International Journal of Sciences

Research Article Volume 2 - September 2013 (9)

Evaluation and Degradation Chemistry of Orbifloxacin using LC-MS

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Abstract: Orbifloxacin (ORBI) was subjected to different ICH (Q1A(R2)) prescribed stress conditions of thermal stress, hydrolysis, oxidation and photolysis. It was stable to dry heat (60 ºC) and photolysis (UV-VIS) in solid form. It showed extensive decomposition under hydrolytic and photolytic conditions in acid, base and neutral solutions. Degradation was also observed in oxidative condition. Degradation products of ORBI formed under different forced conditions were characterized through LC–MS studies. In total, eleven major degradation products (DP1-DP11) were detected. Successful separation of drug and degradation products formed under various stress conditions was achieved on a analytical column SymmetryShield Waters RP18, 5 µm, 250 x 4.6 mm using 5% acetic acid and methanol as mobile phase in a gradient mode at flow rate of 0.5 ml/min. The peaks were detected using a PDA detector set at 290 nm. The method was extended to LC–APCI-MS for characterization of the degradation products and the pathways of decomposition were proposed.

Keywords: Orbifloxacin; Chemistry degradation; SIAM, LC-APCI-MS; Stability

1. Introduction

Orbifloxacin (ORBI) is a synthetic fluoroquinolone broad-spectrum antimicrobial drug that has been developed especially for use in veterinary medicine (Nakamura, 1995; Matsumoto et al., 1998, 1999; Ihrke et al., 1999; O'neil, 2006; Marín et al., 2007, 2008; Reynolds, 2007; Goudah and Abo−El−Sooud, 2008). ORBI is widely used in treatment of skin, soft tissue, urinary tract, gastrointestinal and respiratory infections in animals (Nakamura, 1995; Matsumoto et al., 1999; Haines et al., 2001; Ganière et al., 2004; Martinez et al., 2006; Davis et al., 2006; Scott et al., 2006; McKay et al., 2007). Chemically, it is 1-cyclopropyl-5,6,8-trifluoro-1,4-dihydro-7-(*cis*-3,5-dimethyl-1-piperazinyl)-4-oxoquinoline-3-carboxylic acid (Fig. 1.). The mechanism of the bactericidal effect of ORBI is based on the inhibition of the DNA gyrase of the bacteria, the enzyme that produces a negative supercoil in DNA and thus permits transcription and replication (Rang et al., 2003).

The drug substance monograph of ORBI in British Pharmacopoeia lists seven impurities (A–G) (BP, 2011). All same seven impurities are also mentioned in the drug monograph by the United States Pharmacopeia (USP, 2011). However, the listed impurities are not classified into process impurities and degradation products. So the intrinsic degradation profile of drug under prescribed stress conditions is still undisclosed.

The studies on degradation behavior to explain the degradation pathway and characterization of all degradation products of ORBI are limited in the literature. Morimura et al., in 1995a, investigated the degradation kinetics of ORBI as a function of pH, temperature and buffer concentration. Two degradation products (DP), under acidic and alkaline conditions were isolated and their structures were elucidated by MS. In the same year, these authors (Morimura et al., 1995b) also studied the photodegradation kinetics of ORBI in aqueous solution at various pH values under irradiation with sunlight or a chemical lamp and, in 1997 (Morimura et al., 1997a), they identified three photodegradation products. Another study involved orbifloxacin and its conversion to a photoproduct substituted with a chloride at the 8 position by its photoreaction in aqueous solution containing chloride ion (Morimura et al., 1997b). However these authors did not report all possible degradation conditions, so some degradation products were not identified. Recently, Cazedey et al. (2011) developed a stability-indicating method for ORBI in tablets, though this study did not attempt the identification of the degradation structures.

In the quality control of the pharmaceutical products identification and quantification of the active ingredient and its impurities are very important because of safety and efficacy reasons. Impurities and potential degradation products that can be present in medicines can change the chemical, pharmacological,

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and toxicological properties of the product (Zivanovic et al., 2006).

Stress and stability testing provides evidence for the quality of the bulk drug and its final drug product when they are exposed to environmental factors such as pH, temperature and humidity and help to determine the intrinsic stability of the molecule by establishing the degradation pathways. The International Conference on Harmonization (ICH) guidelines (2003), suggest stress studies on a drug to establish its inherent stability characteristics not only for identification of degradation products but also understanding the stability of drug molecule. So, it is of great importance to know the complete degradation profile of ORBI, which is not yet reported in the literature.

