RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Carbon dots as nanoantennas for anti-inflammatory drug analysis using surface-assisted laser desorption/ionization time-of-flight mass spectrometry in serum

Gangaraju Gedda[3], Sunil Pandey[1, 2], Mukesh Lavkush Bhaisare[1], Hui-Fen Wu*[1, 2, 3, 4, 5]

[1]Department of Chemistry, National Sun Yat-Sen University, Kaohsiung, 70, Lien-Hai Road, Kaohsiung, 80424, Taiwan

[2]Center for Nanosciences and Nanotechnology, National Sun Yat-Sen University, 70, Lien-Hai Road, Kaohsiung, 80424, Taiwan

[3]Doctoral Degree Program in Marine Biotechnology, National Sun Yat-Sen University, Kaohsiung, 80424, Taiwan

[4]School of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, 800, Taiwan

[5]Institute of Medical Science and Technology, National Sun Yat-Sen University, Kaohsiung, 80424, Taiwan

*Corresponding author, Phone: +886-7-5252000-3955; Fax: +886-7-5253908
E-mail: hwu@faculty.nsysu.edu.tw (Prof. HF Wu)

Abstract

Carbon dots (C-dots)-assisted laser ionization/desorption time-of-flight mass spectrometry (CALDI-TOF MS) is a relatively new approach for the detection of analytes. We have attempted to harness UV absorption capacity of C-dots (especially at 337 nm) as a matrix for the detection of a widespread use anti-inflammatory drug Mefenamic acid (MFA). Due to the surface modification, excellent water solubility and ultra-small size, C-dots can play a cardinal role in the detection of low molecular weight compounds. In comparison with 2,5-dihydroxy benzoic acid (DHB), C-dots were found to be an outstanding matrix to avoid background signals and fragmentation of the MFA signals. The C-dots is a perfect matrix for the detection of MFA in both positive and negative ion modes from the serum with the low detection limit of 0.51 ng and 0.46 ng, respectively.

Keywords: Carbon dots, Matrix-assisted laser desorption/ionization time-of- flight mass spectroscopy, Mefenamic acid, 2, 5-Dihydroxybenzoic acid.

1. Introduction

Mefenamic acid (MFA) is an important non-steroidal anti-inflammatory drug (NSAID) which is often prescribed for the treatment of osteoarthritis, rheumatoid arthritis and as painkillers in various diseases[1, 2]. On the other side, acute hepatic necrosis, morbidity, and mortality are caused by excessive doses of this drug [3, 4]. So, due to public health concerns the detection of this drug appears very important. Many analytical tools have been used to detect this drug due to its widespread use. An extensive literature regarding its determination in pharmaceutical formulations and biological fluids has been performed using titrimetric analysis, proton nuclear magnetic resonance (PNMR), atomic absorption spectrometry (AAS), spectrophotometry, liquid chromatography (LC), etc[3, 5-9]. Unfortunately, these methods can not directly analyze MFA from clinical samples or biological fluids and require complicated sample preparation process and separation methods. Therefore, it is essential to develop a new and rapid method for direct detecting this drug from clinical samples.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a rapid analytical tool for the analysis of biomolecules[10, 11]. In MALDI- TOF MS, the matrix plays key functions and acts as energy mediator for absorbing and transferring the laser energy to analytes for effective ionization and desorption[12]. α-Cyano-4-hydroxycinnamic acid (CHCA), sinapic acid (SA) and 2,5-dihydroxy benzoic acid (DHB) are commonly used matrices. However, MALDI-TOF MS is difficult to analyze small molecular weight compounds owing to generating large number of fragment or clustering signals from the organic matrices, which severely interfering the analyte peaks. Additionally, non-homogeneous co-crystallization of matrix with sample and sweet spot effect are major drawbacks leading to poor reproducibility. Nanoparticles-assisted laser desorption/ionization (NALDI-TOF MS) mass spectrometry has been intensively used[12]. Many nano materials have been used including CdSe, Pt, HgTe, ZnO, etc[13-16]. But most of these materials are extremely toxic and complicated synthesis process. Here we introduced Carbon dots (C-dots)-assisted laser ionization/desorption time-of-flight mass spectrometry (CALDI-TOF MS) to analyze low molecular weight of anti-inflammatory drug MFA.

C-dots are recently born of nanomaterials in the dynasty of carbon based materials at nanoscale[17, 18]. Due to their extraordinary attributes, C-dots received exceptional fame in biological applications such as drug delivery[19, 20], sensors[21-23], fluorescent probes[24, 25], and biological

imaging[22, 26-30]. C-dots have high quantum yield as well as exceptional bio-compatibility unlike semiconductor quantum dots (such as CdTe, CdSe and CdS) which exhibits extrême toxicity towards biological system[31]. Recently, carbon based materials such as graphene and graphene oxide and carbon nanotube have exhibited their capacity as an ideal matrix for the detection of vital analytes using MALDI-TOF-MS[32-35]. In our work, we have successfully used citric acid derived C-dots as the matrix for detection of anti-inflammatory drug MFA using MALDI-TOF MS. Several instances where the ability of the C-dots have been proved as an efficient matrix for the detection and quantification of biological analytes[36, 37]. In this work, we aim to detect low molecular weight of NSAID, MFA (MW 241) without any background interferences in the low mass range. This efficiency can be attributed to relative optical stability of the C-dots prepared at low temperature without using any corrosive reagents like acids. Sensitive detection and quantification of drug delivery MFA can offer tremendous advantages during quantification of drugs in the biological fluids in various pharmacological studies such as understanding the pharmaco-dynamics of drugs after administration in body. In addition, we also demonstrated the sensitive detection of NSAID drug from biological fluids with background free conditions using MALDI-TOF MS. The interferences of biological peaks have been diminished using our C-dots as a matrix. We also improved C-dots synthesis with simple and easy procedures.

