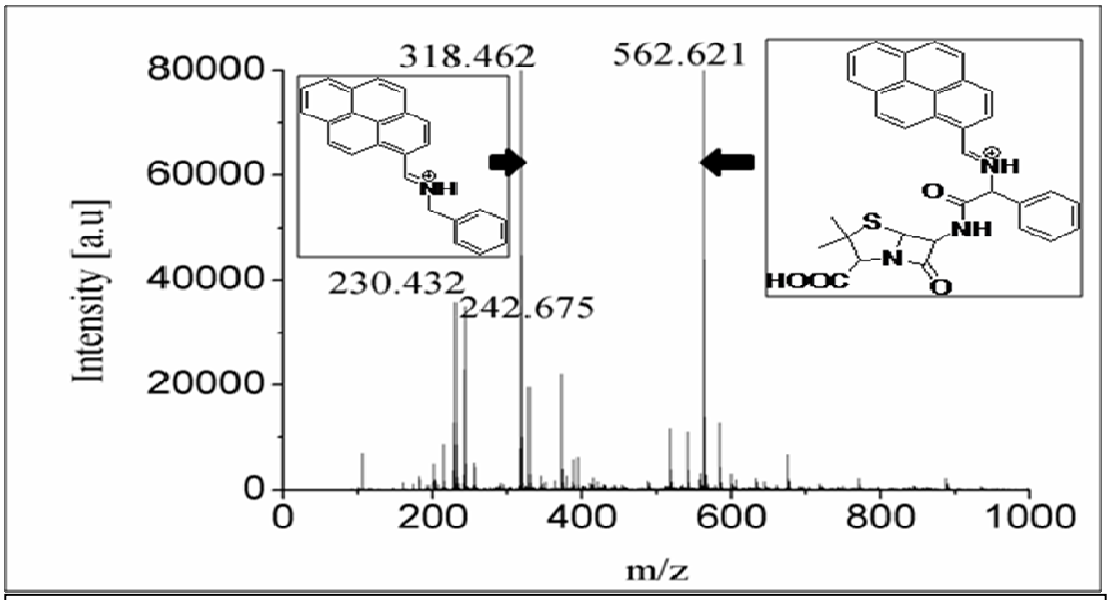

Fig 4: LDI-TOF Mass Spectrum of capture complex of ampicillin (m/z= 562) in positive ion mode

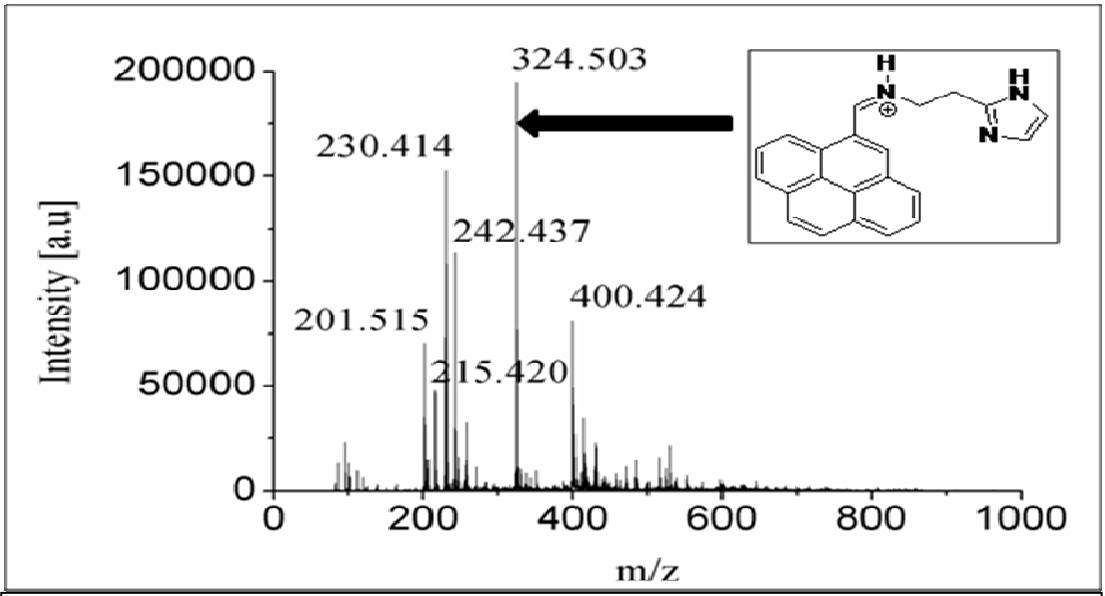

Fig 5: LDI-TOF Mass Spectrum of capture complex of histamine (m/z= 324) in positive ion mode

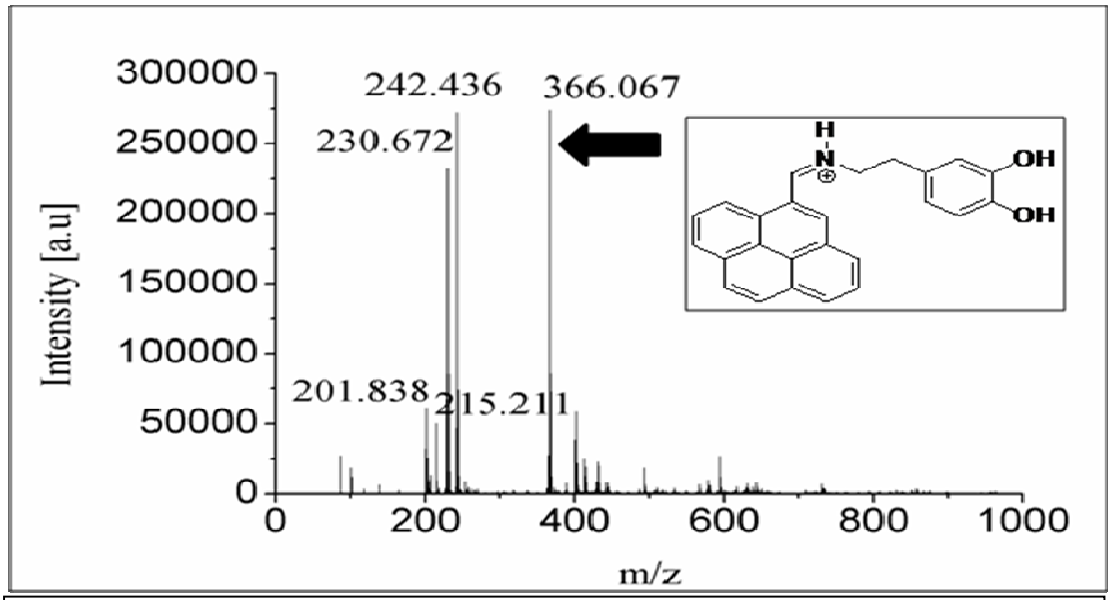

Fig 6: LDI-TOF Mass Spectrum of capture complex of dopamine (m/z= 366) in positive ion mode

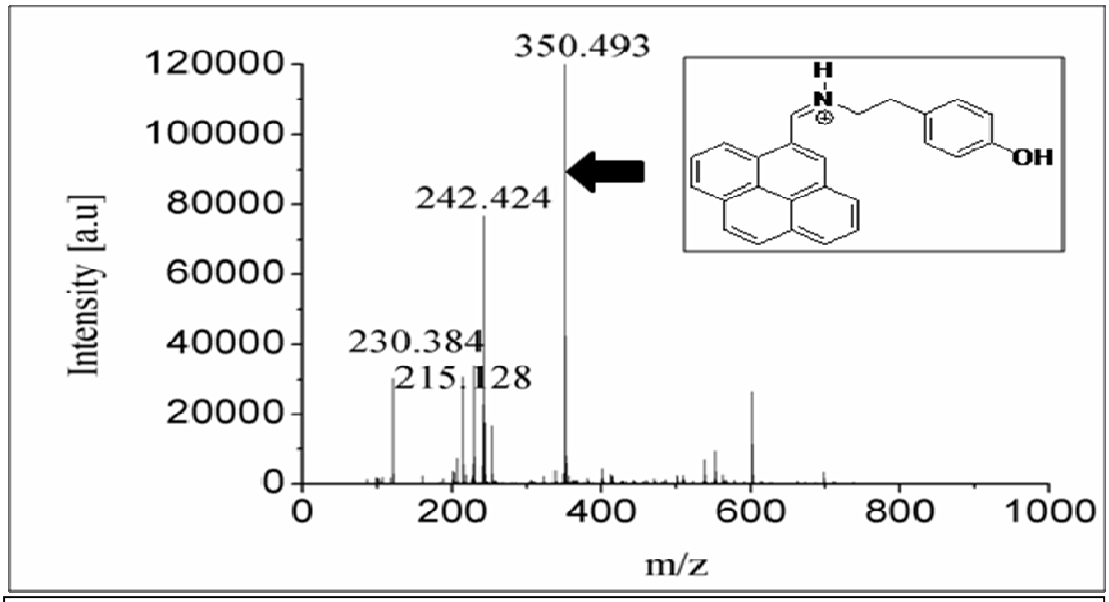

Fig 7: LDI-TOF Mass Spectrum of capture complex of tyramine (m/z= 350) in positive ion mode

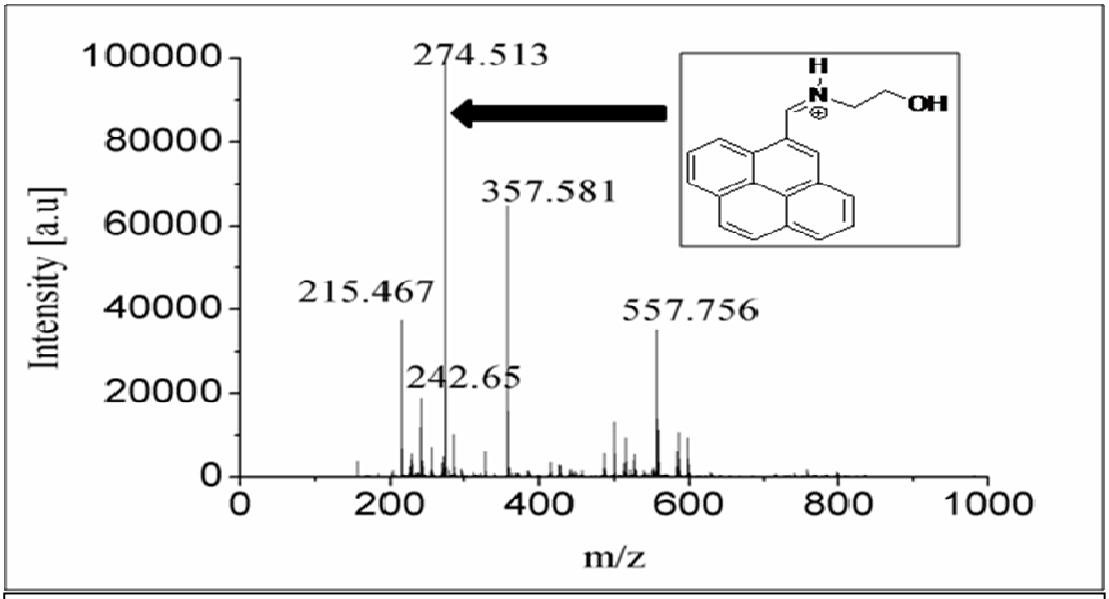

Fig 8: LDI-TOF Mass Spectrum of capture complex of Ethanolamine (m/z= 274) in positive ion mode

Table 2 (Bisimine):-

Bis amine Compound	**Expected bis imine peak (m/z)**	**Obtained Peaks (m/z)**
Ethylene diamine	484	485, 242, 215
1,3-diamino propane	498	499, 242, 215