Hence, LC-UV-PDA and LC-MS techniques were used to develop a sensitive, accurate and reproducible method for the determination and identification of ORBI and its degradation products, formed under ICH recommended stress conditions of hydrolysis, oxidation, heat and photolysis (ICH, 2003, 2006a, 2006b), along with method validations (ICH, 2005).

An integral aim of the present study was to investigate the complete degradation behavior of the ORBI. It was done through a systematic investigation involving: (i) forced decomposition of the drug under a variety of stress conditions (hydrolysis and photolysis (acid, base and neutral solutions), oxidation, thermal stress and photolysis of solid form), (ii) optimization of LC conditions to separate the drug and its degradation products on a reversed-phase C18 column and development of a LC–MS compatible method, (iii) method validation, (iv) conduct of LC–MS studies to establish fragmentation profiles of the drug and the degradation products, (v) elucidation of structures of degradation products through comparative study of mass and literature data, and (vi) ascertaining degradation pathway of the drug based on the information collected.

2. Experimental

2.1. Reagents and chemicals

Orbifloxacin standard (assigned purity 99.8%) was obtained from Sigma-Aldrich (Seelze, Germany). Analytical grade reagents and HPLC grade solvents were used. Acetic acid, methanol, sodium hydroxide pellets (NaOH), concentrated hydrochloric acid (HCl) were supplied by Merck (Darmstadt, Germany). Hydrogen peroxide (H_2O_2) was obtained from Acros Organics (New Jersey, USA). The drug product of orbifloxacin, i.e. Orbax™ tablet (Schering-Plough Animal Health Ind. Com. Ltda., São Paulo, Brazil) with a label claim of 22.7 mg drug was purchased commercially. All chemicals were used without further purification.

Purified HPLC grade water was obtained by reverse osmosis and filtration through a Milli-Q™ system (Millipore, Milford, MA, USA). This was used to prepare all solutions.

2.2. Forced degradation studies

In a typical degradation study, 10–30% degradation of the active drug is sufficient, but not so severe as to generate secondary products. Stress studies were carried out according to ICH guidelines Q1A(R2). All solutions for degradation studies were prepared to obtain a final concentration of 1.0 mg/ml. In all degradation studies the average peak area of ORBI and its degradation products after application (100 µg/ml) of three replicates was obtained. All the prepared samples were passed through 0.45 µm membrane filter and then transferred to LC vials for analysis.

2.2.1. Acid, base and neutral hydrolytic degradation

Acid, base and neutral decomposition studies were performed by storing the solutions of drug (1 mg/ml) in 0.1 N HCl, 0.1 N NaOH and water, respectively, at 80 ºC for 8 days. The resultant solutions were first neutralized to prevent secondary decomposition and then diluted with 5% acetic acid: methanol (80:20, v/v) (diluent) to obtain 100 µg/ml solutions.

2.2.2. Oxidative degradation

To study hydrogen peroxide induced degradation the drug solution (1.0 mg/ml) was exposed to 3% H_2O_2 at room temperature, in the dark, for a period of 24 hours. The resultant solution was diluted with diluent to obtain 100 µg/ml solutions.

2.2.3. Photochemical degradation

The photochemical stability of the drug was studied by exposing the acid, base and neutral solutions of ORBI (1.0 mg/ml) as well as solid API using a Petri plate container to expose the drug to ultraviolet (UV) (at least 200 W h/m^2) and fluorescence (VIS) (minimum 1.2 Milion Lux hours) lights for 8 days at 25 ºC/60% relative humidity (RH). The solutions were diluted or prepared with diluent to obtain a solution of 100 µg/ml.

2.2.4. Dry heat degradation

The solid API was placed in an oven at 60 ºC for 15 days to study dry heat degradation. At the time of assay, the solid sample was dissolved in the diluent to a concentration of 100 µg/ml.

2.2.5. Accelerated and long term stability conditions

Tablet powder samples were stored into stability chambers without light influence under 30 ºC/75% RH and 40 ºC/75% RH for 6 months to assess the

accelerated and long term stability. At the time of assay, the solid samples were dissolved in the diluent to a concentration of 100 µg/ml.

2.3. Liquid chromatography (LC-UV-PDA) conditions

Chromatographic separation was performed on a Shimadzu LC system that includes a binary pump (LC20AD), a degasser (DGU-20A_5), a column heater oven (CTO-20AC), a photodiode array (PDA) detector (SPD-M20A), an autosampler (4 °C) (SIL-20AC) and a system controller (CBM-20A). The HPLC column was a SymmetryShield Waters RP18, 5 µm (250 mm x 4.6 mm). The chromatographic data were recorded using a computer system with LCsolution data acquiring software (also from Shimadzu).The system was programmed at 50 ºC for the column oven, 290 nm for detection wavelength and 20 µl for injection volume in a flow rate of 0.5 ml/min. A gradient elution was used to elute ORBI and all possible DP from the column. The gradient program used was: solvent A: 5% acetic acid; solvent B: methanol (time/%B): 0/5, 30/50, 35/5.