Using citric acid derived C-dots; we explored the superiority of C-dots over the conventional organic matrix of DHB for detection limit of MFA. Furthermore, these C-dots work as an ideal matrix for the detection of the analytes in both positive and negative modes. This makes C-dots as a vital alternative to the conventional organic matrices for the detection of low molecular weight compounds. Additionally, we detected MFA in human serum at a concentration of 0.51 ng (In the positive ion mode) and 0.46 ng (In the negative ion mode).

2. Material and Methods

Acetonitrile (ACN), anhydrous Citric acid, and chloroform were purchased from J. T. Baker, USA. 2, 5-dihydroxybenzoic acid (DHB) and trifluoroacetic acid (TFA) were procured from Acros Organics and Sigma-Aldrich (St. Louis, MO, USA) respectively. Mefenamic acid was obtained from Sigma (St. Louis, MO, USA). All the experiments were performed using ultra-

pure deionized water (18MΩ Milli-Q water purification system, Millipore Inc., Bedford, MA, USA).

2.1 Synthesis of C-dots

Citric acid (2 g) was dissolved in 10 mL ultra-pure pure water and stirred at room temperature to make a homogeneous solution. This solution was refluxed for 12 hrs at 60 ºC till the solution turned yellowish red. In order to purify the C-dots solution, 10 mL of the same was subjected to micron-filtration using 0.45µm nylon filter. Bright yellowish green solution was obtained after the filtration that was considered for the experiment. After preliminary confirmation under UV light (λ=365 nm) to check the bright blue fluorescence, the solution was scrutinized by UV-Vis Spectroscopy (Perkin Elmer Lambda 25, USA) to comprehend its optical properties. Same solution of C-dots was studied using fluorescence spectroscopy (Hitachi, F-2700, Japan) at various (300 nm – 480 nm) excitations.

2.2 Characterization

TEM images of C-dots were obtained by using transmission electron microscope (TEM-3010, JEOL, Tokyo, Japan). The UV-visible spectra of C-dots were recorded by using double beam UV–visible spectrophotometer (Hitachi U-3501, Tokyo, Japan). The fluorescence emission spectra of C-dots were recorded by using fluorescence spectrophotometer (Hitachi, F-2700). The Raman spectra of C-dots were recorded by using Raman Spectrometry (Perkin-Elmer, California, USA). X-ray diffraction (XRD) patterns were obtained on XRD spectroscopy (Bruker D8 advance, Germany). FTIR Spectra were recorded with Fourier transform infrared spectrometer (Bruker FT-IR IFS-48, Germany).

2.3 MALDI-TOF Mass Spectrometry

The positive and negative ion mass spectra of MFA were recorded in reflector-ion mode by using MALDI-TOF-MS Microflex (Bruker Daltonics, Bremen, Germany). The accelerating voltage was set at 20 kV. The mass spectrometer was equipped with a pulsed nitrogen laser operated at 337 nm with 4 ns duration pulses at 10.0 Hz and the laser influence was fixed at 51.25 mJ. Mass spectra were obtained as an average of 200 laser shots for analysis.

2.4 Sample preparation and MALDI-TOF MS analysis

A stock standard solution of MFA (1 mM) was prepared by dissolving 2.41 mg of substance in 10 mL of chloroform. Aliquot solutions were prepared by the dilution of stock standard solution with deionized water. The matrix solution (DHB, 50 mM) was prepared in acetonitrile and water (7:3, v/v) containing 0.1% TFA.

For MALDI- TOF MS analysis, analytes and water soluble C-dots/DHB were mixed at a ratio of 2:1(v/v). 1.0 µL of this mixture was spotted onto a stainless steel target plate and allowed to air dry at ambient temperature for further mass analysis.

2.5 Quantification of Mefenamic acid in serum by C-dots assisted LDI-TOF MS.

The blood samples were collected from drug free healthy volunteers. The collected blood samples were kept in room temperature to blood clot after coagulation the precipitate material was removed by centrifuged at 3000 rpm for 10 minutes. The obtained supernatant (serum) was separated and deprotonation by acetonitrile.

To quantify the MFA in serum, initially 50 µg/µL of MFA was directly dissolved in acetonitrile and it is considered as a stock solution. Working standards were prepared and stock solutions were spiked directly into serum to give final concentration of 10 ng/µL, 25 ng/µL, 50 ng/µL, 75 ng/µL and 100 ng/µL.

3. Results and Discussion

3.1 Synthesis and Characterization

Citric acid is one of the most widely used sources for fabrication of highly fluorescent C-dots [38, 39] due to its perfect stoichiometric ratio of C, H, and O (1:2:1). To scrutinize the optical properties of C-dots, we performed UV-Vis studies as shown in Fig. 1A. The sharp peak at 249 nm was observed followed by a fat peak around at 337 nm due to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi$ transition of electrons associated with aromatic groups of C-dots[40, 41]. For C-dots to be an efficient matrix, it must absorb the nitrogen laser (λ= 337 nm) which is used in MALDI-TOF MS to ionize the molecules to be detected. An absorption peak (Fig.1A) between 320 to 360 nm and a maximum around 337 nm makes C-dots qualify the primary prerequisite to be the MALDI matrix.

The fluorescence spectra of C-dots at different excitation values (300 nm to 480 nm with an increase of 20 nm) show excitation dependent emission spectra as shown in Fig.1B. Interestingly, the prepared C-dots showed up conversion from 450 nm to 475 nm (at 300 to 380 nm excitation) as well as down conversion from 475 to 560 nm (at 400 nm- 480 nm excitation). The maximum emission (460 nm) was obtained with the excitation wavelength of 380 nm excitations. Raman spectrum of C-dots (Fig.2A) exhibits two broad peaks of G band (sp^3 hybridization) and D (sp^2 hybridization) bands observed at 1540 and 1340 cm^{-1} respectively. Fig. 2B explains the XRD pattern of C-dots. It exhibits a broad peak at 20° corresponding to plane (002) and the interlayer spacing 4.2 Å, has been confirmed as amorphorous nature.[30, 42, 43] . The explained graphitic nature of the C-dots with an inner layer spacing of ~4.12 Å is in strong agreement with our findings. Fig. 2C displays the high resolution TEM image of C- dots, which showed that the average particle size range is 3-5 nm. FTIR spectra (Fig.2D) shows the functional groups associated with the surface of C-dots. The strong absorption band at 3394 cm^{-1} is due to O-H stretching. Absorption bands at 3072 cm^{-1} and 2980 cm^{-1} belong to sp^2 and sp^3 C-H stretching vibrations. Bands at 1743 and 1615cm^{-1} were attributed to carboxyl groups and aromatic C=C vibrations, many weak bands were observed in the range of 1024 cm^{-1} and 1153 cm^{-1} corresponding to C-O-C stretching vibrations and C-OH stretching mode, respectively. The bands in the range of 1000–1300 cm^{-1} belong to C–OH stretching and –OH bending vibrations. Presence of such functional groups explains the functionalization of C-dots containing –COOH and –OH these functional groups improve the hydrophilicity and stability of the C-dots in aqueous systems.