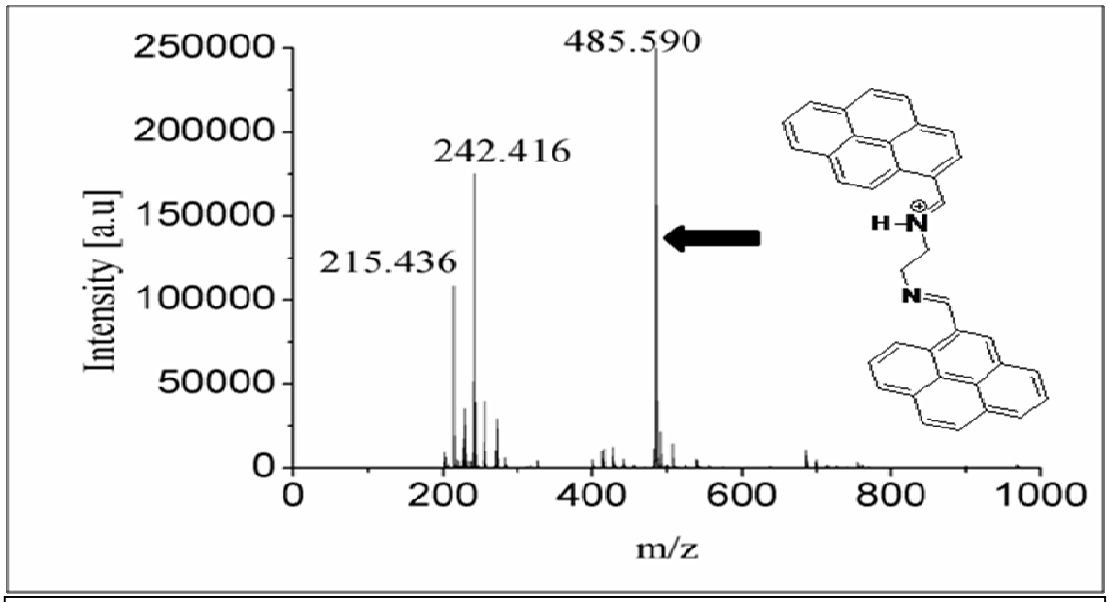

Fig 9: LDI-TOF Mass Spectrum of capture complex of Ethylenedimine (m/z= 485) in positive ion mode

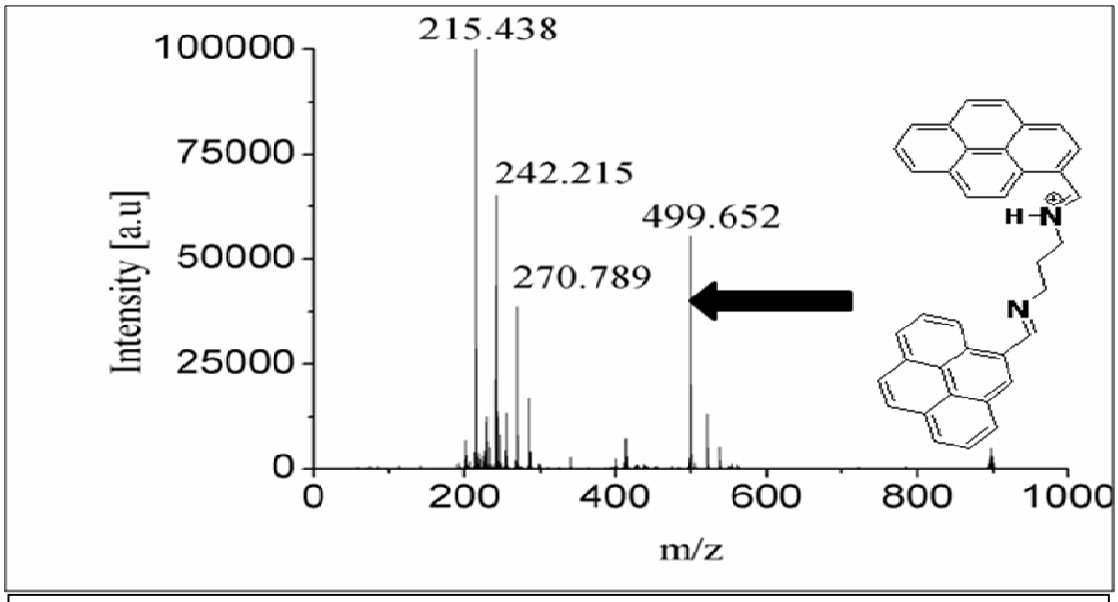

Fig 10: LDI-TOF Mass Spectrum of capture complex of 1, 3-diamino propane (m/z= 499) in positive ion mode

Explanation of peaks:-

In every case, we obtained (imine +1) peak which arises due to formation of iminium species.

Besides molecular ion peaks of iminium complex, we also obtained some fragmention at m/z= 242, m/z= 215, m/z= 201. These peaks are fragmentation peak of iminium complex.

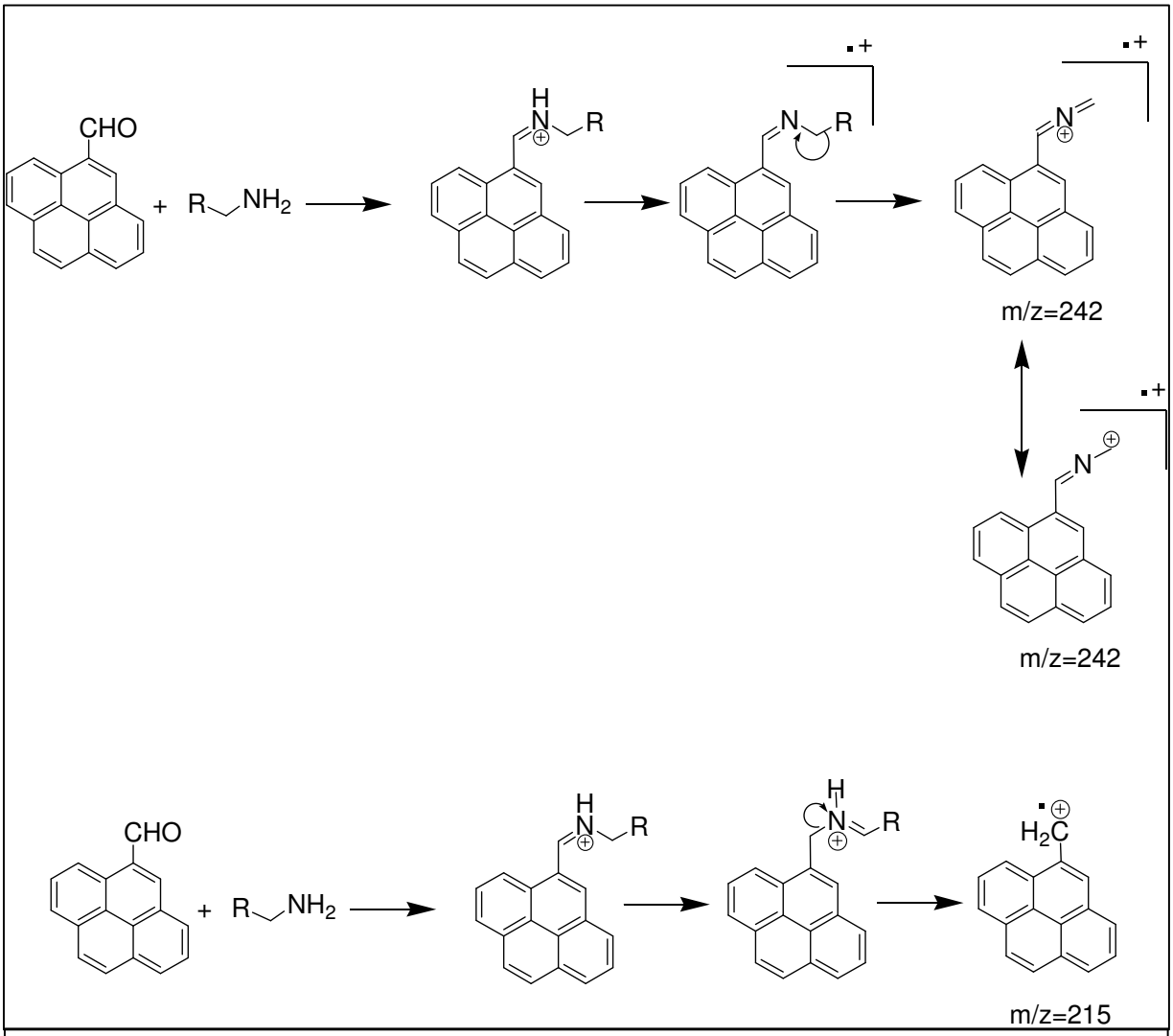

Fig 11: Schematic diagram showing different ion fragment of iminium complex

Dopamine sensitivity detection:-

Sensitivity of dopamine was determined by incubating dopamine solution of different concentration with fixed concentration of 1-pyrenecarboxaldehyde. A series of solutions 1mM 1-pyrenecarboxaldehyde (in Methanol) and varying concentration of dopamine of (50µM, 25µM, 15µM, 10µM, 5 µM, 2 µM, and 1 µM) were incubated at room temperature for 2 hours. 1µL aliquot from the reaction mixture were taken from each reaction mixture and analyzed by a Time of Flight Mass Spectrometer (TOF-MS) in positive ion mode. Dopamine was clearly detectable up to 2µM (m/z = 366).

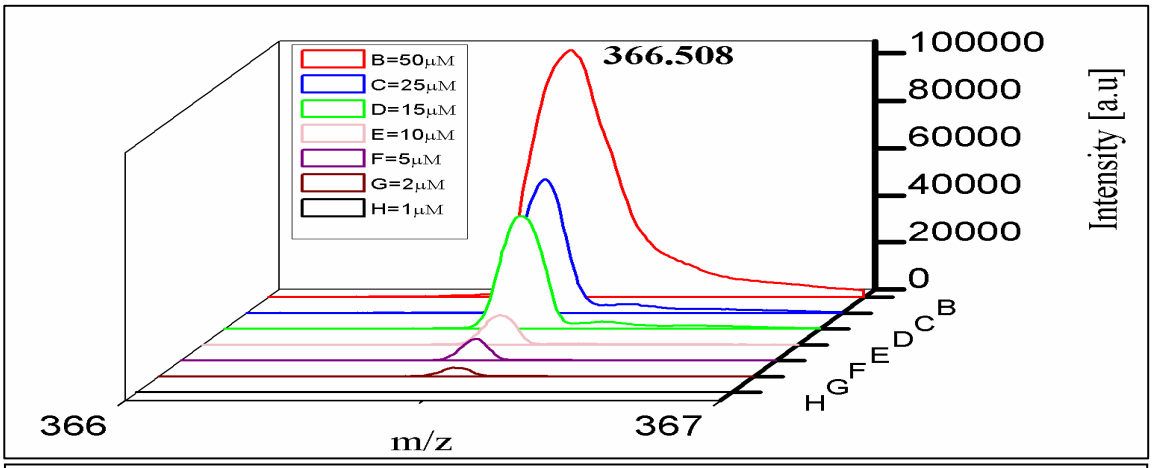

Fig 12a: Comparison of LA-LDI-TOF Mass spectra of capture complex of dopamine at different concentrations

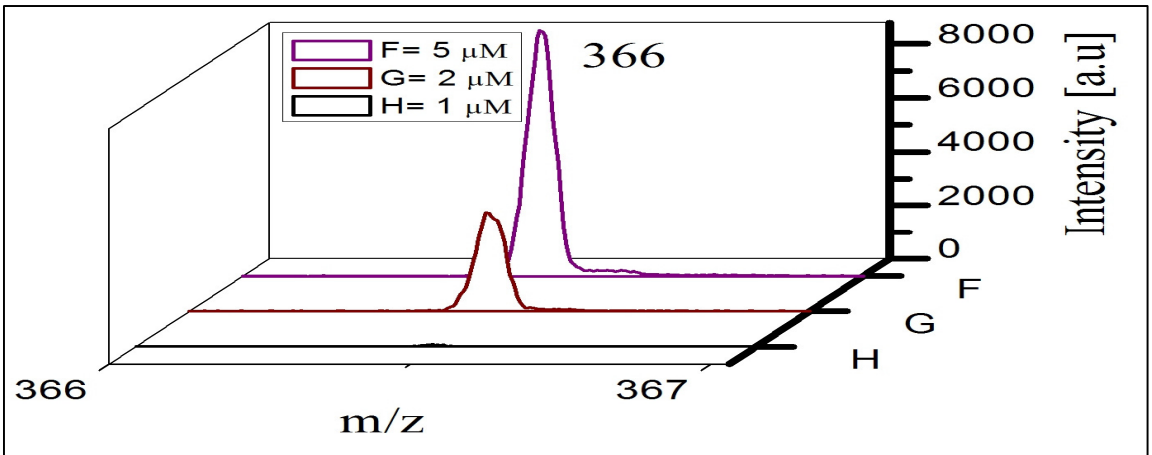

Fig 12b: Comparison of LA-LDI-TOF Mass spectra of capture complex of dopamine at different concentrations (Expanding intensity scale for lower concentrations)

Detection of dopamine, tyramine, and histamine from their mixture in presence of excess amount of glucose:-

Dopamine hydrochloride, tyramine hydrochloride, histamine hydrochloride were neutralized with excess amount of Et_3N. A series of solutions 3mM 1-pyrenecarboxaldehyde (stock solution in methanol) and varying concentration of dopamine, tyramine, histamine (100µM, 50µM, 25µM, 10µM, 5µM) were incubated for 2 hours at room temperature in presence of excess glucose. 1µL aliquot from the reaction mixture were taken from each reaction mixture and analyzed by a Time of Flight Mass Spectrometer (TOF-MS) in positive ion mode. These three amines were detected by LDI MS from the mixture up to 10µM.