2.4. Mass spectrometer (LC-MS) conditions

LC instrument was coupled to a Shimadzu LCMS-2010EV single-stage quadrupole mass spectrometer used in positive ion mode to determine the molecular weights of the DP. MS was operated by alternating between two events. The first event was used to study all possible degradation products generated by forced degradation studies of ORBI with a full-spectrum analysis (total ion current - TIC) in the mass range m/z 50–650, while the second event was used to assess the major degradation products by selected ion monitoring (SIM). This set-up is optimized to improve the sensitive detection of the degradation products that were formed in the forced degradation studies of ORBI.

The MS was operated with an atmospheric pressure chemical ionization (APCI) interface using nitrogen as a nebulizer gas at a flow rate of 1.5 l/min. The curved desolvation line (CDL) and heat block temperatures were both 400 ºC. A detector voltage of 1.5 kV, interface voltage of 3.5 kV and an injection volume of 5 µL were settled. The MS data acquisition and analysis were performed using the LCMS Solutions software (Shimadzu). The chromatographic conditions used for LC–MS analysis were the same as that for LC–UV-PDA analysis.

2.5. Validation parameters

Validation of method was performed according to the ICH guideline for validation of analytical procedures (ICH, 2005). For the calibration, standard solutions at different concentration levels (1.0 to 100.0 µg/ml, n = 6) were prepared by dissolving the API in 5% acetic acid: methanol (80:20, v/v). Calibration curves were constructed using analyte standard peak area ratio versus concentration of analyte.

The LOD and LOQ were established using the signal-to-noise methodology described in ICH (2005). The LOD values were calculated by using a signal-to-noise ratio of three (the ratio between the peak intensity and the noise intensity was used), while LOQ values were calculated by using a signal-to-noise ratio of 10. To assess precision intra-day and inter-day, six standard solutions at work concentration (100.0 µg/ml) were prepared and analyzed. The procedure was repeated on three consecutive days to determine inter-day-variability.

Accuracy of the proposed method was established by recovery experiments. This study was employed by addition of known amounts of ORBI to the pool of degraded samples in three levels (n = 3 at each concentration). For evaluating selectivity in LC, the degradation products formed when ORBI was exposed to hydrolytic, photolytic, oxidative and thermal stress were analyzed.

3. Results and discussion

3.1. Optimization of chromatographic conditions

Satisfactory separation of degradation products was achieved using a C18 column, thus, during the optimization cycle, several conditions were tried on a SymmetryShield Waters RP18, 5 µm, 250 x 4.6 mm. Various mobile phases like methanol and acetic acid, acetonitrile and acetic acid in different proportions were tried in a gradient mode. To detect the drug and degradation products with sufficient peak intensity, the wavelength of 290 nm was selected. It was found that methanol was the best organic modifier needed to elute and separate all peaks within 35 min with sufficient resolution. A mobile phase consisting of 5% acetic acid (solvent A) and methanol (solvent B) in a gradient mode was started at 5% of B, then increased to 50% within 30 min, after that %B returned to 5% within 5 min. A flow rate of 0.5 ml/min, injection volume of 20 µL and column temperature at 50 ºC were also used to obtain a good separation of ORBI and its degradation products.

To characterize degradation products by LC–MS studies, the developed LC method was used without modification. The obtained *m/z* values scans (TIC) were acquired over the mass range from *m/z* 50 to 650 in positive APCI mode. After that, the selected fragments were confirmed by using SIM mode, which allowed to obtain peaks with higher resolution. Based on the molecular weights, the fragmentation pattern of other quinolones and literature, the presence of degradation products was confirmed and also, structures could be proposed. The degradation

pathway was outlined based on the results.

3.2. Validation

The method was validated and good linearity was found in the concentration range of 1.0–100.0 µg/ml (r = 0.9993) of ORBI. The %RSD for intra- and inter-day precision was 0.48 and 0.43, respectively. Also, good recoveries were obtained when stressed samples were spiked with known concentration of the drug at 25, 50 and 75 µg/ml with mean recovery of 99.99%. The limits of detection (LOD) and quantification (LOQ) were found to be 0.7 and 5 ng/ml, respectively. The resolution (R), plates (N), tailing (T) and capacity (k') factors were calculated and the chromatographic parameters are given in Table 1. It can be seen from Table 1 that all the peaks were well resolved (Bonfilio et al., 2012).