3.2. Mefenamic acid analysis by C-dots assisted.

C-dots possess excellent features to serve as a matrix for detection of low molecular weight compounds such as MFA in MALDI-TOF MS. For the ideal applicability of C-dots as an MALDI matrix, Initially, we analyzed MFA using conventional organic matrix DHB (Fig. S1). Spectrum displayed in Fig. S1A and Fig. S1B corresponding to DHB matrix as blank and MFA with DHB. Fig. S1A exhibits background signals like *m/z* 127.6, 139.8, 196, 226.9, **241.9**, 323.7, 339.1, 377, 468.2, 496.4 and 533.7. As similarly observed in Fig. S1B except *m/z* 242.9 $[M+H]^+$ correspond to MFA. It's quite difficult to find out real difference in signal between two spectra's

due to peak observed very close to the control spectra. The strong backgrounds may be originated from the ionization of matrix ions within the selected mass range. Many undesirable signals from DHB which are interferences for the MFA analysis. So DHB it unsuitable for the analysis MFA. Therefore, we proposed using C-dot as a matrix for MFA detection by MALDI-TOF MS. Furthermore, since most of the application in MALDI-TOF MS are restricted to positive ion mode. It may be due to most of the conventional matrices contain carboxylic acidic groups. These acidic groups help to transfer proton to analytes. However, there are few studies on negative ion mode. In this study, we also demonstrate the successful detection of MFA in positive and in negative ion mode by C-dots. This advantage makes the current approach (C-dots method) to be suitable for future biological study.

Fig. 3 display the detection of MFA (1 μg) using C-dots assisted LDI-TOF MS. In contrast to use DHB as a matrix, C-dots exhibited no background in both positive and negative mode signals as evident from Fig. 3(A) and Fig. 3(B). This may be due to surface orchestration of hydroxyl and carboxylic acids that C-dots become highly soluble in water and this may be a significant feature in situ energy transfer to MFA. A prominent signal appears at *m/z* 242 $[M+H]^+$ and 240.4 $[M-H]^-$ in positive and negative mode respectively, it was displayed in Fig. 3(C) and Fig.3(D). The sharpness of the peak (and hence the least full width half maxima (FWHM=1.2)) signify the resolution of the peak. Additional fragmentation of the peak is avoided due to high stability and low sublimation of C-dots under vacuum. The main advantage of these C-dots in MALDI-TOF MS is it can be used as a matrix in both positive and negative mode due to acidic and basic functional groups on the surfaces of the C-dots.

To evaluate the potential of C-dots as the matrix, we compared the detection of the MFA by laser desorption-ionization mass spectrometry (LDI-TOF MS) without using any matrix. C-dots assisted LDI-TOF MS and their results were shown in Fig.S2&Fig.S3. These results indicating that LDI-TOF mass spectrum of MFA generated some background noises with lower intensities (Fig.S2A and Fig.S3A). On the other hand, MFA detection with C-dot as the matrix could reduce background noises and significantly enhanced signal intensities (Fig.S3B and Fig.S3B).

3.3. Quantitative determination of MFA in serum

MFA detection in serum requires cumbersome separation procedures, which can be skipped by our method using C-dots as the ideal matrix. The detection of MFA in biological fluids, the serum was pretreated with acetonitrile to remove proteins with large molecular weight. Fig.4 explains the MALDI-TOF MS spectra of MFA (1µg) in human serum by C-dots assisted LDI-MS. From the background spectrum of human serum, it doesn't show any signal in both positive and negative mode (Fig. 4A) and (Fig. 4B) at low molecular range $m/z \geq 500$ Da. Besides, MFA spiked in human serum generated a strong signal at m/z 242.5$[M+H]^+$ in the positive ion mode (Fig. 4C) and at m/z 240.4 $[M-H]^-$ in the negative ion mode (Fig. 4D).

Furthermore, we demonstrate, the reproducibility for MFA detection using C-dots explained in FigS4 and FigS5. To check the reproducibility, four different samples with equal concentration of MFA(1µg) prepared from stock solution has been spiked in serum and analyzed by MALDI-TOF MS. The observed spectra indicate that the C-dots assisted LDI-TOF MS showing excellent reproducibility. . Additionally, we performed the experiments with varying concentrations of MFA(Fig.5 and Fig.6). These results indicating that by reducing concentration, the signal intensity was also decreased. Its means that the signal intensity depends on the concentration of analyte. Thus the C-dots is suitable for quantitation of MFA and other NSAID drugs of same category. In conventional MALDI-TOFMS, quantitative analysis is still challenging due to unequal distribution of analyte / matrix complex on target plate and hence affecting on the reproducibility of analyte mass signals. Therefore, we further perform the quantitative analysis of MFA in serum by MALDI-TOF MS using C-dots as the matrix.