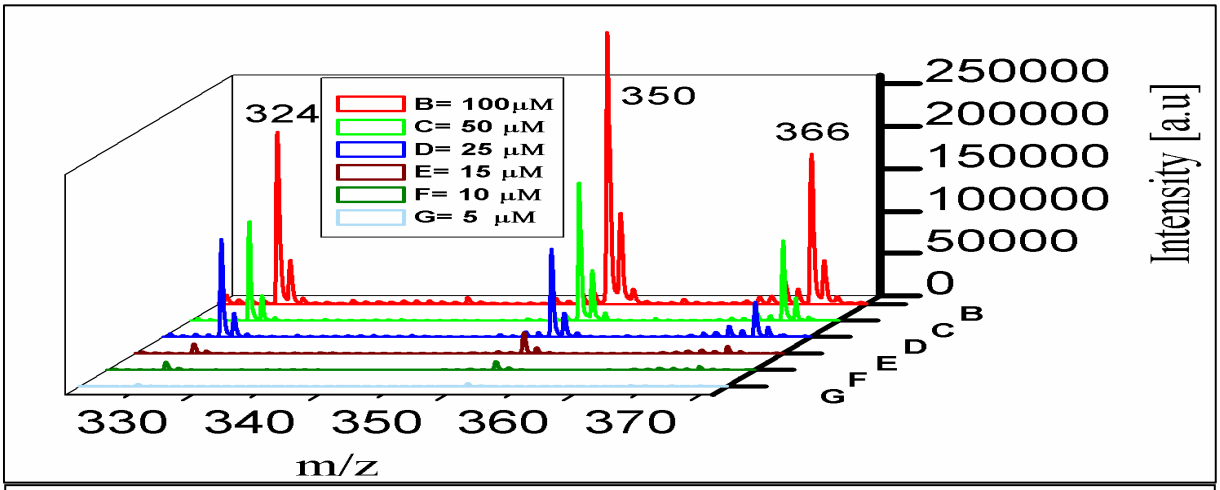

Fig 13a: Comparison of LA-LDI-TOF Mass spectra of capture complex of dopamine, tyramine, and histamine from their mixture at different concentrations in presence of excess glucose

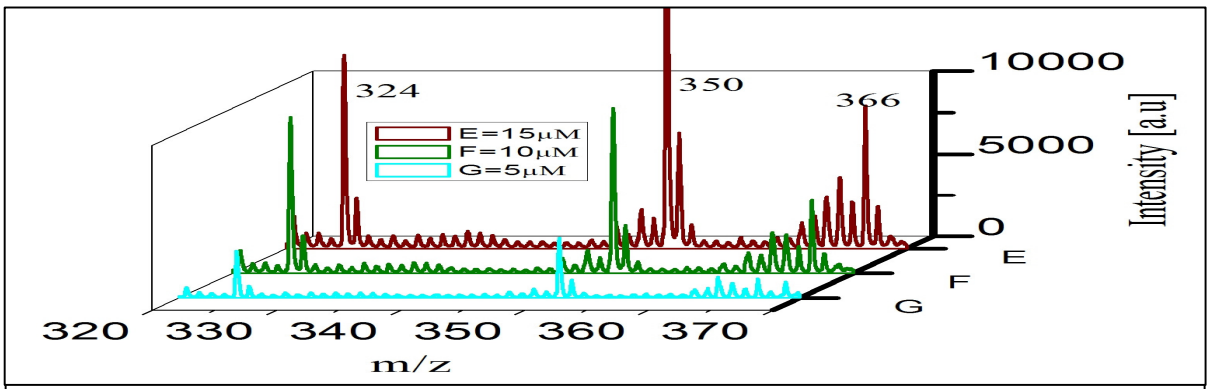

Fig 13b: Comparison of LA-LDI-TOF Mass spectra of capture complex of dopamine, tyramine, and histamine from their mixture at different concentrations in presence of excess glucose (Expanding intensity scale for lower concentrations)

Detection of amino acid:-

We have screened several amino acids for our experiments-

- α- amino acid :- Glycine, alanine, valine, methionine, phenyl alanine, tryptophan, tyrosine,lysine, glutamic acid, aspartic acid
- β-amino acid :- β-alanine
- γ-amino acid :- Gabapentin
- ε-amino acid :- 6-amino caproic acid

We performed our experiment for screening these four types of amino acids in different ways-

1. **Without doing any neutralization**: - 0.1mM aqueous solution of amino acid was incubated with 0.1mM of 1-pyrenecarboxaldehyde for 6 hours.
2. **Neutralisiling amino acids with Et_3N:-** Aqueous solutions amino acids were treated with excess amount of Et_3N. 0.1mM aqueous solution of amino acid (neutralized by Et_3N) and 0.1mM 1-pyrenecarboxaldehyde(stock solution in methanol) was incubated for 4 hours at room temperature and 1μL aliquot from the reaction mixture were taken from each reaction mixture and analyzed by a Time of Flight Mass Spectrometer (TOF-MS) in positive ion mode.
3. **Neutralising amino acids with NaOH: -** Aqueous solutions amino acids were treated with equivalent amount of NaOH. 0.1mM aqueous solution of amino acid (neutralized by NaOH) and 0.1mM 1-pyrenecarboxaldehyde (stock solution in methanol) was incubated for 4 hours at room temperature and 1μL aliquot from the reaction mixture were taken from each reaction mixture and analyzed by a Time of Flight Mass Spectrometer (TOF-MS) in positive ion mode.

The summarized results are given in the table-

Table 3 (Amino acids):-

Amino acid	Expected imine peak (m/z)	Obtained Peak (m/z)		
		Neutralised by NaOH	**Neutralised by Et_3N**	**Without any neutralisation**
α-amino acids				
Glycine	287	332.379 (Iminium + 44) peak	No significant peak	No significant peak
Alanine	301	346 (Iminium + 44) peak)	No significant peak	No significant peak
Valine	329	374 (Iminium + 44) peak	No significant peak	No significant peak
Methionine	361	406 (Iminium + 44) peak	No significant peak	No significant peak
Tyrosine	393	438 (Iminium + 44) peak	No significant peak	No significant peak
Tryptophan	416	461 (Iminium + 44) peak	No significant peak	No significant peak
Lysine	358	403 (Iminium + 44) peak	No significant peak	No significant peak
Glutamic acid	359	360 (Iminium) peak	No significant peak	No significant peak
Aspartic acid	345	346 (Iminium) peak	No significant peak	No significant peak
β –amino acid				
β-alanine	301	346 (Iminium + 44) peak	No significant peak	No significant peak
γ- amino acids				
Gabapentin	383	428 (Iminium + 44) peak	384 (Imine + 1) peak	No significant peak
ε -amino caproic acids				
6-amino caproic acid	343	388 (Iminium + 44) peak	344 (Imine + 1) peak	344 (Imine + 1) peak

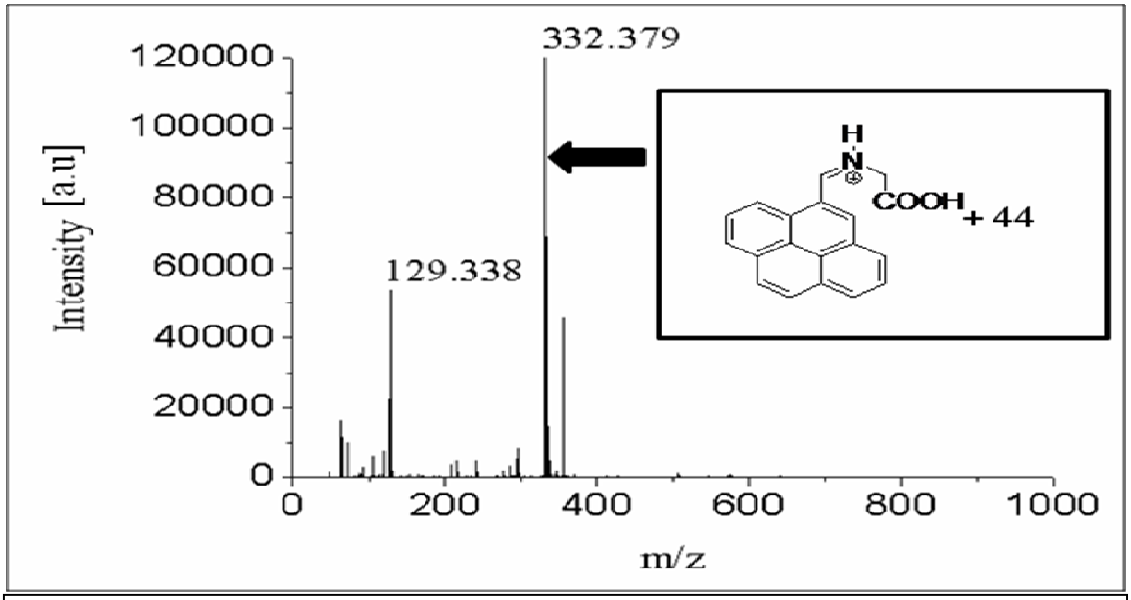

Fig 14: LDI-TOF Mass Spectrum of capture complex of glycine (m/z= 332) in positive ion mode (in presence f NaOH)

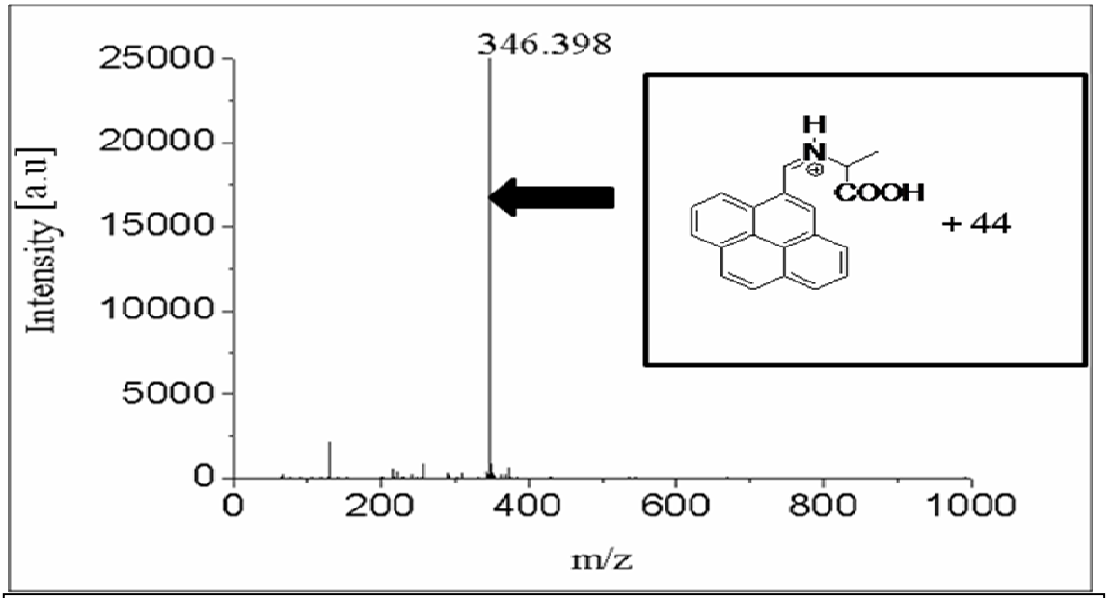

Fig 15: LDI-TOF Mass Spectrum of capture complex of alanine (m/z= 346) in positive ion mode (in presence of NaOH)

