Vol. 6, No. 2, 2015
ISSN 2233-4203/ e-ISSN 2093-8950
LETTER www.msletters.org | Mass Spectrometry Letters

Soft Ionization of Metallo-Mefenamic Using Electrospray Ionization Mass Spectrometry

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Received March 15, 2015; Revised May 15, 2015; Accepted May 26, 2015
First published on the web June 30, 2015; DOI: 10.5478/MSL.2015.6.2.43

Abstract: Detection of mefenamic acid (M, non-steroidal anti-inflammatory drug, NSAIDs) and its metallodrug was investigated using electrospray ionization mass spectrometry (ESI-MS) and fluorescence spectroscopy. ESI-MS data (500 μL, 1×10-3 M) revealed high detection sensitivity for the drug and metallodrug. ESI-MS spectra revealed peaks at 242, 580, and 777 Da corresponding to [M+H]+, [63Cu(M-H)2(H2O)2+H]+, and [56Fe(M-H)3+H]+, respectively. The metal:mefenamic ratios of ESI-MS spectra are in complete agreement with the fluorescence spectroscopy results (1:2 for Cu(II) and 1:3 for Fe(III)). ESI is a soft ionization technique that can be used on labile metallo-mefenamic acids and is promising for the detection of these species in environmental samples and biological fluids.

Keywords: Mefenamic Acid, Metallodrug, ESI-MS, Fluorescence spectroscopy

Introduction

Interactions of metals and biomolecules such as proteins, drugs, cells and others are very important for separation, as biological agents, etc.[1-7] Probing metallodrugs (interactions between metals and drugs) is critical to address biological concerns due to the toxicity of the new species.[8] Among the different techniques available, electrospray ionization mass spectrometry (ESI-MS) is a soft ionization technique that can be used to study metallodrugs and their interactions with biomolecules.[9-13] ESI-MS requires a small volume of the analyte, low concentration, and is tolerated by contaminants such as buffers and salts.[14-16] Furthermore, it is often used for protic and aprotic solvents.[17] The application of ESI-MS for the analysis of transition metal complexes was reviewed in Ref..[18] ESI-MS can also be used for metallodrug-protein interactions[19] and for labile drugs.[20] ESI-MS has been employed to determine the binding or dissociation constants of zinc finger peptides (ZFPs),[21] to characterize a Ru(III) anticancer drug,[22] for Protein metalation processes,[23] and also to characterize antiproliferative activity[24] and cytotoxic metallodrugs.[25] A review of the applications of hyphenated techniques for metal-based pharmaceuticals has been published.

Mefenamic acid (MFA) is a non-steroidal anti-inflammatory drug (NSAID) used to treat pain, including menstrual pain. It was marketed as Ponstel and Postan in the USA and UK, respectively. Kidney and liver deficiencies may cause accumulation of the drug and its metabolites in the excretory system. It is also important to note that overdoses of MFA produce metabolite accumulation that causes acute hepatic necrosis, inducing morbidity and mortality in humans. Therefore, detection of this drug is valuable due to environmental, medical and forensic science concerns. Thus, detection of the drug has been investigated using various techniques, such as high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC–MS), spectroph-otometric methods, and capillary electrochromatography.[27-36] A new analytical technique is required with a better limit of detection (LOD), good performance and high speed.

The detection of mefenamic acid and its metallodrugs using electrospray ionization as a simple and effective analytical tool is described for the first time in this report.

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The coordination chemistry for mefenamic acid with the transition metals "Cu(II) and Fe(III)" is also reported. The metallodrugs were confirmed using fluorescence spectroscopy. The molar ratios determined using electrospray ionization mass spectrometry (ESI-MS) and fluorescence spectroscopy are in good agreement.

Experimental

Chemicals. $CuSO_4.5H_2O$ and ponstel drug (used as pure Mefenamic acid without any additives) were purchased from Sigma Company (China). $FeCl_3.6H_2O$ was purchased from Riedel-de Haën (Seelze, Germany). Methanol (HPLC grade) was purchased from Merck (USA). All the chemicals were used directly without further purification. De-ionized water (18 M purified Millipore water, USA) was used to prepare all of the solutions.