Quantitative calibration curves (Fig. S2) were established in the range of 10-100 ng/µL. Fig. S2A and Fig. S2B, showed calibration curves for $[MFA+H]^+$ and $[MFA-H]^-$ with the regression equation y=6.524x+33.048 and y=6.4698x+35.1881,respectively. These showed linear regression (R^2= 0.9967) in the positive mode and R^2 = 0.9977 in the negative mode. The calibration curve has been used to find out the limit of detection (**LOD=*3Sa/b***). *Sa* is the standard deviation of the regression line and *b* slope of the calibration curve[44]. From the Fig. S2A and Fig. S2B calculated LOD in positive and negative modes which are 0.51 ng and 0.46 ng, respectively. The proposed method exhibits low detection limit for MFA in serum. These results

suggested that C-dots are an excellent alternative for rapid quantitative analysis of MFA in MALDI-TOF MS.

4. Conclusions

Owing to their exceptional physicochemical properties, C-dots is an ideal matrix for the detection of small molecules in biological fluids in the MALDI-TOF MS. MFA; a potential non-steroidal anti-inflammatory drug can be efficiently and sensitively detected using C-dots with both positive and negative ion modes with LODs of 0.51 ng and 0.46 ng, respectively. In comparison to the conventional matrix such as 2,5-dihydroxybenzoic acid (DHB), C-dots possess extremely high stability to analyze for MFA. MFA could be detected in human serum even with trace amount by employing C-dots as the matrix.

Acknowledgments

Authors acknowledge Ministry of science and technology of Taiwan for financial support to fulfill this project. We also thank Mr. Bo-sgum Wu (Department of Chemistry, National Sun-Yat Sen University) for the purchases of chemicals and materials.

References

1. J. Bonnar and B. L. Sheppard, *BMJ*, 1996, **313**, 579-582.
2. F. A. Aly, S. A. Al-Tamimi and A. A. Alwarthan, *Anal.Chim.Acta.,* , 2000, **416**, 87-96.
3. A. Santini, H. Pezza and L. Pezza, *Sens.Actuators B.Chem.,* 2007, **128**, 117-123.
4. A. Beiraghi, K. Pourghazi, M. Amoli-Diva and A. Razmara, *J. Chromatogr.B* 2014, **945**, 46-52.
5. I. Niopas and K. Mamzoridi, *J.Chromatogr.B.,*1994, **656**, 447-450.
6. O. Çakırer, E. Kılıç, O. Atakol and A. Kenar, *J.Pharm.Biomed.Anal.,*, 1999, **20**, 19-26.
7. E. Dinç, C. Yücesoy and F. Onur, *J. Pharm. Biomed. Anal.,* 2002, **28**, 1091-1100.
8. R. E. Williams, L. Cottrell, M. Jacobsen, L. R. Bandara, M. D. Kelly, S. Kennedy and E. A. Lock, *Biomarkers.*, 2003, **8**, 472-490.
9. S. Jawla and S. Jain, *Int.J.Pharm.Sci.Drug Res.,* 2010, **2**, 45-47.

10. S. L. Cohen and B. T. Chait, | *Anal.Chem.,*, 1996, **68**, 31-37.
11. S.-f. Ren, L. Zhang, Z.-h. Cheng and Y.-l. Guo, *J.Am Sot.Mass.Spectrom.,*, 2005, **16**, 333-339.
12. C.-K. Chiang, W.-T. Chen and H.-T. Chang, *Chem.Soc.Rev.,*, 2011, **40**, 1269-1281.
13. H. Kawasaki, T. Yonezawa, T. Watanabe and R. Arakawa, |*J. Phys.Chem.C*.,, 2007, **111**, 16278-16283.
14. T. Watanabe, H. Kawasaki, T. Yonezawa and R. Arakawa, *J. Mass Spectrom.,* 2008, **43**, 1063-1071.
15. K. Shrivas, S. K. Kailasa and H. F. Wu, *Proteomics.*, 2009, **9**, 2656-2667.
16. C.-K. Chiang, Z. Yang, Y.-W. Lin, W.-T. Chen, H.-J. Lin and H.-T. Chang, *Anal. Chem.,*2010, **82**, 4543-4550.
17. A. P. Demchenko and M. O. Dekaliuk, *Methods.Appl.Fluoresc.,*, 2013, **1**, 042001.
18. C. Ding, A. Zhu and Y. Tian, *Acc. Chem. Res.,*, 2013, **47**, 20-30.
19. S. Pandey, M. Thakur, A. Mewada, D. Anjarlekar, N. Mishra and M. Sharon, *J. Mater. Chem. B.,*2013, **1**, 4972-4982.
20. Q. Wang, X. Huang, Y. Long, X. Wang, H. Zhang, R. Zhu, L. Liang, P. Teng and H. Zheng, *Carbon.*, 2013, **59**, 192-199.
21. H.M.Gonçalves,A.J.DuarteandJ.C.EstevesdaSilva,*Biosens. Bioelectron.,*, 2010, **26**, 1302-1306.
22. A. Zhu, Q. Qu, X. Shao, B. Kong and Y. Tian, *Angew.Chem., 2*2012, **124**, 7297-7301.
23. Q. Niu, K. Gao, Z. Lin and W. Wu, *Anal. Methods.,* 2013, **5**, 6228-6233.
24. H. X. Zhao, L. Q. Liu, Z. De Liu, Y. Wang, X. J. Zhao and C. Z. Huang, *Chem. Commun.*, 2011, **47**, 2604-2606.
25. Y. Dong, R. Wang, G. Li, C. Chen, Y. Chi and G. Chen, *Anal.Chem.,* 2012, **84**, 6220-6224.
26. L. Cao, X. Wang, M. J. Meziani, F. Lu, H. Wang, P. G. Luo, Y. Lin, B. A. Harruff, L. M. Veca and D. Murray, *J. Am.Chem.Soc.,* 2007, **129**, 11318-11319.
27. S. Ray, A. Saha, N. R. Jana and R. Sarkar, *J.Phys.Chem.C.,* 2009, **113**, 18546-18551.
28. S.-T. Yang, L. Cao, P. G. Luo, F. Lu, X. Wang, H. Wang, M. J. Meziani, Y. Liu, G. Qi and Y.-P. Sun, *, J.Am.Chem.Soc.,* 2009, **131**, 11308-11309.