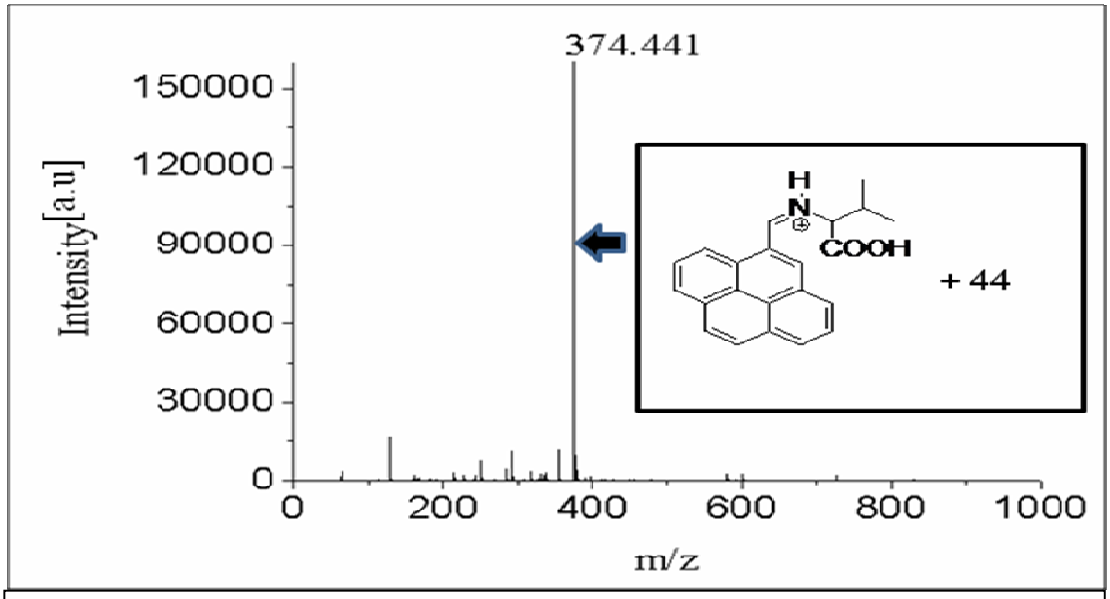

Fig 16: LDI-TOF Mass Spectrum of capture complex of valine (m/z= 374) in positive ion mode (in presence of NaOH)

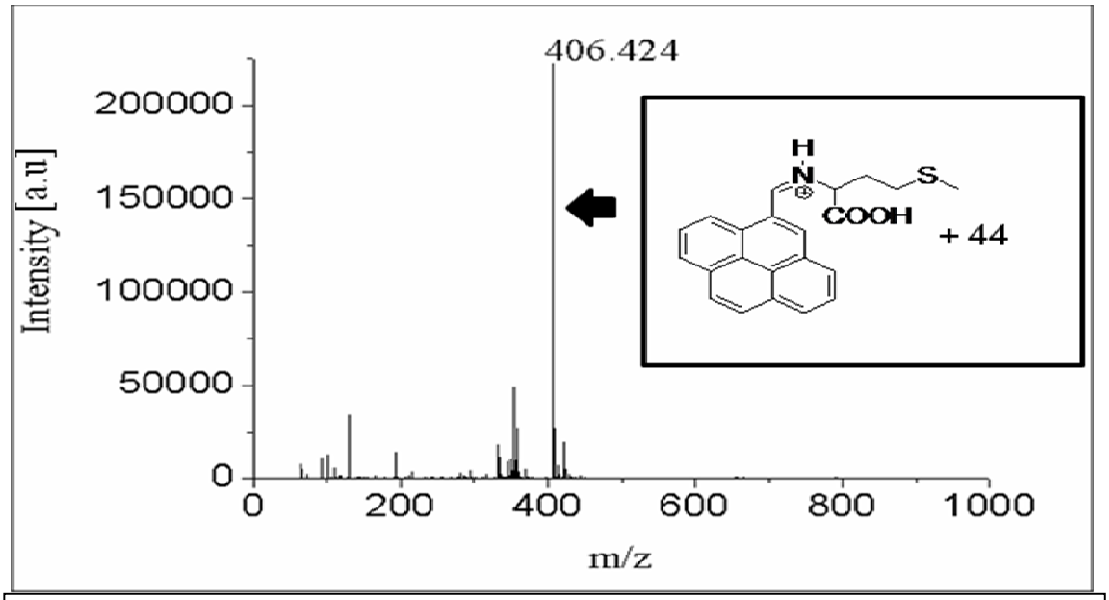

Fig 17: LDI-TOF Mass Spectrum of capture complex of methionine (m/z= 406) in positive ion mode (in presence of NaOH)

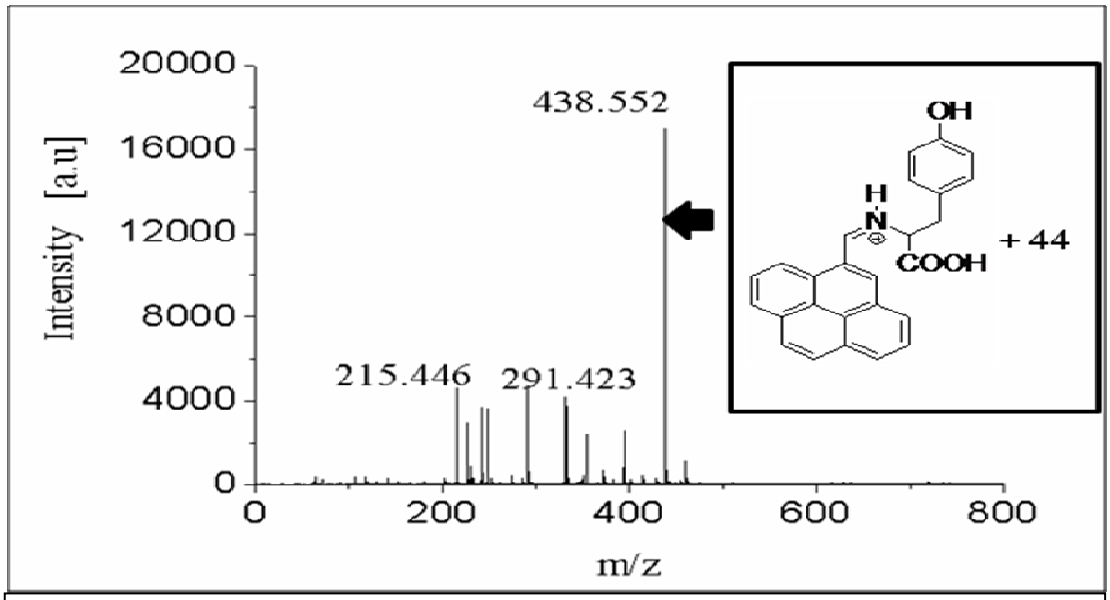

Fig 18: LDI-TOF Mass Spectrum of capture complex of tyrosine (m/z= 438) in positive ion mode (in presence of NaOH)

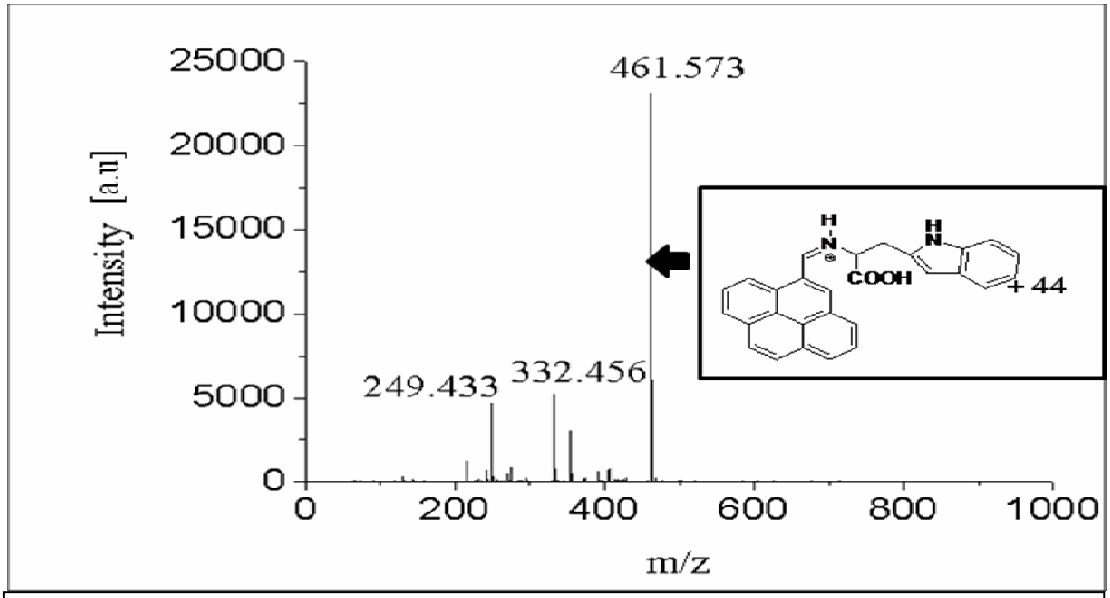

Fig 19: LDI-TOF Mass Spectrum of capture complex of tryptophan (m/z= 461) in positive ion mode (in presence of NaOH)

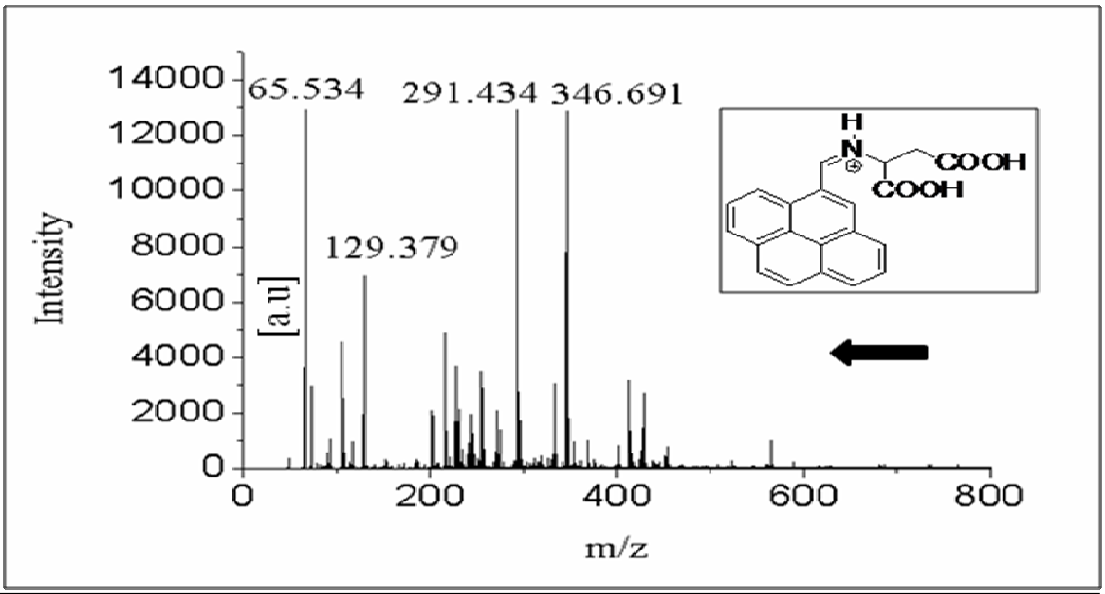

Fig 20: LDI-TOF Mass Spectrum of capture complex of aspartic acid (m/z= 346) in positive ion mode (in presence of NaOH)

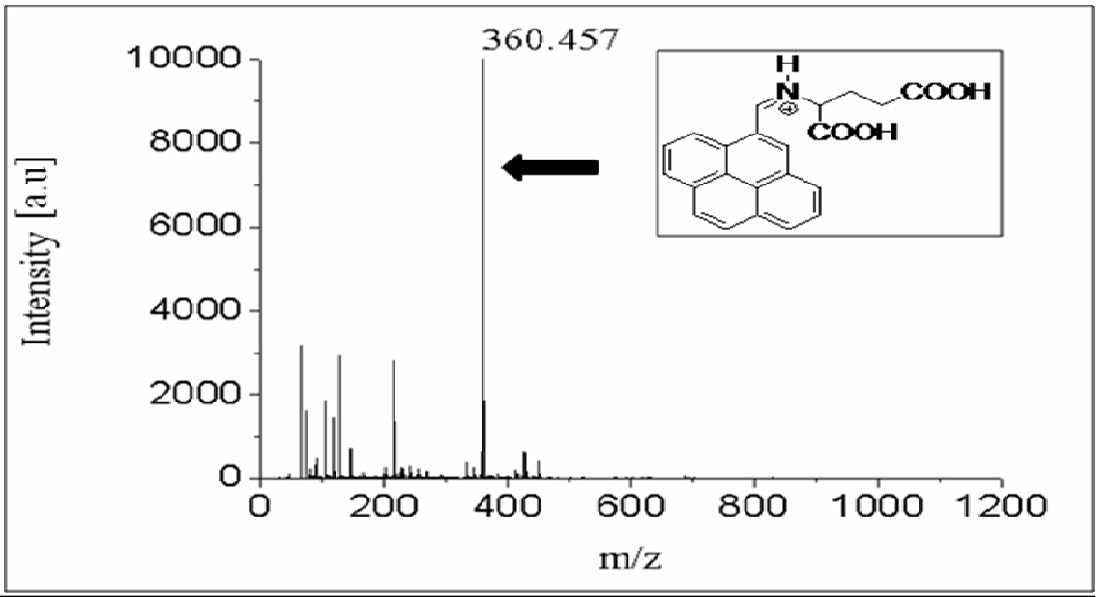

Fig 21: LDI-TOF Mass Spectrum of capture complex of glutamic acid (m/z= 360) in positive ion mode (in presence of NaOH)