3.3. Stress decomposition behavior

The pH influence at a high temperature was verified for ORBI. The degradation rate in base solution was higher as compared to that of acid or neutral solutions. The drug was found to be highly labile to basic hydrolysis at 80 ºC for eight days with around 34% of degradation. In this condition four degradation products were visible in MS detector (m/z, retention time: 328, 10.2 min (DP1); 354, 12.6 min (DP2); 344, 18.3 min (DP3); 394, 18.9 min (DP4)). At the same temperature and time conditions, ORBI in acid and neutral solutions demonstrated 12.85% and 7.58% of degradation, respectively. In acid hydrolysis, one major DP was observed (m/z = 352 (DP5)) with retention time of 11.6 min and in neutral hydrolysis also one major DP6 was found in 13.5 min (m/z 356).

Degradation was also observed in accelerated (40 °C ± 2 °C/75% ± 5% UR) and long term (30 °C ± 2 °C/75% ± 5% UR) stability conditions of tablet powder sample with degradation rate in 6 months of 15.28% and 6.37%, respectively. One major degradation product was identified in both assays. The impurity was identical to the one found in the neutral hydrolysis (DP6) with similar retention time and mass.

ORBI was found to be unstable to oxidative degradation. Under oxidative conditions, using 3% hydrogen peroxide, in 24 hours of exposure, around 17% of drug was degraded (Table 2). Two major degradation products were detected, in retention time of 10.2 min (DP1) and 12.9 min (DP7).

Photodegradation of the drug, similarly to other fluoroquinolones, was pH dependent. The faster degradation rate of ORBI was found in aqueous solution (pH 7), with half-life time ($t_{1/2}$) of approximately eight hours. In base solution, $t_{1/2}$ was about 24 hours. After this time, degradation of ORBI was the most aggressive with almost 95% of degradation in this medium. The lowest degradation rate of ORBI was found in acid solution. Under UV-VIS light in acid medium, only after 72 hours 40% of degradation was observed.

Photodegradation studies of ORBI generated four major degradation products. The major degradation products found under UV-VIS lights in neutral photolysis showed mass of m/z 338 (DP8), 394 (DP9), 412 (DP10) and 390 (DP11) at retention times of 12.2, 13.0, 15.7 and 32.7 min, respectively.

There was no significant degradation of solid API on exposure to dry heat or UV-VIS lights, which indicated that solid form of the drug remained stable on exposure to thermal and photolytic stress (Table 2).

The degradation behavior of ORBI under various stress conditions was investigated by LC. Typical chromatograms are shown in Fig. 2. The chromatographic parameters were determined and given earlier in Table 1.

In total, eleven degradation products (DP1-DP11) were formed under different conditions. Four degradation products (DP1-DP4) were generated on subjecting the drug to base hydrolysis, and one from acidic hydrolysis (DP5). Neutral hydrolysis, accelerated and long term stability studies yield also one degradation product (DP6). DP2 already described as an impurity originated from base hydrolysis was also found in oxidative conditions. Another degradation product checked in oxidation of ORBI was named as DP7.

The photodegradation of ORBI in acid, base and neutral conditions were studied and showed four degradation products (DP8 –DP11).

The peaks not tagged in Fig.2 were not coded because they were not detected by LC–MS.

3.4. Degradation products structure characterization

The analysis of the degradation products was carried by LC and LC–MS. ORBI was subjected to LC–MS with atmospheric pressure chemical ionization to know the fragmentation pattern of drug.

The molecular ion $(M+H)^+$ was observed in the LC-MS spectrum at a mass of 396 m/z. A key step in elucidating degraded structures is to understand the fragmentation pattern of the active drug substance.

LC-MS total ion chromatograms were obtained for ORBI at each stress condition (i.e. acid, base, neutral

hydrolysis and photolysis, oxidation and thermal degradation). The peaks corresponded to the parent drug and its degradation products. For each peak, a signal representing a distinct $[M+H]^+$ value was observed (Fig. 3). Definitive mass-to-charge value assignments were then made for each component under the corresponding APCI conditions. This data led to tentative structural assignments.

The molecular ion peak DP1 (m/z 328 amu) formed by base hydrolysis of the ORBI propose the formation of 5,6,8-trifluoro-7-{[(1*Z*)-2-(methylamino)prop-1-en-1-yl]amino}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid. It suggested that the parent ion lost the cyclopropyl moiety at 1-N and occurs the opening of piperazinyl ring with perm of a methyl and addition of a duple bond. This structure was also found in oxidative studies of ORBI.