29. S.-T. Yang, X. Wang, H. Wang, F. Lu, P. G. Luo, L. Cao, M. J. Meziani, J.-H. Liu, Y. Liu and M. Chen, *J. Phys.Chem.C.,* 2009, **113**, 18110-18114.
30. A. Sachdev, I. Matai, S. U. Kumar, B. Bhushan, P. Dubey and P. Gopinath, *RSC Adv.,*, 2013, **3**, 16958-16961.
31. A. Sachdev, I. Matai and P. Gopinath, *RSC Adv.,* 2014, **4**, 20915-20921.
32. S. f. Ren and Y. l. Guo, *, Rapid Commun.Mass Spectrom.,* 2005, **19**, 255-260.
33. X. Dong, J. Cheng, J. Li and Y. Wang, *Anal. Chem.,* 2010, **82**, 6208-6214.
34. Y. Liu, J. Liu, P. Yin, M. Gao, C. Deng and X. Zhang, *J. Mass Spectrom.,*2011, **46**, 804-815.
35. H. Bai, Y.Pan, W.Tong, W.Zhang, X. Ren, F. Tian, B. Peng, X. Wang, Y. Zhang and Y. Deng, *Talanta.*, 2013, **117**, 1-7.
36. P.C. Hsu, Z.Y. Shih, C.-H. Lee and H.T. Chang, *Green Chem.,*, 2012, **14**, 917-920.
37. S. Chen, H. Zheng, J. Wang, J. Hou, Q. He, H. Liu, C. Xiong, X. Kong and Z. Nie, *Anal. Chem.,* 2013, **85**, 6646-6652.
38. F. Wang, S. Pang, L. Wang, Q. Li, M. Kreiter and C.-y. Liu, *Chem.Mater.,* 2010, **22**, 4528-4530.
39. F. Wang, Z. Xie, H. Zhang, C. y. Liu and Y. g. Zhang, *Adv.Funct.Mater.,*2011, **21**, 1027-1031.
40. A. Jaiswal, S. S. Ghosh and A. Chattopadhyay, *Chem.Commun.*, 2012, **48**, 407-409.
41. X. Zhai, P. Zhang, C. Liu, T. Bai, W. Li, L. Dai and W. Liu, *Chem. Commun.*, 2012, **48**, 7955-7957.
42. J. Zhang, W. Shen, D. Pan, Z. Zhang, Y. Fang and M. Wu, *New J. Chem.,* 2010, **34**, 591-593.
43. A. Mewada, S. Pandey, S. Shinde, N. Mishra, G. Oza, M. Thakur, M. Sharon and M. Sharon, *Mater. Sci. Eng. C.,*2013, **33**, 2914-2917.
44. A. Shrivastava and V. B. Gupta, *Chron.Young.Sci.,*, 2011, **2**, 21.

Figure captions

Fig. 1 UV absorption spectra of (A) C-dots, and PL emission spectra(B) showed upward and downward conversion exhibited by excitation from 300 nm to 480 nm

Fig. 2 Raman spectrum of (A) C-dots, XRD Spectrum (B), TEM image with size distribution histogram in inset (C), FT-IR spectrum (D).

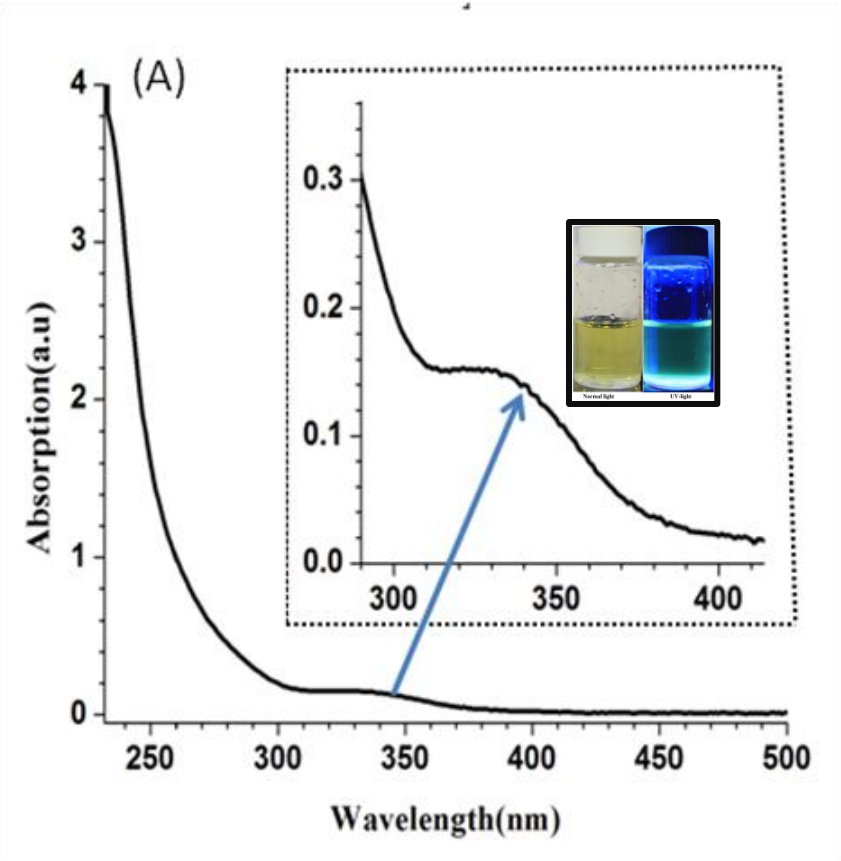
Fig. 3 MALDI-MS spectra of MFA using C-dots as the matrix detected in positive mode and negative mode,(A) and (B) C-dots control as matrix (C) and (D) exhibits detection of MFA using C-dots as matrix in positive and negative mode..