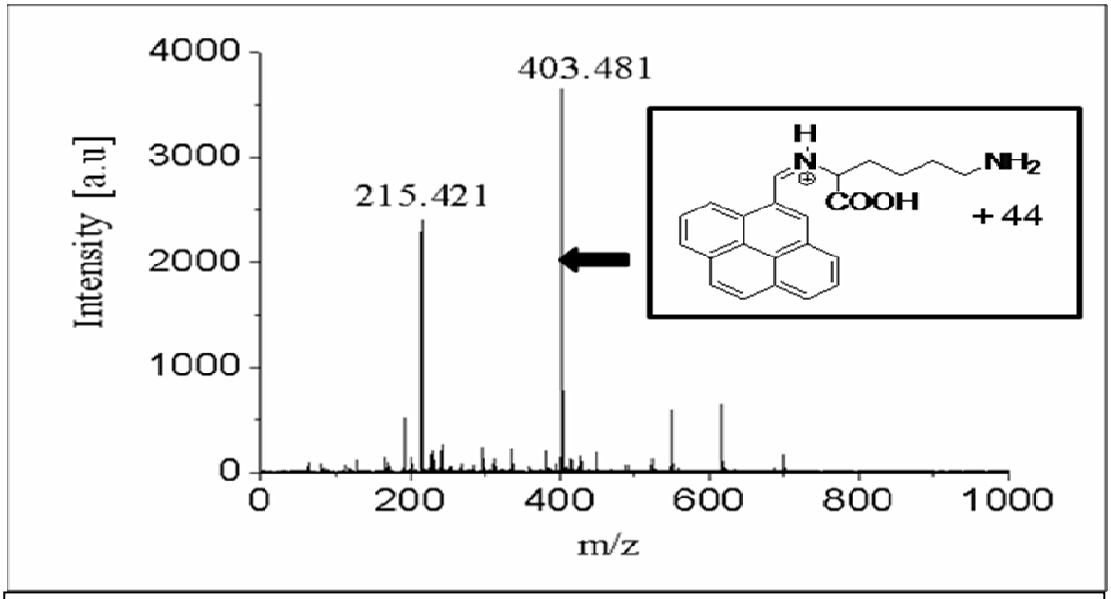

Fig 22: LDI-TOF Mass Spectrum of capture complex of lysine (m/z= 403) in positive ion mode (in presence of NaOH)

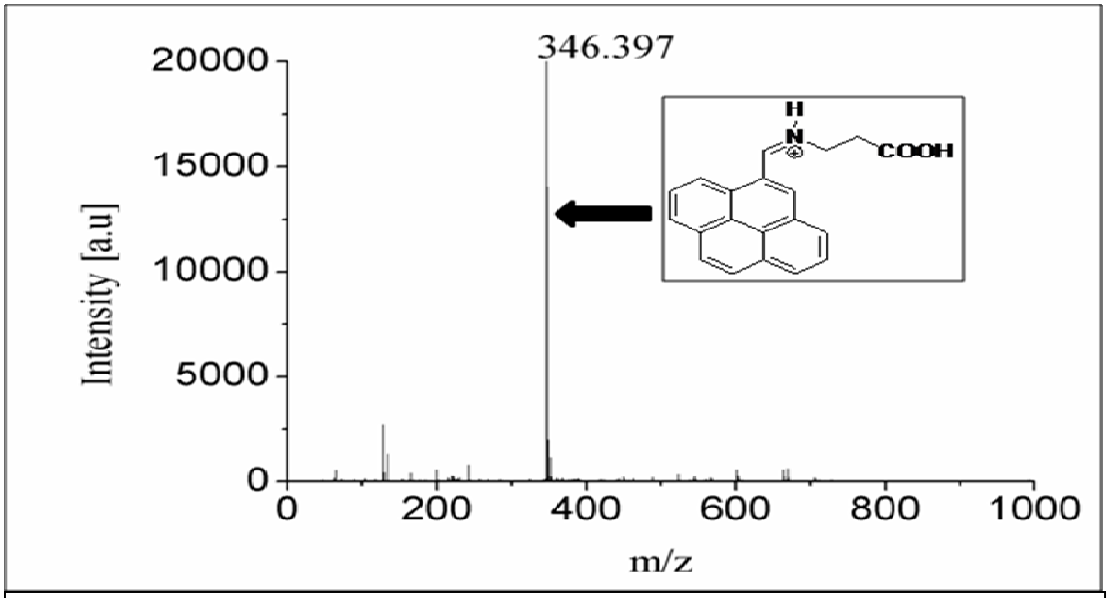

Fig 23: LDI-TOF Mass Spectrum of capture complex of β-alanine (m/z= 346) in positive ion mode (in presence of NaOH)

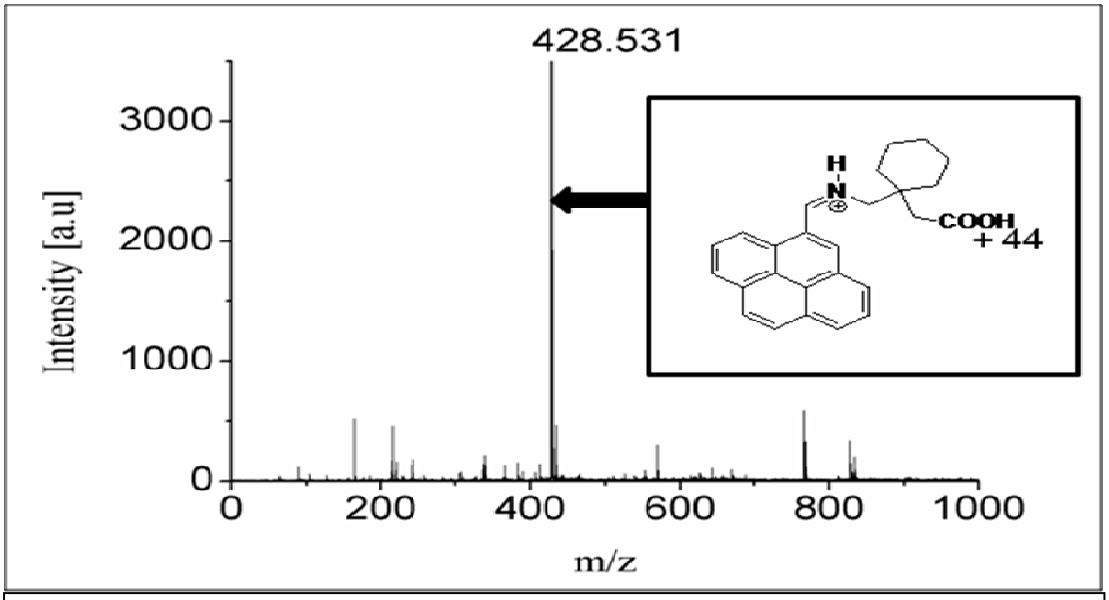

Fig 24: LDI-TOF Mass Spectrum of capture complex of gabapentin (m/z= 428) in positive ion mode (in presence of NaOH)

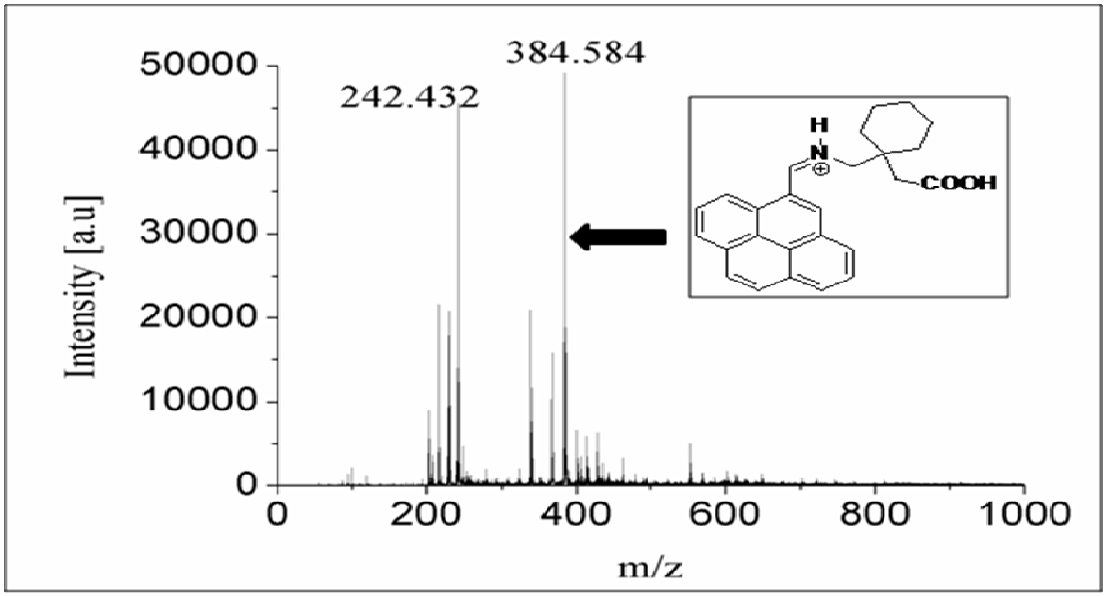

Fig 25: LDI-TOF Mass Spectrum of capture complex of gabapentin (m/z= 384) in positive ion mode (in presence of Et_3N)

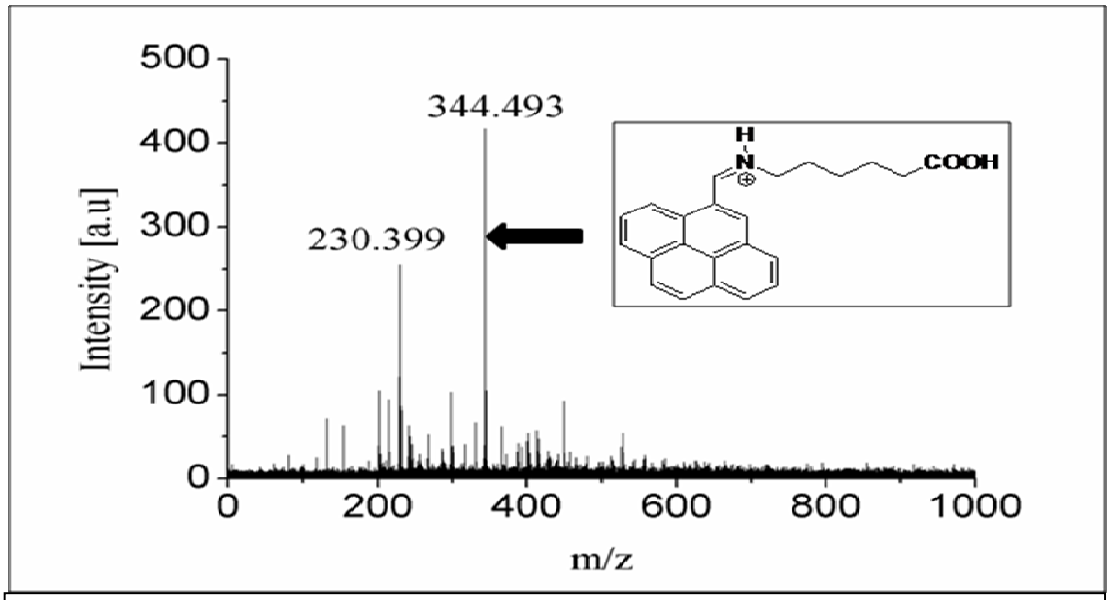

Fig 26: LDI-TOF Mass Spectrum of capture complex of 6-aminocaproic acid (m/z= 344) in positive ion mode (in presence of Et_3N)