In case of DP2, the experimental m/z value was 354 amu and it is suggested that elemental composition is $C_{18}H_{22}F_3N_3O_3$. A difference of 42 amu between the fragment ion at m/z 354 and precursor ion (m/z 396) could be attributed to loss of –COOH at position 3 and the substitution of ketone at position 4 for a hydroxyl moiety during base hydrolysis.

The fragment ion at *m*/*z* 344 (DP3) formed by loss of 52 amu is proposed to be due to elimination of 1-cyclopropyl and the opening of piperazinyl ring with permanence of a ethyl moiety.

The cleavage of C-F bond in 5-position with introduction of a hydroxy moiety led to DP4 also in the base hydrolysis of ORBI molecule. Further, the structure of DP4 was matched with impurity D in British (BP, 2011) and in the USP Pharmacopoeia (USP, 2011) it was named as *cis*-1-cyclopropyl-7-(3,5-dimethyl-1-piperazinyl)-6,8-difluoro-1,4-dihydro-5-hydroxy-4-oxo-3-quinolinecarboxylic acid. Morimura et al. (1995a) also described this structure.

A difference of 44 amu between the fragment at m/z 352 and precursor ion m/z 396 suggested that DP5 was formed by decarboxylation at the 3-position, during the acid hydrolysis, to form 1-cyclopropyl-5,6,8-trifluoro-1,4-dihydro-7-(*cis*-3,5-dimethyl-1-piperazinyl)-4-oxoquinoline. In fact, this degradation product was also previously described by British Pharmacopoeia (BP, 2011), USP (USP, 2011), and Morimura et al. (1995a).

Two chemicals structures were proposed to be DP6. For the first one it was suggested by the cleavage of bond between cyclopropyl moiety and 1-N atom of pyridine ring. The second structure was proposed based on opening of 7-dimethylpyperazine ring, as shown in Figure 4.

The oxidation of ORBI molecule was indicated mainly from the difference of 28 amu between mass of the drug and the oxidized product. On this basis, the structures of the DP7 was worked out to be 1-cyclopropyl-5,6,8-trifluoro-4-oxo-7-piperazin-1-yl-1,4-dihydroquinoline-3-carboxylic acid indicating the loss of two methyl moieties.

There were two possible structures for the ion of *m*/*z* 338 (DP8), which resulted from acid, base and neutral photolysis, as shown in Fig. 4. These were due to probability of photodecomposition of the dimethylpiperazinyl moiety at 7-position with the cleavage of C-F bond at the 8-position, and another is the elimination of a cyclopropyl group at the 1-position with the cleavage of a C-F bond at the 8-position. These structures were further supported by Morimura et al. (1997a) reports on mass characterization of ORBI. In BP (2011) (impurity B) and USP (2011) only the structure obtained due to the decomposition of the dimethylpiperazinyl ring at the 7-position and fragmentation of C-F bond at 8-position was reported.

The DP9 generated by neutral hydrolysis showed m/z of 394 amu. Although this product presents the same specific mass that DP4, it shows different relative retention time what indicates different structures. It is suggested that the DP9 structure is due to the addition of a double bond in the piperazinyl ring characterizing the loss of 2 amu.

The extra mass of 16 amu from precursor ion indicated that there might be an substitution of fluorine by chlorine atom. The mass spectra of DP10 also reveals that the molecular peak of the protonated degraded ion has isotope peaks at m/z 412 and 414 (chlorine isotopic pattern) as can be seen in (Fig. 3).

As shown in Fig. 2, DP11 resolved latest, indicating its lower polarity in comparison to ORBI and other degradation products. Thus, it is suggested that the three fluorine atoms were replaced by hydroxyl moieties
Some peaks were not detected by LC–MS, possibly due to poor ionizability (Fig. 2).

3.5. Degradation pathway and mechanisms
The structural elucidation of degradation products revealed three main susceptible sites on ORBI molecule, viz., the bond between nitrogen and cyclopropyl group at the 1-position, the bond at the carbon and carboxylic acid at the 3-position and the 7-piperazinyl ring. However, cleavage in other bonds like 4, 5 and 8-positions were also susceptible to degradation reactions.

All data are based on molecular weight and fluoroquinolones literature. Additional study will be done to confirm the chemical structures of orbifloxacin degradation products.