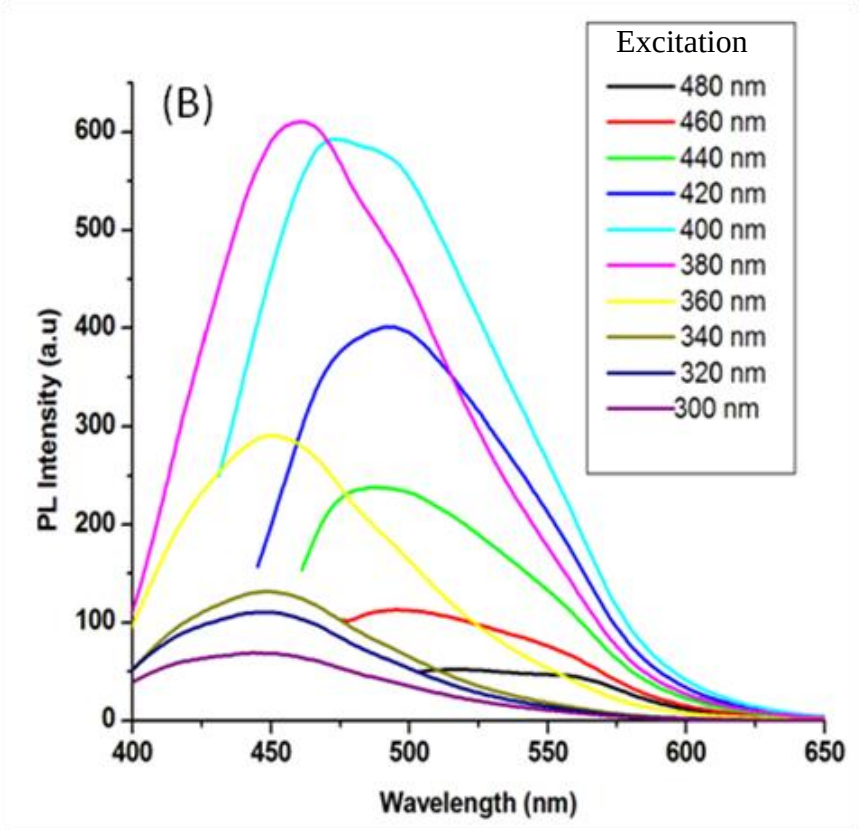
Fig. 4 MALDI-MS spectra shows detection of MFA in serum with C-dots as matrix in positive and negative mode, (A) and (B) shows serum alone in positive and negative mode. (C) and (D) explain detection of MFA with C-dots as the matrix in positive and negative mode. .

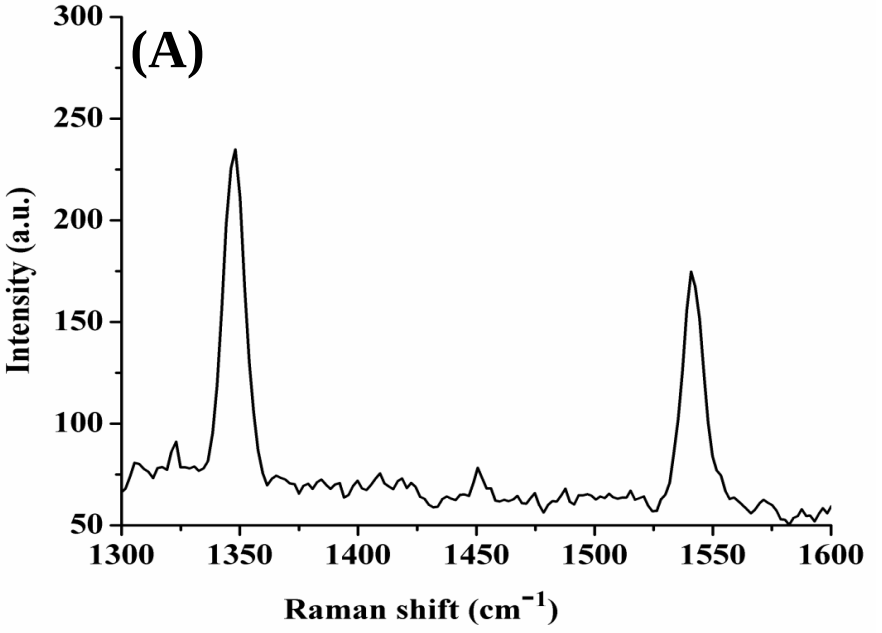
Fig. 5 MALDI-MS spectra of different concentration of MFA in serum in positive mode with C-dots as matrix.

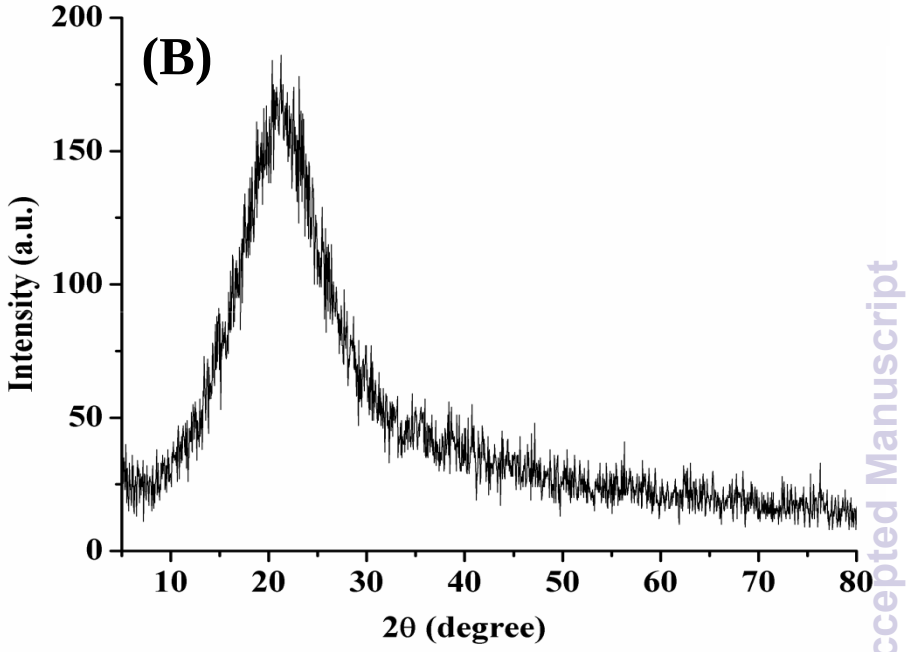
Fig. 6 MALDI-MS spectra for different concentration of MFA in serum in negative mode with C-dots as matrix.