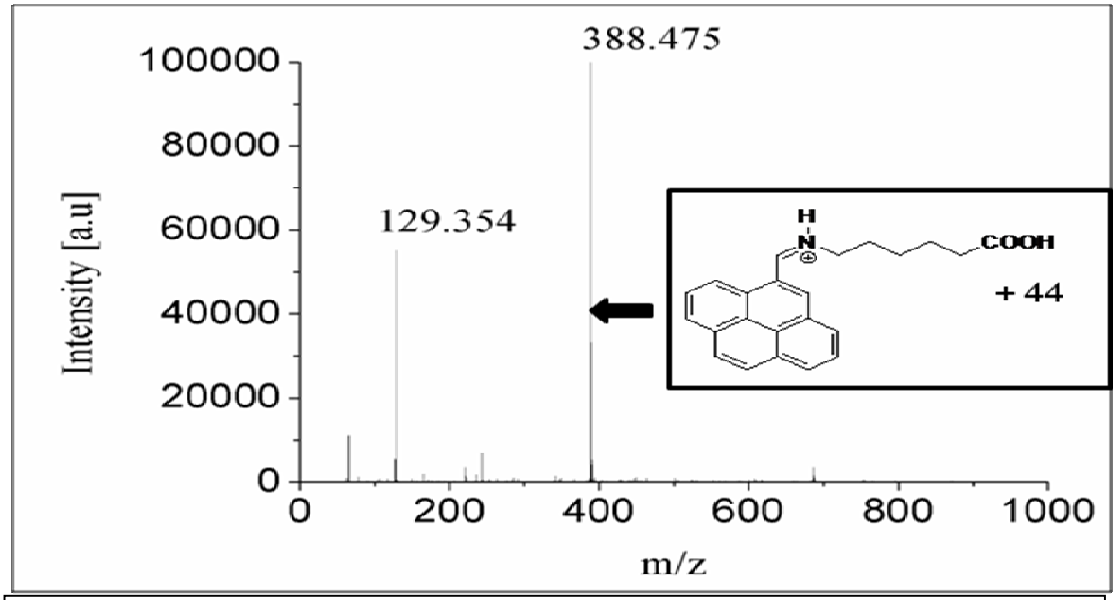

Fig 27: LDI-TOF Mass Spectrum of capture complex of 6-aminocaproic acid (m/z= 388) in positive ion mode (in presence of NaOH)

Detection of amino acids from their mixture:-

Aqueous solutions glycine, alanine, valine were neutralized by aqueous NaOH solution. 0.1mM aqueous solution of each amino acid (neutralized by NaOH) and 0.3mM 1-pyrenecarboxaldehyde solution was incubated for 4 hours at room temperature in presence of 10 folds excess of glucose, tartaric acid, ascorbic acid, citric acid, α- ketoglutaric acid and 1μL aliquot from the reaction mixture were taken from each reaction mixture and analyzed by a Time of Flight Mass Spectrometer (TOF-MS) in positive ion mode. Three amino acids were detected from their mixture.

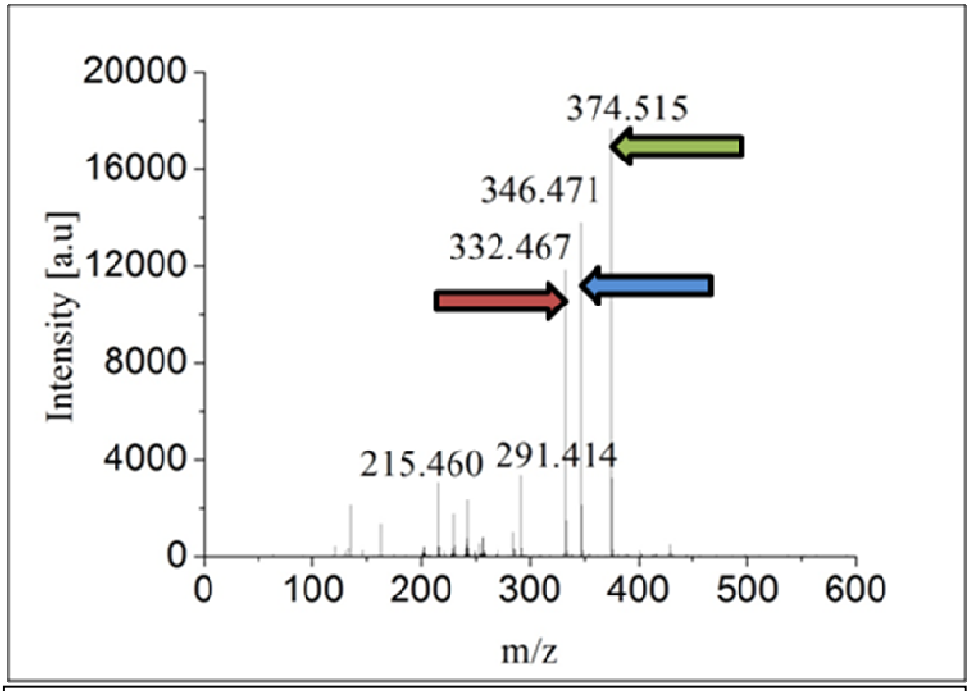

Fig 28: LDI-TOF Mass Spectrum of capture complex of glycine, alanine, valine from their mixture in presence of excess of ascorbic acid, tartaric acid, citric acid, glucose, α-ketoglutaric acid (m/z= 332, 346, 374) in positive ion mode (in presence of NaOH)

Detection of peptides:-

Experimental condition:-

Aqueous solutions peptides were treated with equivalent amount of NaOH. 0.1mM aqueous solution of peptide (neutralized by NaOH) and 0.1mM 1-pyrenecarboxaldehyde solution was incubated for 4 hours at room temperature and 1μL aliquot from the reaction mixture were taken from each reaction mixture and analyzed by a Time of Flight Mass Spectrometer (TOF-MS) in positive ion mode.

Experimental results are given in the table-

Table 4 (peptides):-

Peptide	**Expected imine peak (m/z)**	**Obtained (iminium + 44) peak (m/z)**
Glycyl-L-Leucine	400	445
Glycyl-L-Isoleucine	400	445
Glycyl-Glycyl-L-Leucine	457	502
L-Carnosine(β-Alanyl-L-Histidine)	438	483

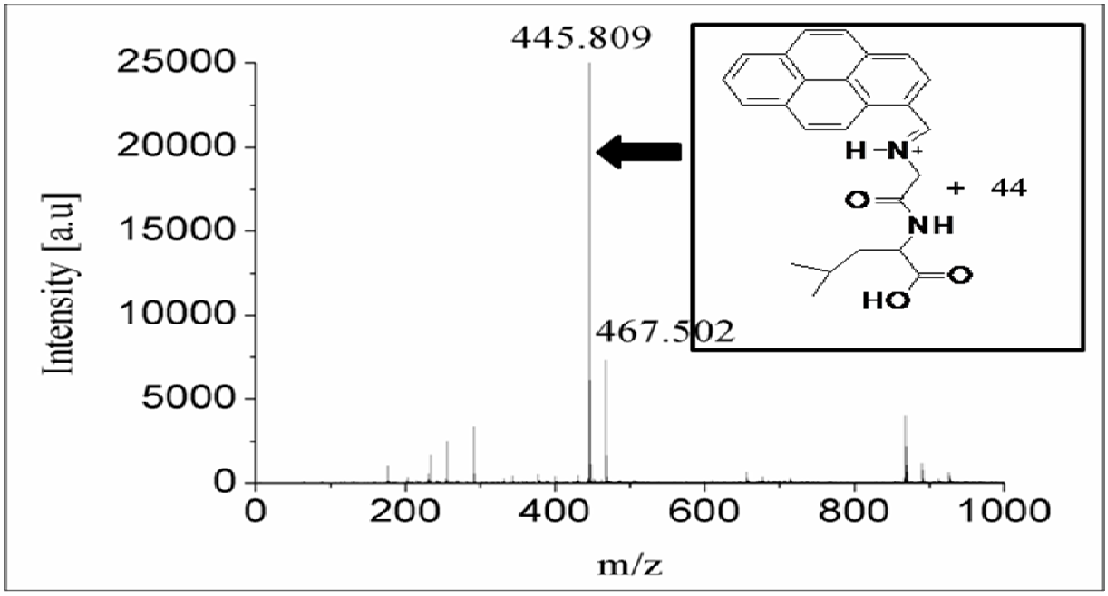

Fig 29: LDI-TOF Mass Spectrum of capture complex of Glycyl-L-Leucine (m/z= 445) in positive ion mode (in presence of NaOH)

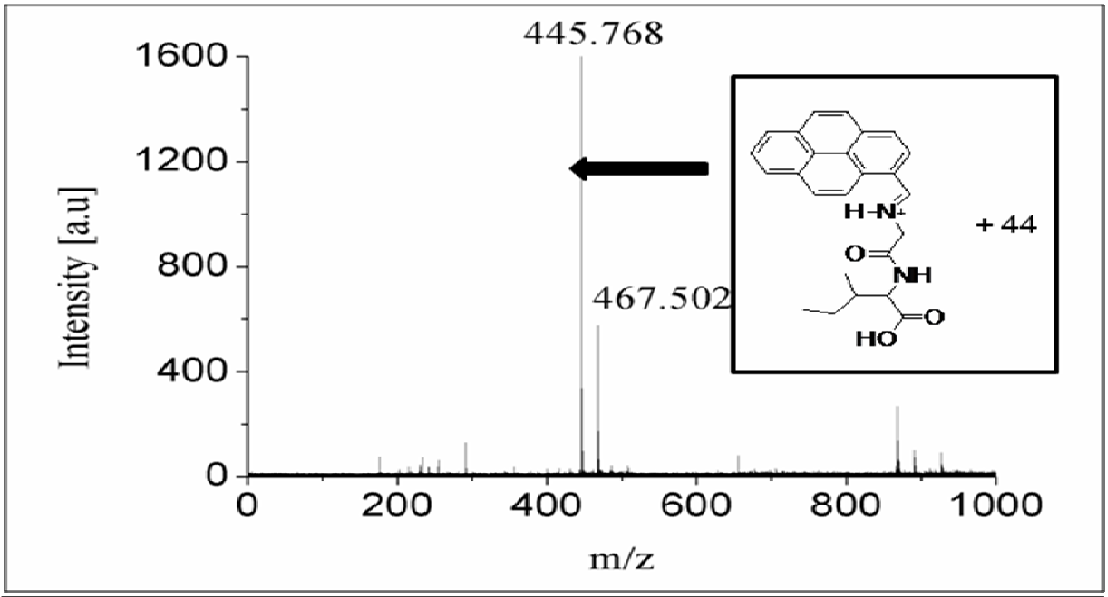

Fig 30: LDI-TOF Mass Spectrum of capture complex of Glycyl-L-Isoleucine (m/z= 445) in positive ion mode (in presence of NaOH)

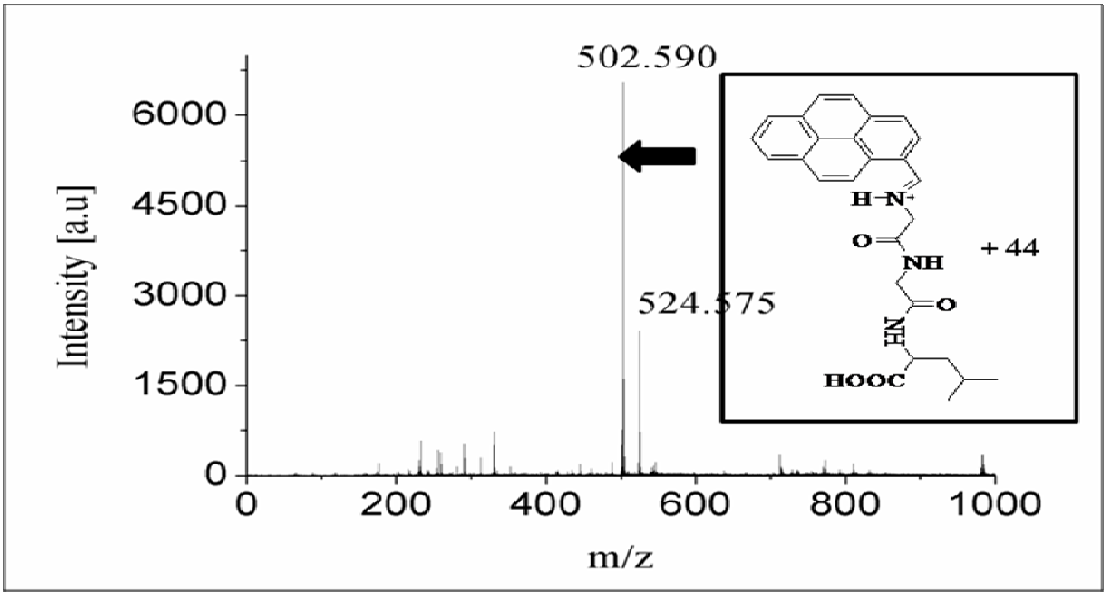

Fig 31: LDI-TOF Mass Spectrum of capture complex of Glycyl-Glycyl-L-Leucine (m/z= 502) in positive ion mode (in presence of NaOH)

