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Analysis of Fluoroquinolones by Electrospray Ionization Quadrupole Time-of-Flight Mass Spectrometry

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In order to analyze fragmentation behaviours of fluoroquinolones, five commercially fluoroquinolones were investigated systematically by electrospray ionization quadrupole time-of-flight tandem mass spectrometry (ESI-Q-TOF-MS/MS) in positive ion mode. In simple MS spectra, the predominant precursor ions $[M + H]^+$ were observed for molecular mass information. MS/MS experiments of the precursor ions $[M + H]^+$ were used for characteristic cleavage fragments analysis to achieve detailed structural information. ESI-Q-TOF-MS/MS has been proven to be a fast, effective and practical tool for the analysis of the fragmentation patterns of fluoroquinolones. It was found that major fragment ions were produced by the cleavage of C-N or C-C bonds of piperazine ring of fluoroquinolones.

Keywords: Fluoroquinolones, Electrospray, ESI-Q-TOF-MS/MS, Fragmentation.

INTRODUCTION

Quinolones are the synthetic antibacterial drugs to rival the β-lactam and the macrolide antibacterials for impact in clinical usage in the antibacterial therapeutic field which were discovered in the 1960s[1,2]. They have a broad antibacterial spectrum of activity against Gram-positive, Gram-negative and mycobacterial pathogens as well as anaerobes[3]. These compounds had been developed in the 1980s with the addition of a fluorine atom at position 6 transforming a quinolone into a fluoroquinolone[4]. Due to their preferable pharmacokinetic properties, low toxicity and broad spectrum of activity against pathogenic microorganisms, various fluoroquinolones have been licensed for the treatment of human and veterinary infective diseases[5-7].

The basic structure of the fluoroquinolones is nalidixic acid nuclear,which has a carbon atom at position 8, a carboxyl group at position 3, a keto group at position 4, a fluorine atom at position 6 and a piperazine moiety at position 7[8]. The presence of these parts is very important for maintaining the antibacterial activity of the compounds and the cleavage of these parts of the molecule leads to the complete loss of its biological activity[9,10].

Electrospray ionization (ESI) is one of the softest ionization techniques, which can be used to analyze a variety of polar compounds, volatile compounds or thermal unstable compounds, such as drugs and their metabolites, natural product, biomacromolecules, *etc*[11]. Recent studies have reported the application of ESI-MS for characterization of fluoroquinolones with higher specificity, less sample consumption and better reproducibility than the other types of ionization[12-14]. Electrospray ionization source was coupled to a high-resolution quadrupole time-of-flight mass spectrometer that offer analysis with a wide dynamic quantification range[15,16]. Electrospray ionization quadrupole time-of-flight tandem mass spectrometry (ESI-Q-TOF-MS/MS) has become a particularly effective way in the analysis of the fragmentation pathways of fluoroquinolones more recently.

In this paper, we used ESI-Q-TOF-MS/MS to systematically analyze fragmentation behaviours of fluoroquinolones in positive ion mode. The following five fluoroquinolones fragmentation patterns were investigated and compared with each other to offer a theoretical basis for their mass spectrometry analysis, organism metabolism or environmental degradation products.

EXPERIMENTAL

HPLC grade methanol was provided by Fisher Scientific (Pittsburgh, PA, USA). Deionized water and mass calibration standard were purchased from Agilent (Santa Clara, CA, USA). Five fluoroquinolones: ofloxacin, levofloxacin, enrofloxacin, norfloxacin and lomefloxacin were obtained from Sigma-Aldrich (St Louis, MO, USA). The samples analysed were dissolved in methanol. Serial dilution with methanol gave solutions which was directly injected into the mass spectrometer.

Mass spectrometry: MS experiments were performed on a Bruker micrOTOF-Q mass spectrometer (Bremen, Germany) with an ESI source. The spray voltage was set to -500 V in positive ion mode. The capillary voltage was fixed

at 4500 V and at 180 °C. The samples were infused into the source at a flow rate of 180 uL/h by means of the integrated syringe pump. Nitrogen was used as a sheath gas(30 Kpa) and the flow rate is 4 L/min. Mass calibration standard was used to obtain maximum sensitivity and the calibration peak is at *m/z* 158.9731, *m/z* 226.9515, *m/z* 362.9263 and *m/z* 430.9138.The suitable collision energy was required to completely dissociate precursor ions $[M + H]^+$.