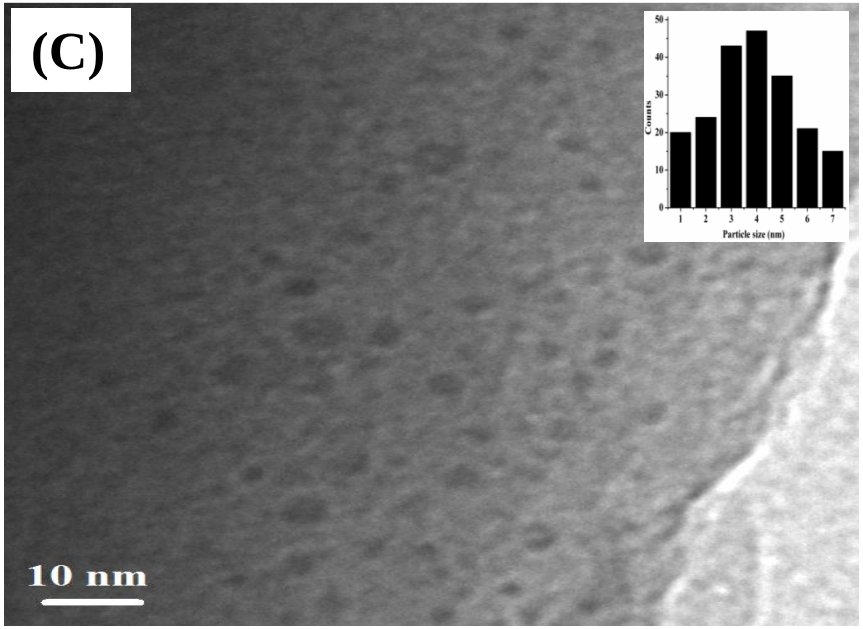
Fig. 1











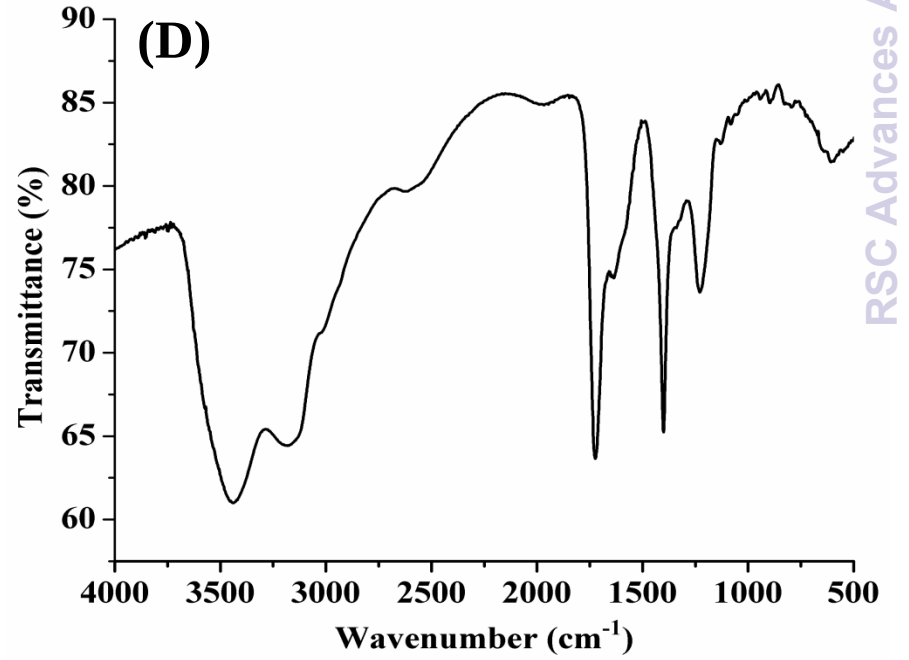
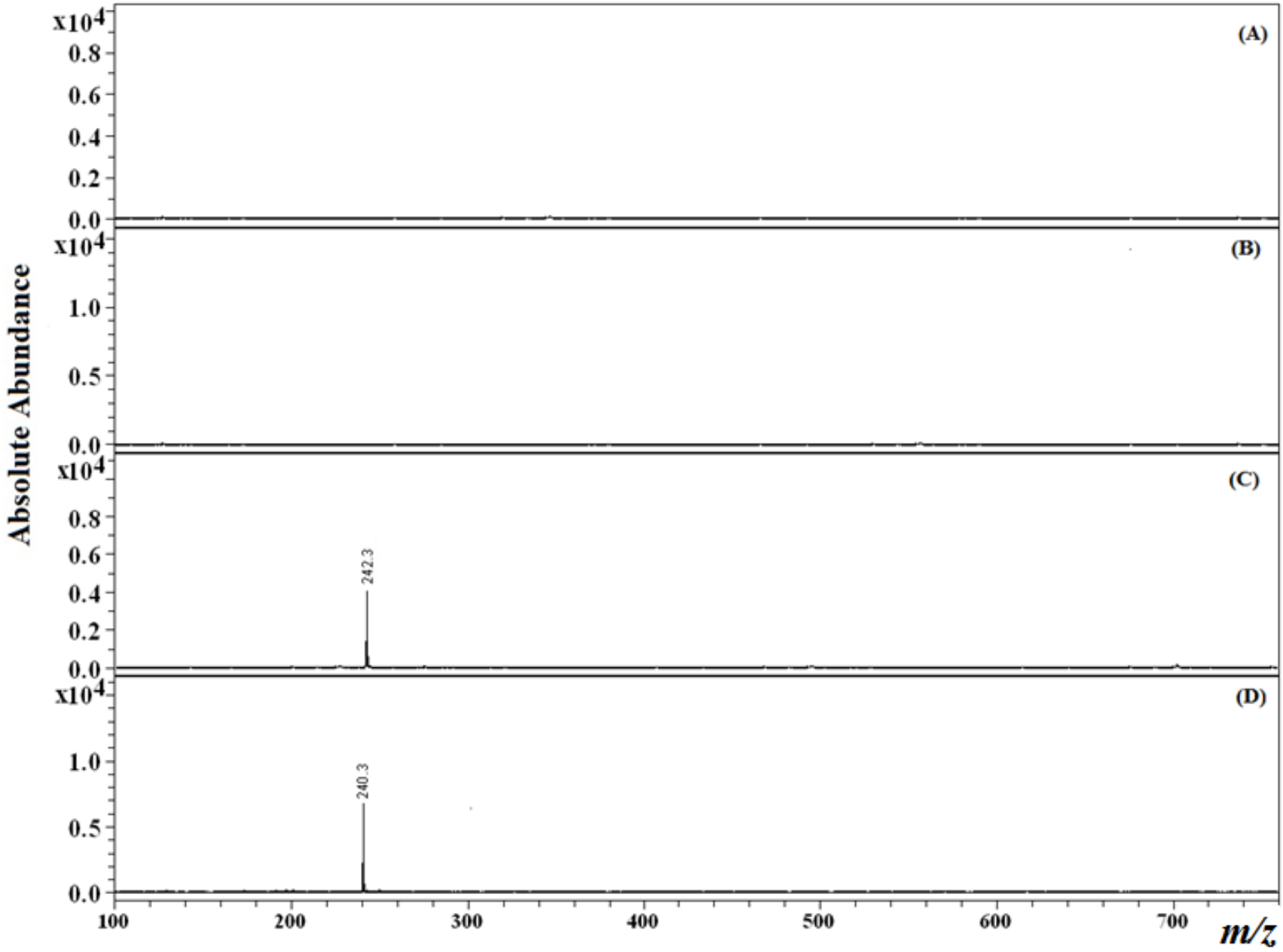