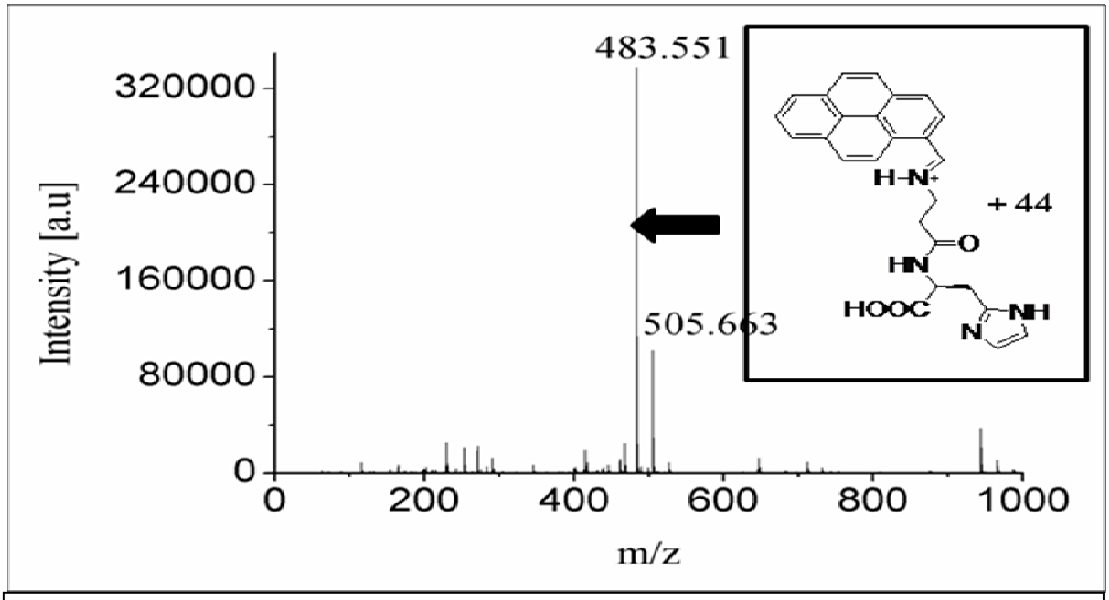

Fig 32: LDI-TOF Mass Spectrum of capture complex of L-carnosine (m/z= 483) in positive ion mode (in presence of NaOH)

Detection of peptides from their mixture:-

Aqueous solutions L-Carnosine and Glycyl-Glycyl-L-Leucine solution were neutralized by NaOH. 0.1mM aqueous solution of each peptide (neutralized by NaOH) and 0.2mM 1-pyrenecarboxaldehyde solution was incubated for 4 hours at room temperature and 1µL aliquot from the reaction mixture were taken from each reaction mixture and analyzed by a Time of Flight Mass Spectrometer (TOF-MS) in positive ion mode. Both peptides were detected from their mixture.

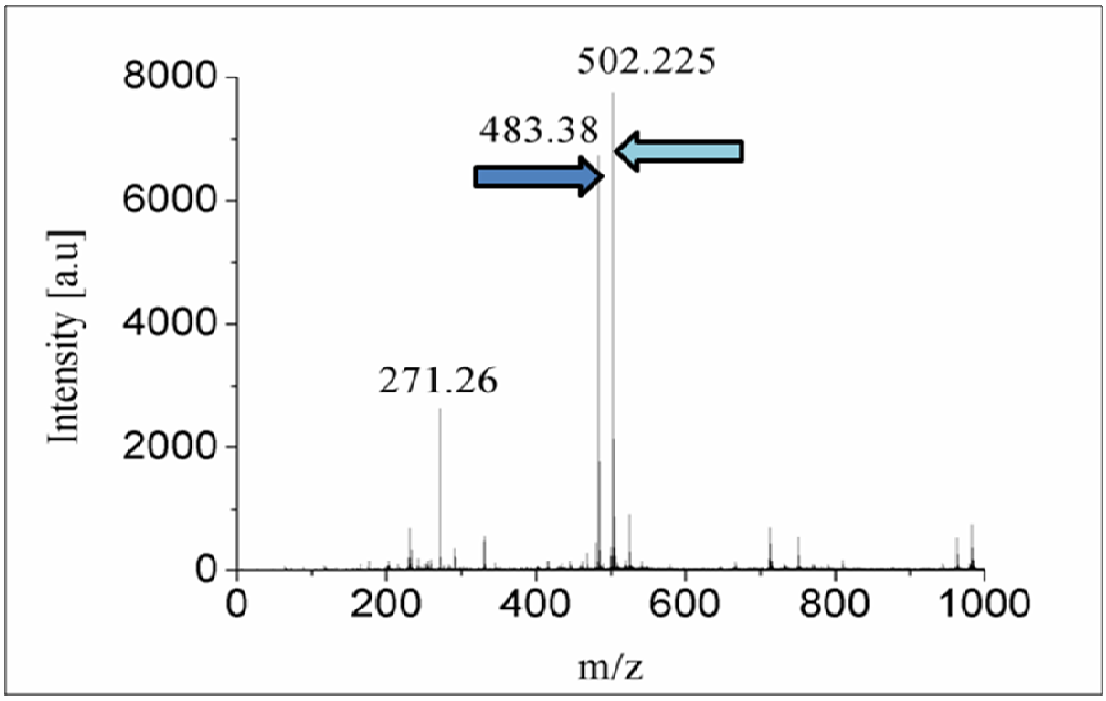

Fig 33: LDI-TOF Mass Spectrum of capture complex of Glycyl-Glycyl-L-Leucine (m/z= 502) and L-Carnosine (m/z= 483) in positive ion mode (in presence of NaOH)

Comparison of LDI Mass spectrum of dopamine with and without matrix:-

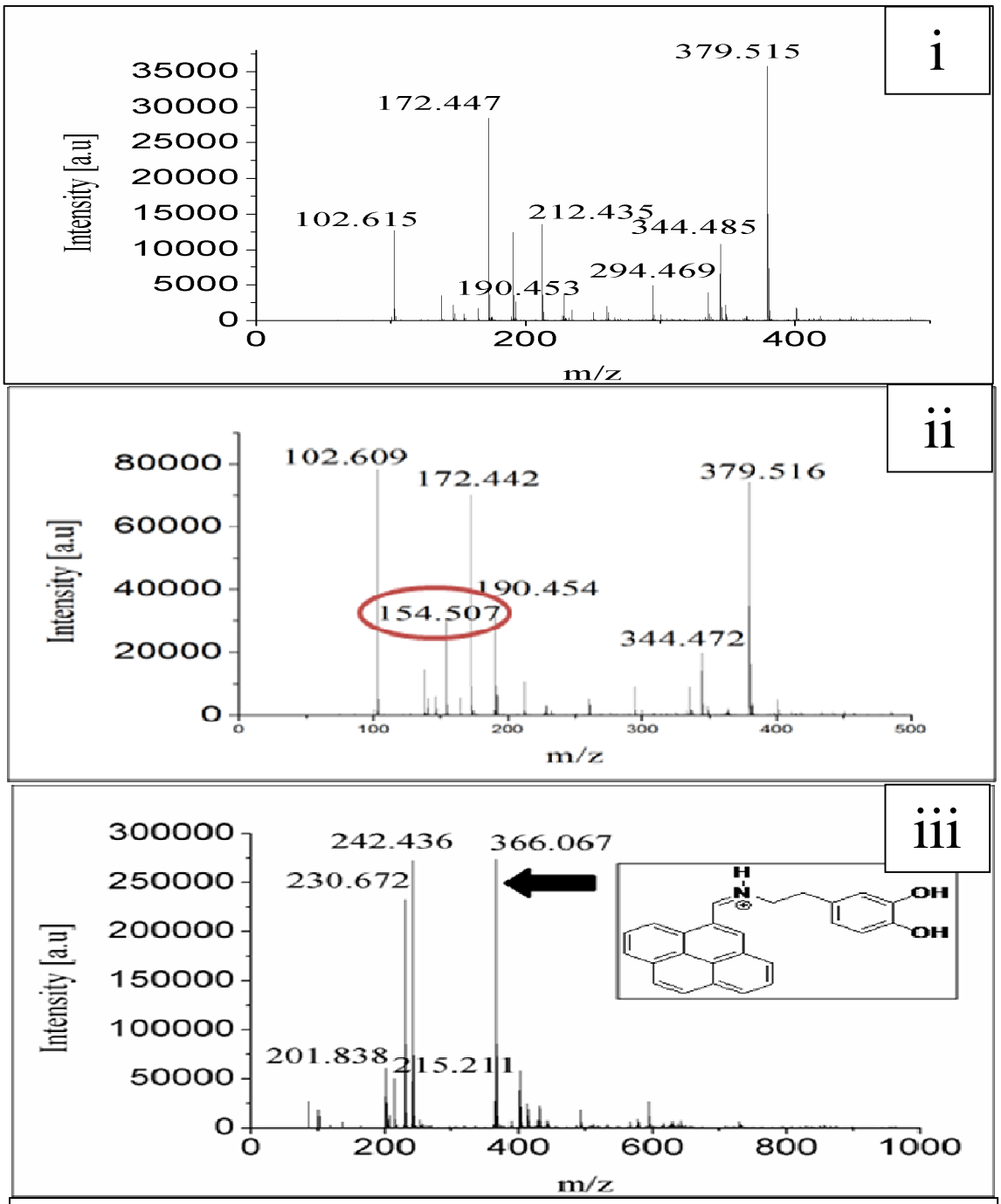

Fig 34: Comparison of LDI Mass spectrum of dopamine with and without matrix in positive ion mode

(i) HCCA matrix (ii) HCCA matrix + Dopamine (m/z= 154) [matrix:analyte= 100:1 molar ratio] (iii) Dopamine + 1-Pyrenecarboxaldehyde (m/z = 366)

Comparison of LDI Mass spectrum of amino acids(in a mixture) with and without matrix:-

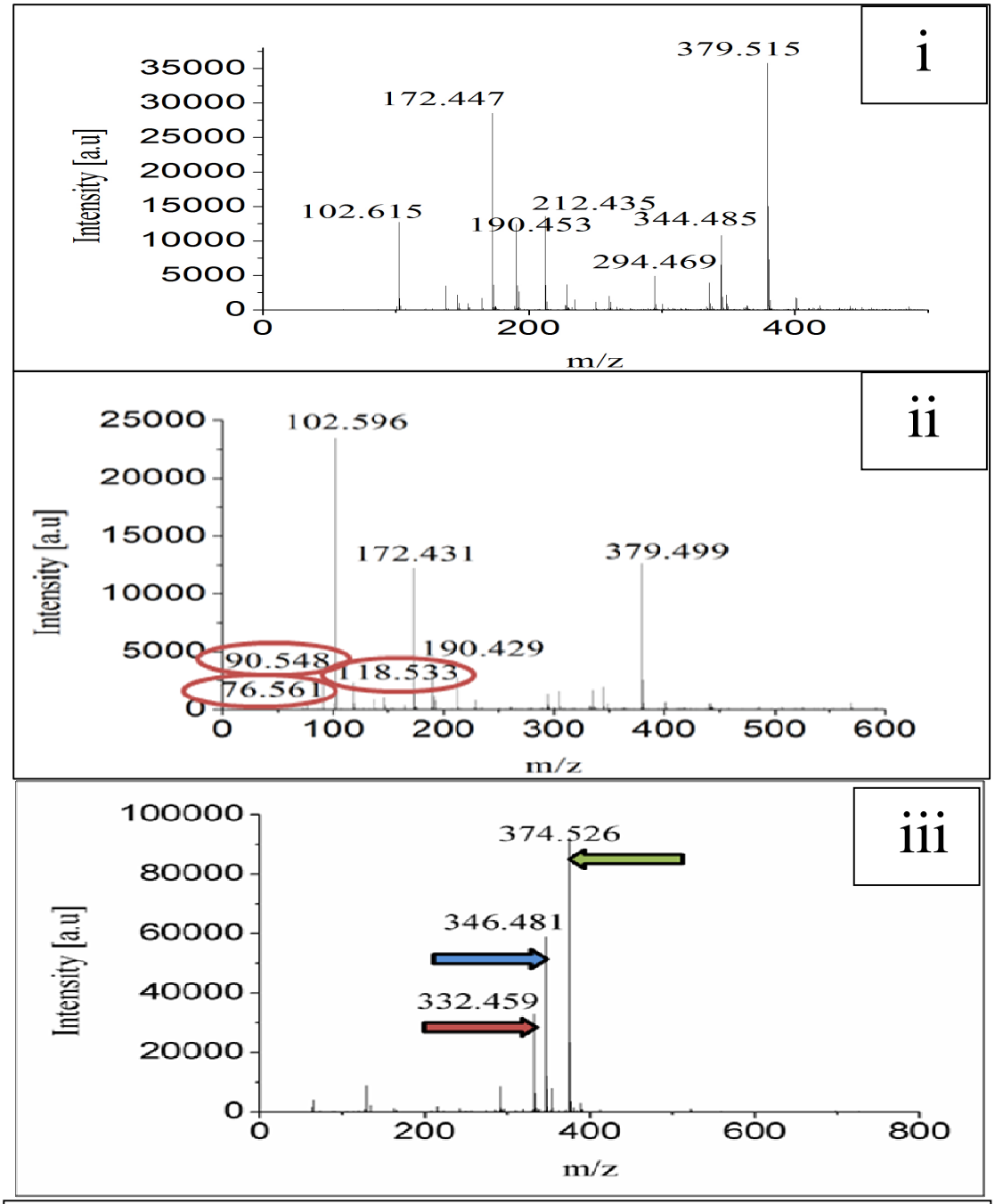

Fig 35: Comparison of LDI Mass spectrum of amino acids (in a mixture) with and without matrix