RESULTS AND DISCUSSION

In positive ion mode, fluoroquinolones under study led to abundant protonated species. However the abundance of $[M + Na]^+$ ions was found to be lower. Structural information was not available in simple MS spectra. Hence MS/MS experiments were essential for characteristic cleavage fragments analysis of the examined compounds $[M + H]^+$ species.

The same results are seen in the MS/MS spectrum of $[M + H]^+$ ion for ofloxacin and levofloxacin [Fig. 1(a)], as they are isomers. The first loss of CO_2 from $[M + H]^+$ ion gives ion at *m/z* 318. Further cleavage of piperazine ring leads to the formation of the ions at *m/z* 261 and *m/z* 247. The ion at *m/z* 233 is produced by the simultaneous cleavage of N(1)-C,C-O bonds and piperazine ring. The accurate mass and elemental compositions of major product ions from ofloxacin and levofloxacin are shown in Table-1. The proposed fragmentation pathways were reported in **Scheme-I**(a).

The MS/MS spectrum of $[M + H]^+$ ion for enrofloxacin [Fig. 1(b)] indicates characteristic ions at *m/z* 342 and *m/z* 316 corresponding to the losses of H_2O and CO_2. In addition to $[M + H\text{-}H_2O]_+$ and $[M + H\text{-}CO_2]^+$, the ion at *m/z* 245 corresponding to the loss of CO_2 followed by the cleavage of piperazine ring is observed, which finally produce even-electron ion at *m/z* 204 and odd-electron ion at *m/z* 203 by the cleavage of N(1)-C bond. The fragment ion at *m/z* 286 is due to the loss of C_4H_8 of piperazine moiety from the ion at *m/z* 342, while the fragment ion at *m/z* 261 can be explained by the cleavage of piperazine ring after the loss of CO [Table-2 and **Scheme-I** (b)].

Fig. 1(c) shows the MS/MS spectrum of $[M + H]^+$ ion for norfloxacin. The spectrum exhibites more abundant ion at *m/z* 231, moderately abundant ions at *m/z* 249, *m/z* 203 and *m/z* 189 and less abundant ions at *m/z* 272 and *m/z* 300. The ion at *m/z* 300 is due to the loss of HF from $[M + H]^+$ ion. The ion at *m/z* 231 produced by the loss of H_2O and the cleavage of N(1)-C bond and piperazine ring yields the ion at *m/z* 203 because of the loss of CO. In this case, the cleavage of piperazine ring and nalidixic acid nuclear is observed, which is different from the cleavage patterns of other compounds, leading to the ion at *m/z* 189 [Table-3 and **Scheme-I** (c)].

TABLE-1
MAJOR PRODUCT IONS FROM $[M + H]^+$ *m/z* 362 FOR OFLOXACIN (COLLISION ENERGY: 25 eV) AND LEVOFLOXACIN (COLLISION ENERGY:28 eV)

Fragment ion	Formula	Observed	Calculated	Error (ppm)
$[M + H]^+$	$C_{18}H_{21}N_3O_4F$	362.1511	362.1511	-0.2
$[M + H\text{-}CO_2]^+$	$C_{17}H_{21}N_3O_2F$	318.1605	318.1612	2.3
$[M + H\text{-}CO_2\text{-}C_3H_7N]^+$	$C_{14}H_{14}N_2O_2F$	261.1031	261.1034	1.1
$[M + H\text{-}CO_2\text{-}C_4H_9N]^+$	$C_{13}H_{12}N_2O_2F$	247.0871	247.0877	2.7
$[M + H\text{-}CO_2\text{-}C_5H_{11}N]^+$	$C_{12}H_{10}N_2O_2F$	233.0735	233.0721	-6.2