ESI(+)-MS measurements were performed using Finnigan MAT ion trap mass spectrometer (Finnigan LCQ-Advantages, San Jose, CA, USA). A microsyringe pump (Harvard Apparatus, Edenbridge, Great Britain) was used to inject the sample solution. All mass spectra were obtained in positive ion mode. The ion trap analyzer was operated at a pressure of ~1.5×10^{-5} Torr, and capillary temperature was 200°C. ESI spray needle voltage was fixed at 4.50 kV. A tube lens offset voltage of 80.0 kV and capillary voltage of 9.59 kV were used to obtain ESI-MS spectra. Sheath gas flow rate (arb.) during the experiments was 18.90. Each mass spectrum was the average of 3 individual scans. All mass spectra were recorded on freshly prepared solutions in the mass range of 0-1000 Da.

The fluorescence spectra were obtained from a fluorescence spectrophotometer (F-2700 Hitachi Co., Japan) equipped with a xenon arc lamp (150 W). The scan speed was set to 120 nm min-1 and 5 nm spectral slit widths were used for excitation and emission in 5 nm step sizes. Emission spectra were recorded using 360 nm excitation. All spectra were visualized using Origin V6.0.

The molar ratio method was applied to determine the stoichiometry of the complex in solution using fluorescence spectroscopy. A stock solution of the drug (1.0×10^{-3} M) was prepared in 50% (v/v) methanol. Metal solutions of Cu(II) and Fe(III) (1.0×10^{-3} M) were prepared in deionized water. The metal concentrations were kept constant while different molar ratios of the drug (1:0.5, 1:1,1:1.5,1:4) were added to the metal solution at pH 7.4 (phosphate buffer solution, PBS) and incubated 10 min before measurements. Fluorescence spectroscopy was measured at λ_{ex} = 360 nm.

ESI-MS measurements. Mefenamic acid (1.0×10^{-3} M) was mixed with the appropriate molar ratio of metal solutions (1.0×10^{-3} M) with 1.0 mL of ammonium acetate buffer solution (pH = 7.4). The stock solution was incubated for 10 min before mass analysis. All spectra were collected at least three times to confirm repeatability.

Results and Discussion

A few analytical tools have been proven to be capable of drug and metallodrug detections. Among these techniques, electrospray ionization mass spectrometry (ESI-MS) is simple, sensitive and can be employed for qualitative and quantitative analysis. The main reason is that the bonds between anticancer metallodrugs and model proteins are labile and easy to destroy. Therefore, ESI-MS can be used for the molecular characterization of these adducts, as described by Messori and co-workers.[37] Mefenamic acid (M) has a nominal mass of 241.2Da. The ESI(+)-MS spectrum (Figure 1) peaks at *m/z* 242.1 correspond to the protonated drug i.e $[M+H]^+$. The inset in Figure 1 represents the peak assignments of the drug related ions. ESI-MS (Figure 1) showed a dehydration peak for the drug at *m/z* 224.9, corresponding to $[M-H_2O+H]^+$. The peak at *m/z* 483.0 corresponds to the drug dimer, i.e $[2M+H]^+$. ESI-MS did not affect the non-covalent bond in the dimer peak (*m/z* 465, $[2M-H_2O+H]^+$). Detection of the dimer species indicates that ESI-MS is a soft ionization technique for non-covalent interactions. The peak assignments and limit of detection are listed in Table 1. The data reveals that ESI-MS is highly sensitive over other techniques as shown in Table 2. The data also shows that mefenamic acid can be detected using fluorescence and mass spectrometry. It is well known that the latter has higher sensitivity compared to other methods.

Mefenamic acid is an N-anthranilic acid derivative with carboxylic and amine groups as the main function groups. Fluorescence emission of Cu(II)-mefenamic acid (Figure 2A) and Fe(III)-mefenamic acid (Figure 2B) can be used to probe the metallodrug formation in the liquid phase. Fluorescence spectra (Figure 2(A-B)) of mefenamic acid display emission were obtained at 475 nm. The emission was shifted to a lower wavelength upon complexation with metals such as Cu(II) (Figure 2A) and Fe(III) (Figure 2B).