Fig. 3



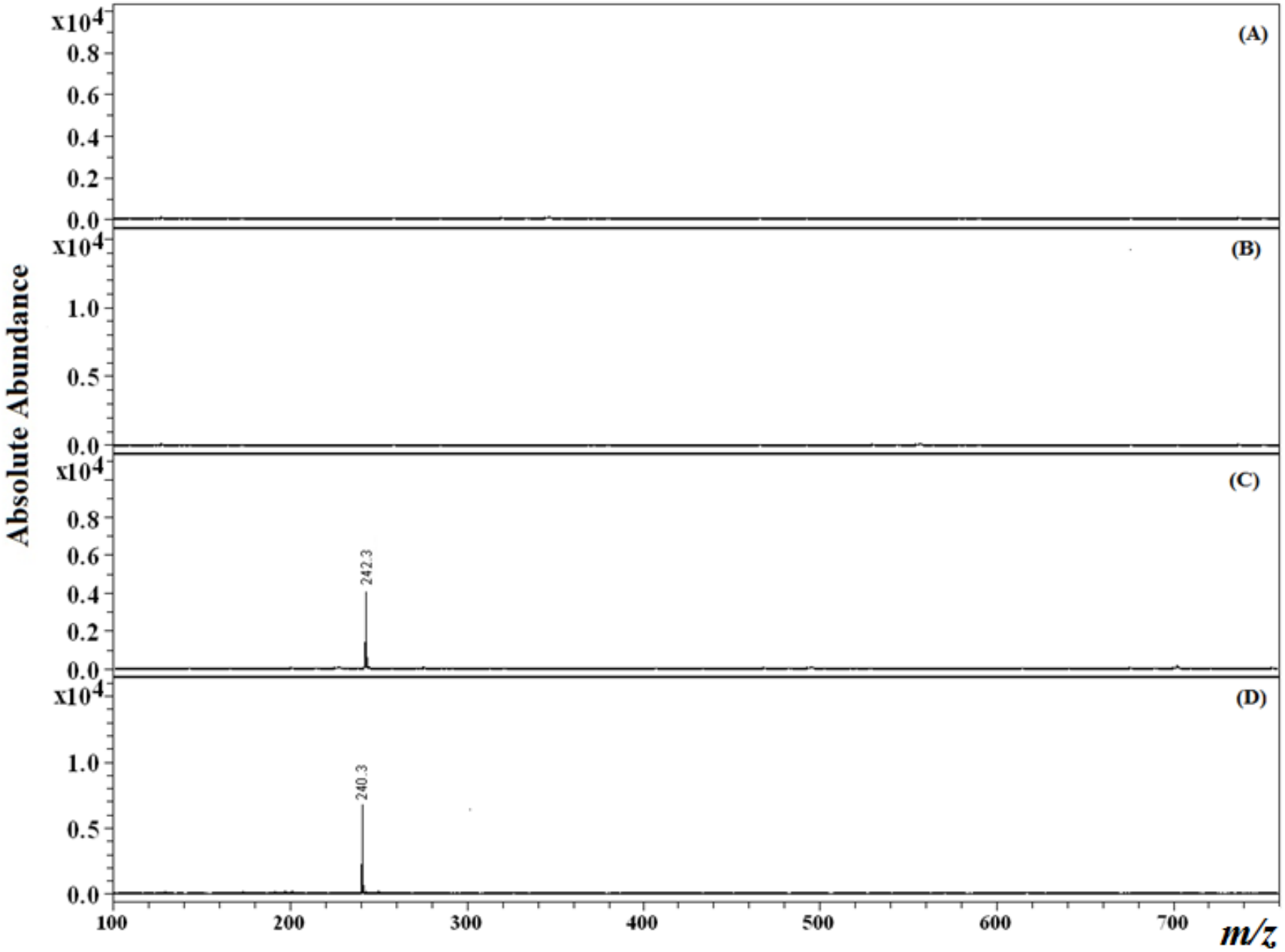


Fig. 5