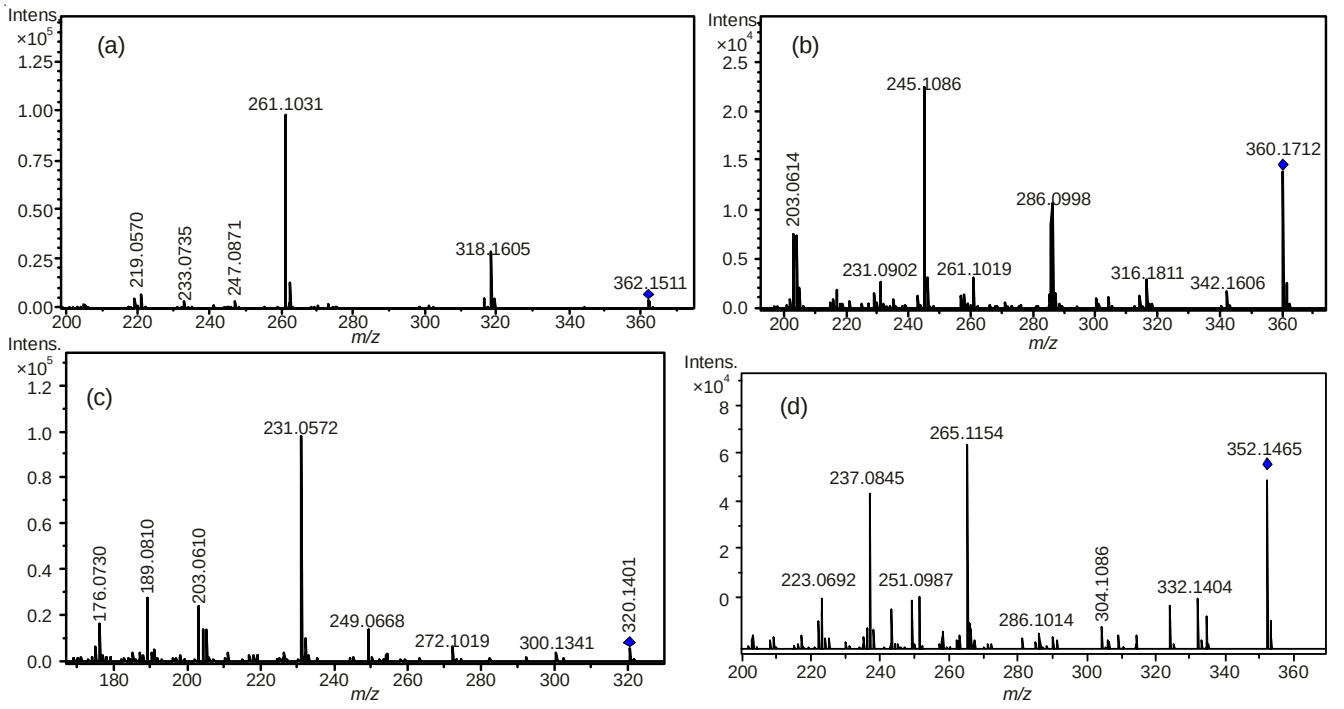


Fig. 1. Product ions scan of the selected precursor $[M + H]^+$ for: (a) ofloxacin(collision energy:25 eV) and levofloxacin(collision energy:28 eV) at *m/z* 362, (b) enrofloxacin(collision energy:30 eV) at *m/z* 360, (c) norfloxacin(collision energy:38 eV) at *m/z* 320, (d) lomefloxacin (collision energy:35 eV) at *m/z* 352

Lomefloxacin, different from the previous four fluoroquinolones, has one fluorine atom at position 6 and position 8, respectively. The spectrum of $[M + H]^+$ ion for lomefloxacin is shown in Fig. 1(d). The ion at *m/z* 249 indicates the loss of H_2O and the cleavage of N(1)-C bond and piperazine ring from $[M + H]^+$ ion, as the same as the pathway of the ion at *m/z* 320 giving the ion at *m/z* 231 for norfloxacin. The ion at *m/z* 332 corresponds to the loss of HF from $[M + H]^+$ ion. The ion at *m/z* 265 is due to the cleavage of N(1)-C(6) and C(3)-N(4) bonds of piperazine ring. The cleavage of N(1)-C bond and the loss of HF are observed, leading to the ion at *m/z* 304. The CO_2 loss from the ion at *m/z* 281 results in the ion at *m/z* 237 [Table-4 and **Scheme-I** (d)].

Conclusion

It has been demonstrated that ESI-Q-TOF-MS/MS is a fast, effective and practical tool for the analysis of the fragmentation patterns of fluoroquinolones. The precursor ions $[M + H]^+$ show characteristic fragmentation behaviours.