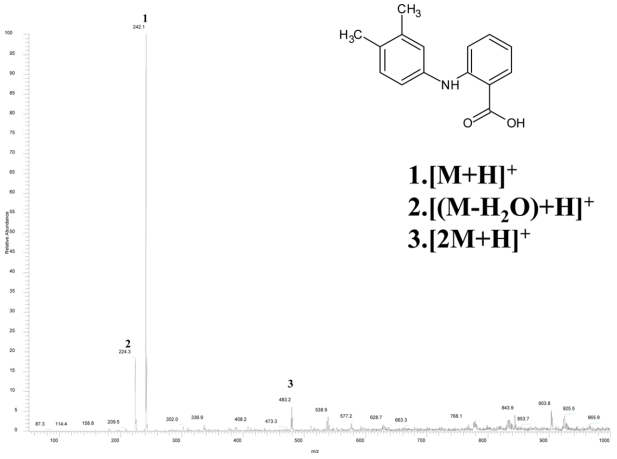

Figure 1. ESI-MS spectrum for mefenamic acid.

The molar ratio of the metal complexes in the metallodrug was determined independently using fluorescence emission as in Figure 2(A-B). The molar ratio using fluorescence emission showed that the ratios were 1:2 and 1:3 for Cu(II) and Fe(III) complexes, respectively (Figure 2C). Mefenamic acid has been reported to react with different metals at ambient temperature, physiological pH (i.e 7.4) and contact time of 10 min 38. The ESI(+)-MS spectrum of Cu(II)-mefenamic acid (Figure 3) shows a low resolution, which may be due to the instability of the complex, or its low volatility. The peak assignments are inserted in Figure 3 and listed in Table 1. The spectrum (Figure 3) shows peaks at 580, 543, and 310 Da corresponding to $[^{63}Cu(M\text{-}H)_2(H_2O)_2+H]^+$, $[^{63}Cu(M\text{-}H)_2+H]^+$ and $[^{63}CuC_{14}H_{14}O_4+H]^+$, respectively. In contrast with the Cu(II)-mefenamic acid complex, the ESI-MS (Figure 4) for the Fe(III)-mefenamic acid complex showed better resolution. The spectrum contained a peak at *m/z* 777.0 Da that was assigned to $[^{56}Fe(M\text{-}H)_3+H]^+$. This peak agreed with the molar ratio data in Figure 2C i.e 1:3. Peak assignments and LOD are listed in Table 1.

Table 1. ESI-MS assignments and LOD for mefenamic acid and their metallodrug

m/z	Assignment	LOD (M)
242.1	$[M+H]^+$	
224.3	$[(M\text{-}H_2O)+H]^+$	
483.2	$[2M+H]^+$	1×10^{-4}
465.0	$[2M\text{-}H_2O+H]^+$	
137.0	[2-Aminobenzoic acid+H]$^+$	
580.0	$[^{63}Cu(M\text{-}H)_2(H_2O)_2+H]^+$	
543.0	$[^{63}Cu(M\text{-}H)_2+H]^+$	1×10^{-3}
310.0	$[^{63}CuC_{14}H_{14}O_4+H]^+$	
777.0	$[^{56}Fe(M\text{-}H)_3+H]^+$	1×10^{-3}

Table 2. Comparison of the present method with other methods for extraction and determination of mefenamic acid

Technique	Matrices	LOD (μg mL^{-1})	Ref.
SBSE-HPLC-DAD	Water	1.5	40
HF-LPME-HPLC	Water	-	41
MCR-ALS-FS	Water	320	42
MAE-SPE-GC-MS	soil	0.9 ng Kg^{-1}	43
HPLC-UV	urine	7	45
ESI-MS	Water	0.1	Here

Note: SBSE-HPLC-DAD, stir bar sorptive extraction- high performance liquid chromatography –diode array detection; HF-LPME-HPLC , Hollow fiber-based liquid phase microextraction-high performance liquid chromatography; microwave assisted extraction-solid phase extraction-gas chromatography-tandem mass spectrometry

ESI-MS, a soft ionization method, generates multiply-charged species of the target analyte, hence, it is very useful for studying metallodrug-protein interactions. It is also extremely effective for investigating interactions between selected metallodrugs and one or a few isolated proteins in the sample. As shown in Table 2, ESI-MS is simple, sensitive and effective for mefenamic acid.[39-45]

It is important to stress that most technique is not recommended for the detection of metallodrug species. This is due to the high sensitivity of the non-covalent bonds, which are very labile and can be destroyed. Thus, ESI-MS was used only for complexes involving metallodrugs.[18] The data revealed that ESI-MS is