(i) HCCA matrix (ii) HCCA matrix + amino acids (m/z= 75, 89, 117) [matrix:analyte= 100:1 molar ratio] (iii) amino acids (glycine , alanine, valine)+ 1-Pyrenecarboxaldehyde (m/z = 332, 346, 374)

Detection of amino acid and peptide by imine formation and followed by reductive amination:-

$NaBH_4$ reduction:-

Aqueous solutions analytes (peptide, amino acid) were treated with equivalent amount of NaOH. 0.1mM aqueous solution of peptide (neutralized by NaOH) and 0.1mM 1-pyrenecarboxaldehyde solution was incubated for 4 hours. Then 1mM aqueous solution of $NaBH_4$ was added to it at 0^oC and incubated at room temperature for 4 hours. 1µL aliquot from the reaction mixture were taken from each reaction mixture and analyzed by a Time of Flight Mass Spectrometer (TOF-MS) in positive ion mode.

$NaCNBH_3$ reduction:-

Aqueous solutions analytes (peptide, amino acid) were treated with equivalent amount of NaOH. 0.1mM aqueous solution of peptide (neutralized by NaOH) and 0.1mM 1-pyrenecarboxaldehyde solution was incubated for 4 hours at room temperature. Then 1mM aqueous solution of $NaCNBH_3$ was added to it at 0^oC and incubated at room temperature for 4 hours. 1µL aliquot from the reaction mixture were taken from each reaction mixture and analyzed by a Time of Flight Mass Spectrometer (TOF-MS) in positive ion mode.

The summarized results are given in the table 5-

Table 5-

Analytes	**Without reduction(obtained imine+45 peak)** **m/z**	**Reduction by $NaBH_4$ (obtained amine+45 peak)** **m/z**	**Reduction by $NaCNBH_3$ (obtained amine+ 45 peak)** **m/z**
Alanine	346	348	348
Glycyl-L-Isoleucine	445	447	447

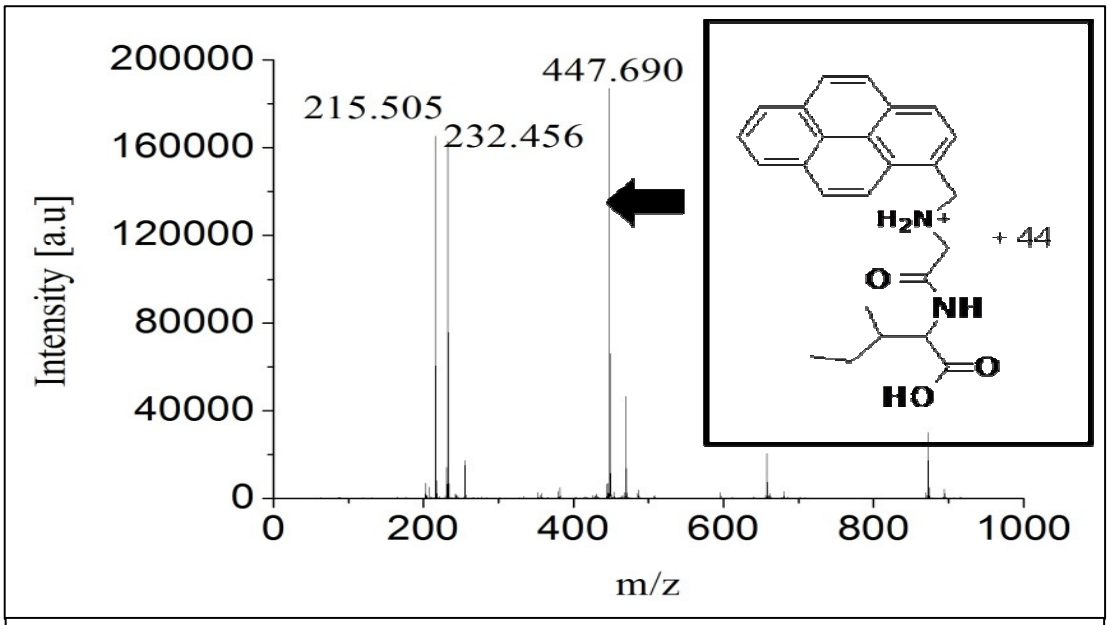

Fig 36: LA-LDI mass spectrum of amine complex of Glycyl-L-Isolysine (m/z= 447) in positive ion mode (reduced by $NaBH_4$)

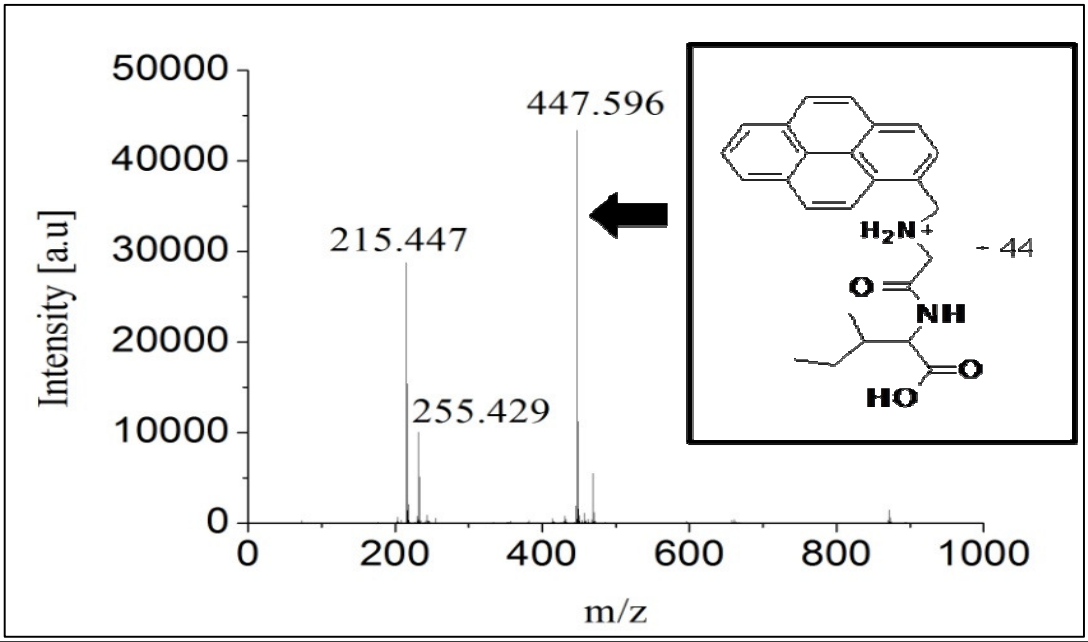

Fig 37: LA-LDI mass spectrum of amine complex of Glycyl-L-Isolysine (m/z= 447) in positive ion mode (reduced by $NaCNBH_3$)

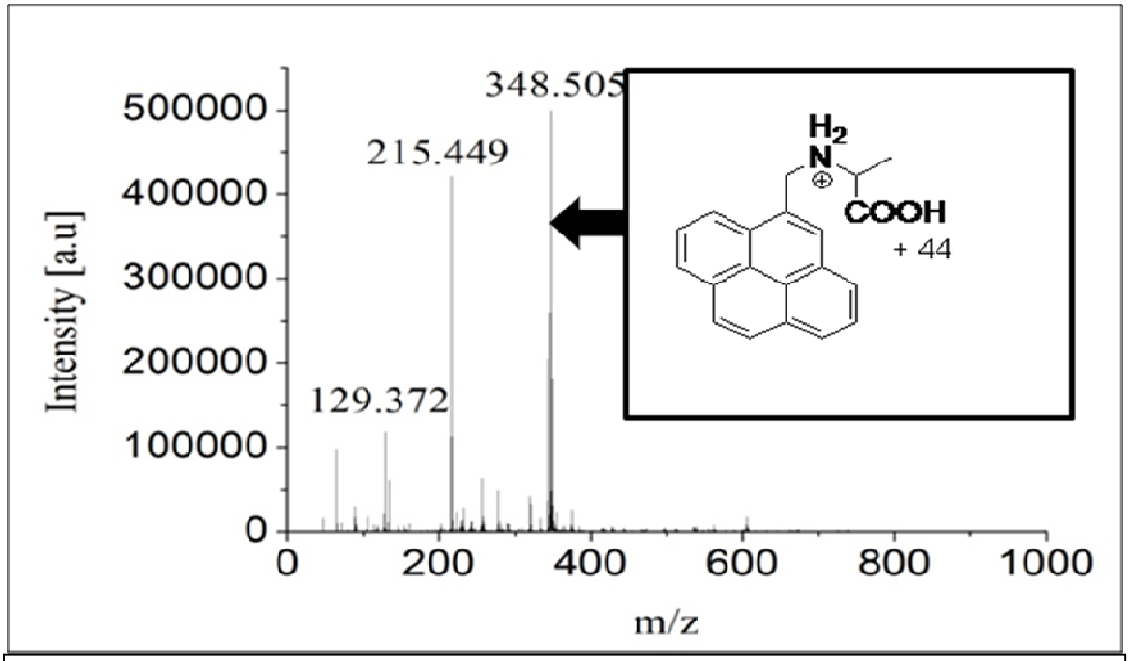

Fig 38: LA-LDI mass spectrum of amine complex of alanine (m/z= 348) in positive ion mode (reduced by $NaBH_4$)

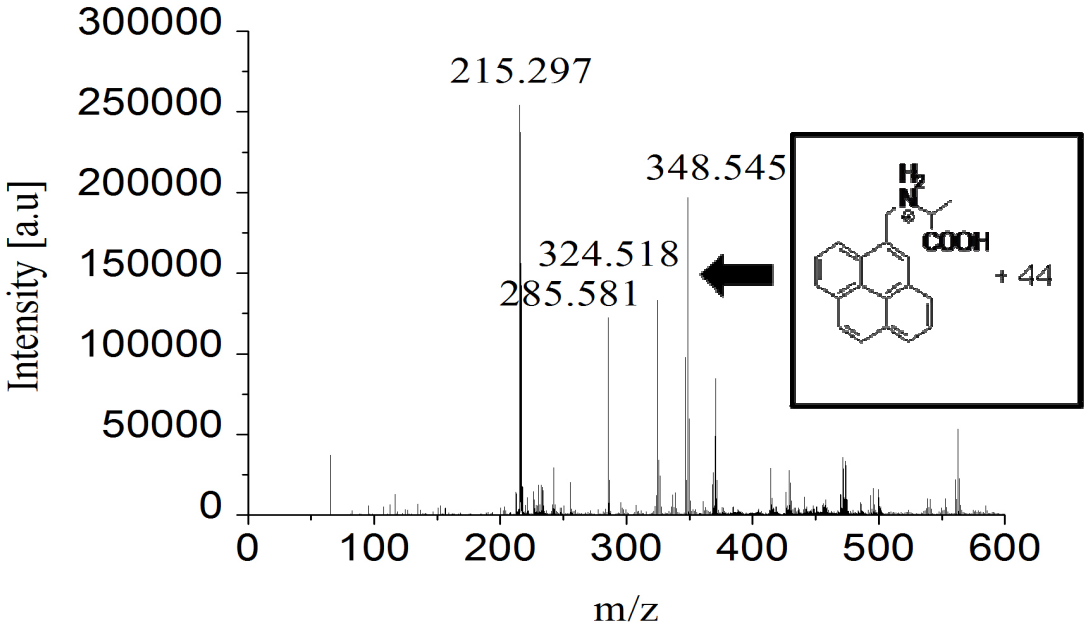

Fig 39: LA-LDI mass spectrum of amine complex of alanine (m/z= 348) in positive ion mode (reduced by $NaCNBH_3$)