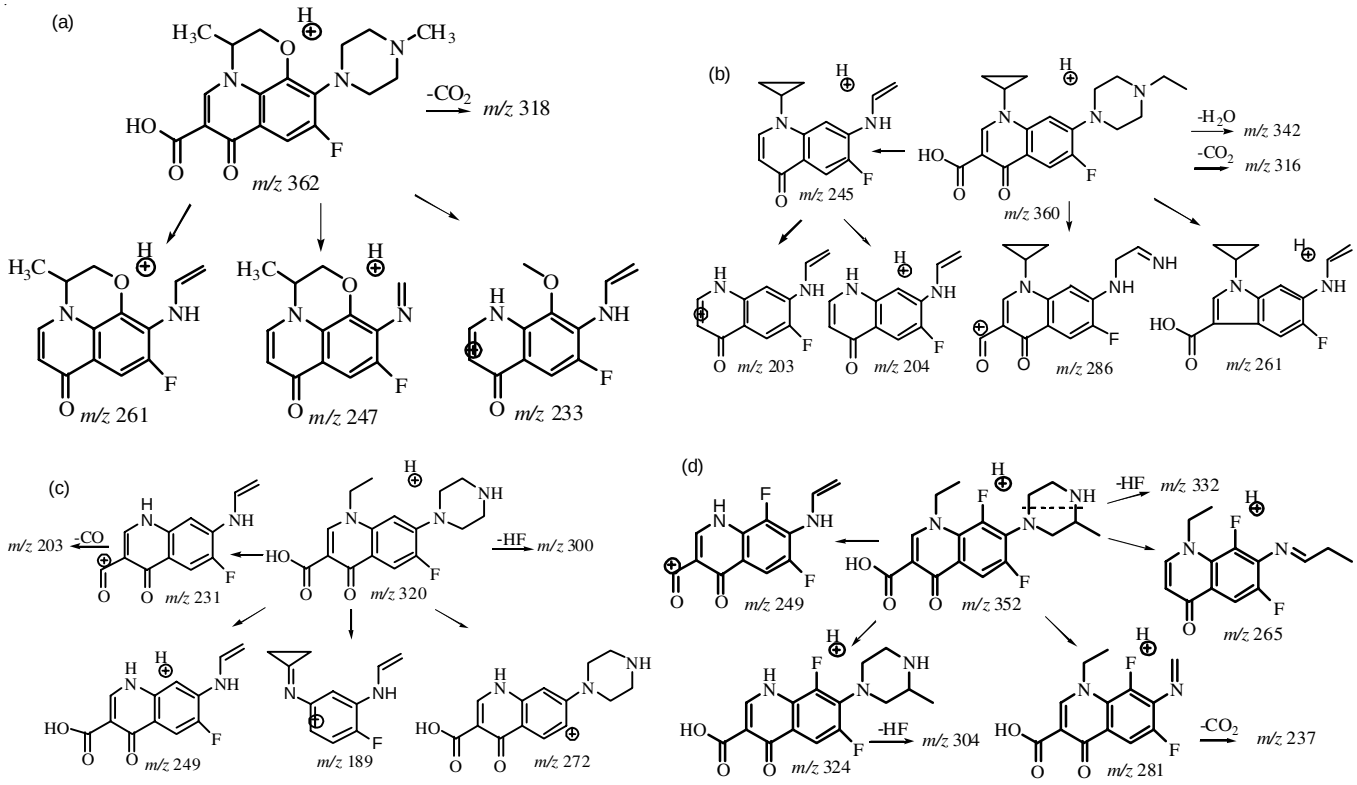
The cleavage of N(1)-C(2) and C(5)-C(6) bonds of piperazine ring of fluoroquinolones is observed for all compounds. The ion at *m/z* 265 corresponds to the cleavage of N(1)-C(6)

TABLE-2
MAJOR PRODUCT IONS FROM $[M + H]^+$ *m/z* 360 FOR ENROFLOXACIN (COLLISION ENERGY:30 eV)