Taking into consideration of these facts, the degradation pathway was proposed (Fig. 4)

4. Conclusions

A validated stability indicating LC method was developed to study the degradation behavior of orbifloxacin under hydrolysis (acid, base and neutral) and photolysis, oxidation and thermal conditions. The method was suitable for extension to LC–MS studies. LC–MS characterization of degradation products was carried out and pathways of decomposition were proposed. A total number and the nature of degradation products formed under different stress conditions were obtained. The drug was found to degrade extensively in all conditions except dry heat and dry photolysis conditions.

Acknowledgement

This work was supported by PACD-FCFAr-UNESP (Araraquara, Brazil), FAPESP (São Paulo, Brazil) and CNPq (Brasília, Brazil).

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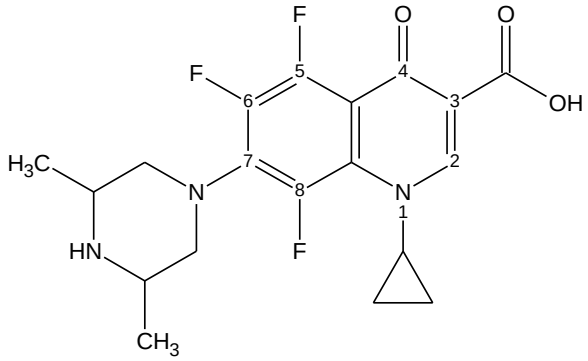

Fig. 1. Numbered chemical structure of orbifloxacin (ORBI)

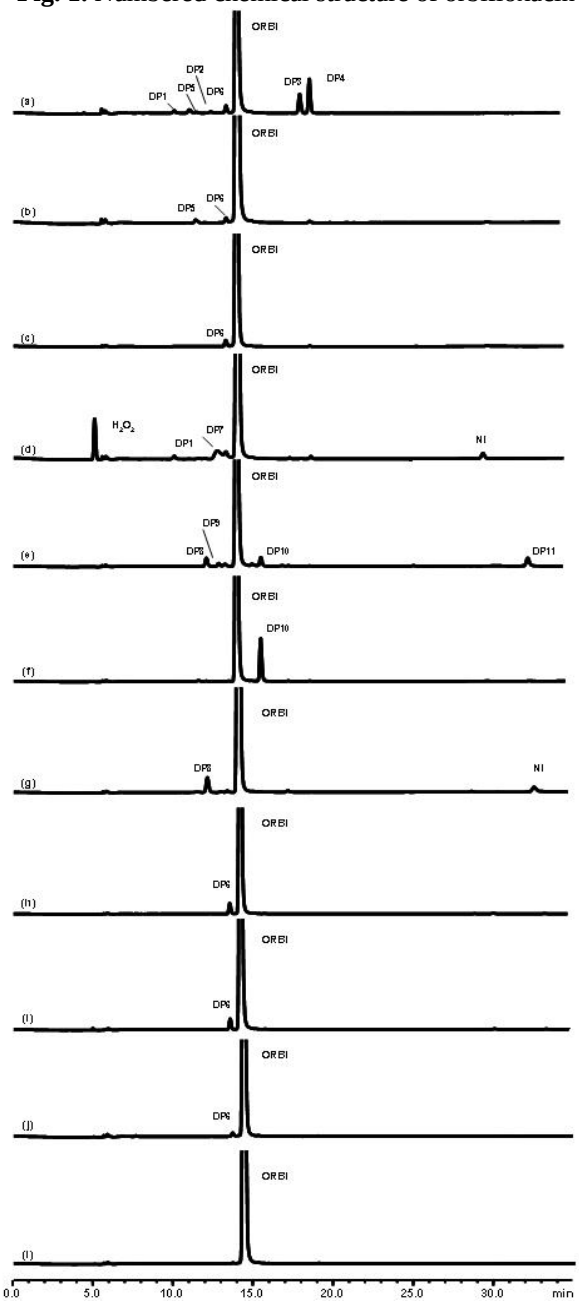

Fig. 2. Typical LC chromatograms of ORBI degradation products formed under (a) base hydrolysis, (b) acid hydrolysis, (c) neutral hydrolysis, (d) oxidative degradation, (e) neutral photolysis, (f) acid photolysis, (g) base photolysis, (h) accelerated stability, (i) long term stability, (j) thermal degradation of solid form, (l) photolytic degradation of solid form (NI: not identified in MS).