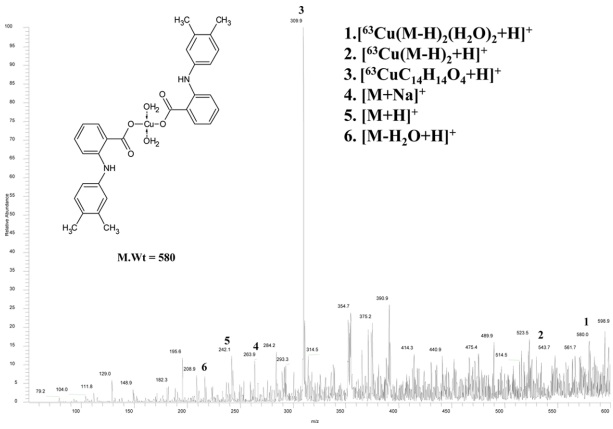

Figure 3. ESI-MS spectrum for Cu(II)-mefenamic acid.

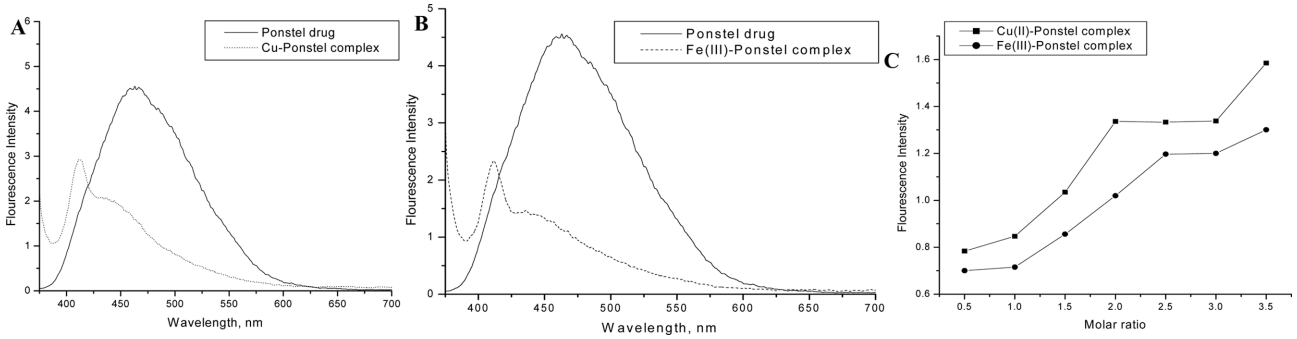

Figure 2. Fluorescence emission of mefenamic acid and their metal complexes, (A) Cu (II) (B) Fe(III) and (C) molar ratio analysis.

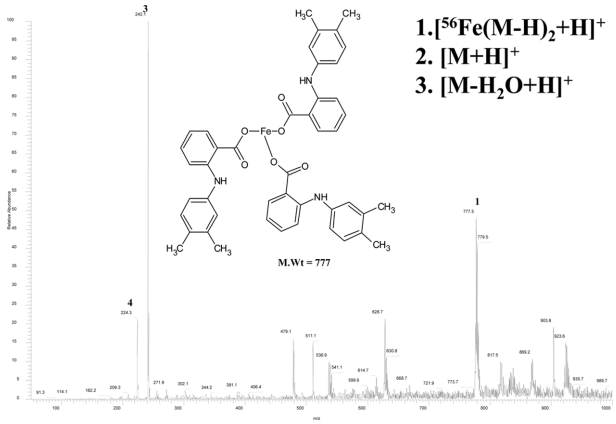

Figure 4. ESI-MS for Fe(III)-mefenamic acid complex.

apparently a simpler, more sensitive and soft ionization approach for metallo-mefenamic detection.

Conclusion

ESI-MS has been introduced as a new analytical tool to characterize a non-steroidal anti-inflammatory drug (mefenamic acid) and its metallodrugs. ESI-MS is simple, sensitive and efficient for metallodrug analysis. The technique also provides high resolution, high throughput, low sample load and soft ionization for metallodrug detection. The ESI-MS data was validated by fluorescence spectroscopy.

Acknowledgements

The authors are grateful to the Ministry of Science and Technology of Taiwan for the financial support.

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