Fragment ion	Formula	Observed	Calculated	Error (ppm)
$[M + H]^+$	$C_{19}H_{23}N_3O_3F$	360.1712	360.1718	1.8
$[M + H\text{-}H_2O]^+$	$C_{19}H_{21}N_3O_2F$	342.1606	342.1612	1.7
$[M + H\text{-}CO_2]^+$	$C_{18}H_{23}N_3OF$	316.1811	316.1820	2.7
$[M + H\text{-}H_2O\text{-}C_4H_8]^+$	$C_{15}H_{13}N_3O_2F$	286.0998	286.0986	-4.2
$[M + H\text{-}CO\text{-}C_4H_9N]^+$	$C_{14}H_{14}N_2O_2F$	261.1019	261.1034	5.7
$[M + H\text{-}CO_2\text{-}C_4H_9N]^+$	$C_{14}H_{14}N_2OF$	245.1086	245.1085	-0.7
$[M + H\text{-}CO_2\text{-}C_4H_9N\text{-}C_3H_5]^+$	$C_{11}H_9N_2OF$	204.0695	204.0693	-0.6
$[M + H\text{-}CO_2\text{-}C_4H_9N\text{-}C_3H_6]^+$	$C_{11}H_8NOF$	203.0614	203.0615	0.6

TABLE-3
MAJOR PRODUCT IONS FROM $[M + H]^+$ *m/z* 320 for NORFLOXACIN (COLLISION ENERGY:38 eV)

Fragment ion	Formula	Observed	Calculated	Error (ppm)
$[M + H]^+$	$C_{16}H_{19}N_3O_3F$	320.1401	320.1405	1.3
$[M + H\text{-}HF]^+$	$C_{16}H_{18}N_3O_3$	300.1341	300.1343	0.7
$[M + H\text{-}HF\text{-}C_2H_4]^+$	$C_{14}H_{14}N_3O_3$	272.1019	272.1030	4.0
$[M + H\text{-}C_2H_5N\text{-}C_2H_4]^+$	$C_{12}H_{10}N_2O_3F$	249.0670	249.0670	0.9
$[M + H\text{-}H_2O\text{-}C_2H_5N\text{-}C_2H_4]^+$	$C_{12}H_8N_2O_2F$	231.0572	231.0564	-3.2
$[M + H\text{-}H_2O\text{-}C_2H_5N\text{-}C_2H_4\text{-}CO]^+$	$C_{11}H_8N_2OF$	203.0610	203.0615	2.6
$[M + H\text{-}C_2H_5N\text{-}C_3H_4O_3]^+$	$C_{11}H_{10}N_2F$	189.0810	189.0823	6.5



Scheme-I: Major fragmentation pathways for: (a) ofloxacin and levofloxacin, (b) enrofloxacin, (c) norfloxacin, (d) lomefloxacin

TABLE-4
MAJOR PRODUCT IONS FROM $[M + H]^+$ *m/z* 352 FOR LOMEFLOXACIN (COLLISION ENERGY:35 eV)

Fragment ion	Formula	Observed	Calculated	Error (ppm)
$[M + H]^+$	$C_{17}H_{20}N_3O_3F_2$	352.1465	352.1467	0.6
$[M + H\text{-}HF]^+$	$C_{17}H_{19}N_3O_3F$	332.1404	332.1405	0.3
$[M + H\text{-}C_2H_4]^+$	$C_{15}H_{16}N_3O_3F_2$	324.1152	324.1154	0.6
$[M + H\text{-}C_2H_4\text{-}HF]^+$	$C_{15}H_{15}N_3O_3F$	304.1086	304.1092	2.0
$[M + H\text{-}C_4H_9N]^+$	$C_{13}H_{11}N_2O_3F_2$	281.0735	281.0732	-1.1
$[M + H\text{-}CO_2\text{-}C_2H_5N]^+$	$C_{14}H_{15}N_2OF_2$	265.1154	265.1147	-2.6
$[M + H\text{-}H_2O\text{-}C_3H_7N\text{-}C_2H_4]^+$	$C_{12}H_7N_2O_2F_2$	249.0479	249.0470	-3.6
$[M + H\text{-}C_4H_9N\text{-}CO_2]^+$	$C_{12}H_{11}N_2OF_2$	237.0845	237.0834	-4.6

and C(3)-N(4) bonds of piperazine ring for lomefloxacin. The ion at *m/z* 189 is due to the distinctive cleavage of piperazine ring and nalidixic acid nuclear for norfloxacin. In addition, the losses of neutral molecules such as CO_2, CO, H_2O and HF are also observed. In the experiments, the fragmentation pathways can be confirmed by the precursor ions $[M + H]^+$ and the cleavage fragments with accurate mass.

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