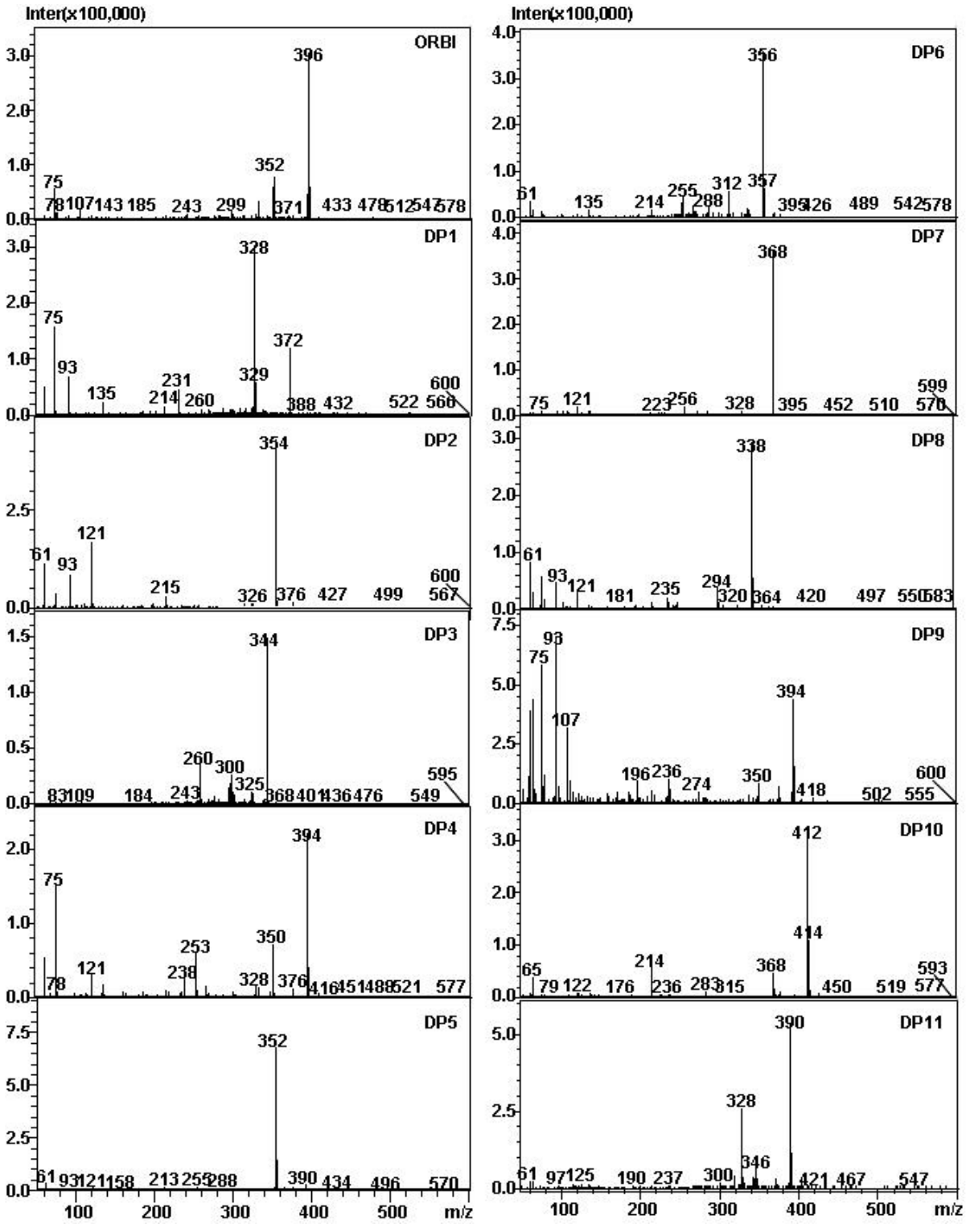

Fig. 3. Mass spectra of ORBI and its degradation products (DP1–DP11).

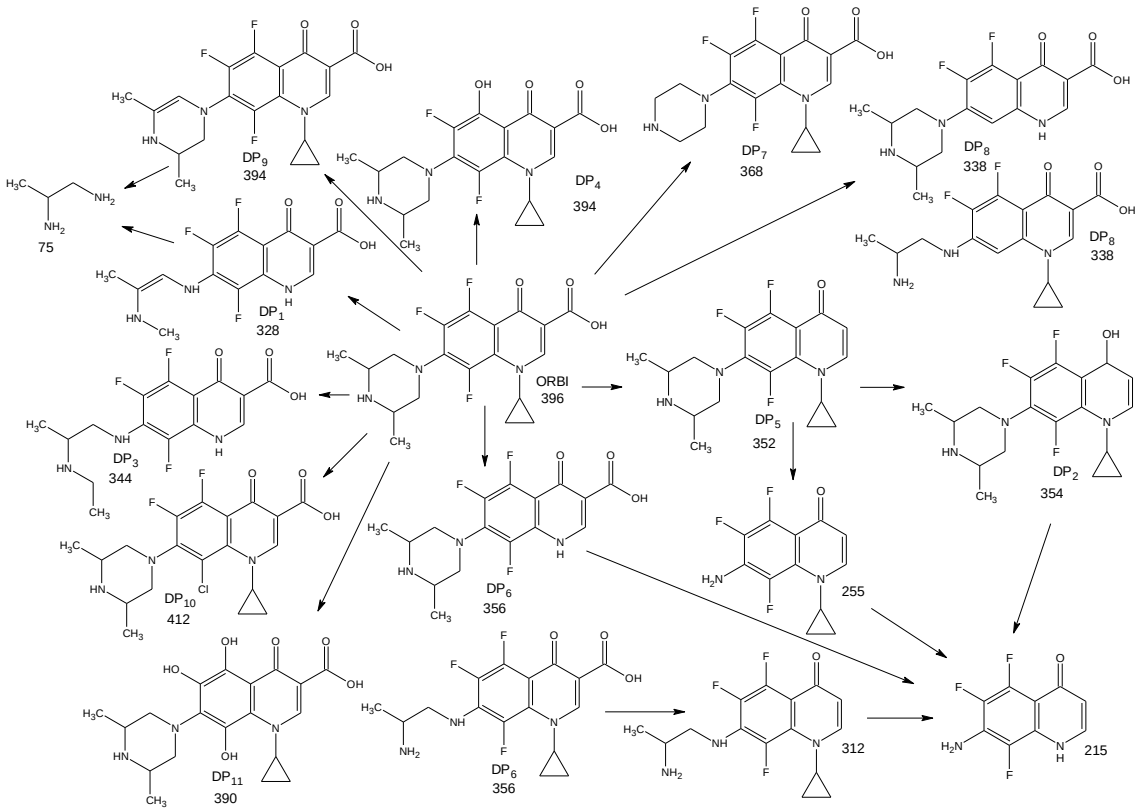

Fig. 4. Degradation pathway of orbifloxacin (ORBI) in acid, base and neutral hydrolysis and photolysis, oxidation and thermal conditions.

Table 1. Parameters of orbifloxacin and its degradation products identified by LC-MS

DP	*Code*	*RT*	*RRT*	*Ion**	*R*	*N*	*T*	*k'*
-	**ORBI**	14.21	1.00	396	2.32	41321	1.35	2.15
1	**DP1**	10.29	0.72	328	15.67	18604	1.24	1.27
2	**DP2**	12.64	0.89	354	2.10	14717	NC	1.79
3	**DP3**	18.37	1.29	344	8.14	60577	1.06	3.05
4	**DP4**	18.99	1.34	394	2.18	76279	1.19	3.19
5	**DP5**	11.69	0.82	352	17.72	13647	1.50	0.99
6	**DP6**	13.52	0.95	356	27.45	32914	NC	1.30
7	**DP7**	12.94	0.91	368	1.81	2990	NC	1.52
8	**DP8**	12.26	0.86	338	22.58	23955	1.28	1.09
9	**DP9**	13.04	0.92	394	2.46	27323	NC	1.22
10	**DP10**	15.71	1.11	412	1.96	50336	1.22	1.67
11	**DP11**	32.73	2.30	390	9.57	118009	1.34	4.57

*$[M+H]^+$ (m/z); DP: degradation product; RT: retention time; RRT: relative retention time; R: resolution; N: plates; T: tailing factor; k': capacity factor; NC: not calculated

Table 2. Percentage data of orbifloxacin (ORBI) degraded in different forced stress studies

Conditions	*% degradation*
Acid hydrolysis (0.1 *M* HCl, 80 °C, 8 days)	12.85
Base hydrolysis (0.1 *M* NaOH, 80 °C, 8 days)	33.67
Neutral hydrolysis (Water, 80 °C, 8 days)	7.58
Oxidative degradation (3% H_2O_2, room temperature, 24 h)	16.74
Acid photodegradation (0.1 *M* HCl, UV-VIS, 12 h)	17.62
Base photodegradation (0.1 *M* NaOH, UV-VIS, 5 h)	16.47
Neutral photodegradation (Water, UV-VIS, 1 h)	14.73
Thermal degradation (Powder, 60 °C, 15 days)	1.76
Photodegradation (Powder, UV/FL, 8 days)	1.82
Accelerated stability studies (40 °C/75% RH, 6 months)	15.28
Long-term stability studies (30 °C/75% RH, 6 months)	6.37