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A validated stability-indicating RP-HPLC method for levofloxacin in the presence of degradation products, its process related impurities and identification of oxidative degradant

M. Lalitha Devi[a,*], K.B. Chandrasekhar[b]

[a] Reference Standard Laboratory, United States Pharmacopeia-India Private Limited, ICICI Knowledge Park, Turkapally, Shameerpet, Hyderabad 500 078, India
[b] Department of Chemistry, Jawaharlal Nehru Technological University of College of Engineering, Anantapur 515002, India

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ABSTRACT

The objective of current study was to develop a validated specific stability indicating reversed-phase liquid chromatographic method for the quantitative determination of levofloxacin as well as its related substances determination in bulk samples, pharmaceutical dosage forms in the presence of degradation products and its process related impurities. Forced degradation studies were performed on bulk sample of levofloxacin as per ICH prescribed stress conditions using acid, base, oxidative, water hydrolysis, thermal stress and photolytic degradation to show the stability indicating power of the method. Significant degradation was observed during oxidative stress and the degradation product formed was identified by LCMS/MS, slight degradation in acidic stress and no degradation was observed in other stress conditions. The chromatographic method was optimized using the samples generated from forced degradation studies and the impurity spiked solution. Good resolution between the peaks corresponds to process related impurities and degradation products from the analyte were achieved on ACE C18 column using the mobile phase consists a mixture of 0.5% (v/v) triethyl amine in sodium dihydrogen orthophosphate dihydrate (25 mM; pH 6.0) and methanol using a simple linear gradient. The detection was carried out at 294 nm. The limit of detection and the limit of quantitation for the levofloxacin and its process related impurities were established. The stressed test solutions were assayed against the qualified working standard of levofloxacin and the mass balance in each case was in between 99.4 and 99.8% indicating that the developed LC method was stability indicating. Validation of the developed LC method was carried out as per ICH requirements. The developed LC method was found to be suitable to check the quality of bulk samples of levofloxacin at the time of batch release and also during its stability studies (long term and accelerated stability).

1. Introduction

Levofloxacin is described chemically as (−)-(S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid. The drug is an antibiotic of the 3rd-generation fluoroquinolone family inhibits the bacterial enzymes DNA gyrase and topoisomerase IV [1]. The fluoroquinolones are quinolones with fluorine at position 6 of naphthyridine ring [2]. Published structure–activity data show that fluorine atom helps to broadens their activity spectrum against both Gram-negative and Gram-positive pathogens [3,4]. Levofloxacin is currently the only respiratory fluoroquinolone approved by the U.S. FDA for the treatment of nosocomial pneumonia. Chemically, levofloxacin is the *S*-enantiomer (L-isomer) of ofloxacin, and has approximately twice the potency of ofloxacin, because the *R*+enantiomer (D-isomer) of ofloxacin is essentially inactive. In addition, the *S*-enantiomer (L-isomer) of ofloxacin has substantially less toxicity. Like other fluoroquinolones, it works by inhibiting DNA gyrase, an enzyme that negatively supercoils DNA. Validation of a levofloxacin HPLC assay in plasma and dialysate for pharmacokinetic studies was published using fluorescence detection [5]. In one publication an HPLC assay and a microbiological assay to determine levofloxacin in soft tissue, bone, bile and serum was described [6]. Analysis of levofloxacin in pharmaceutical preparations by high performance thin layer chromatography was also described [7]. The high performance liquid chromatography tandem mass spectrometry method (HPLC/MS/MS) has been used to determine levofloxacin in human plasma [8]. So far several articles were published for the determination of levofloxacin in metabolites and in biological fluids [9,10]. Most of these reported methods involve troublesome mobile phase (buffers) and difficult

* Corresponding author. Tel.: +91 9849059742.
E-mail addresses: mullapudilalitha@yahoo.co.in, lalli_abhi@yahoo.com (M. Lalitha Devi).

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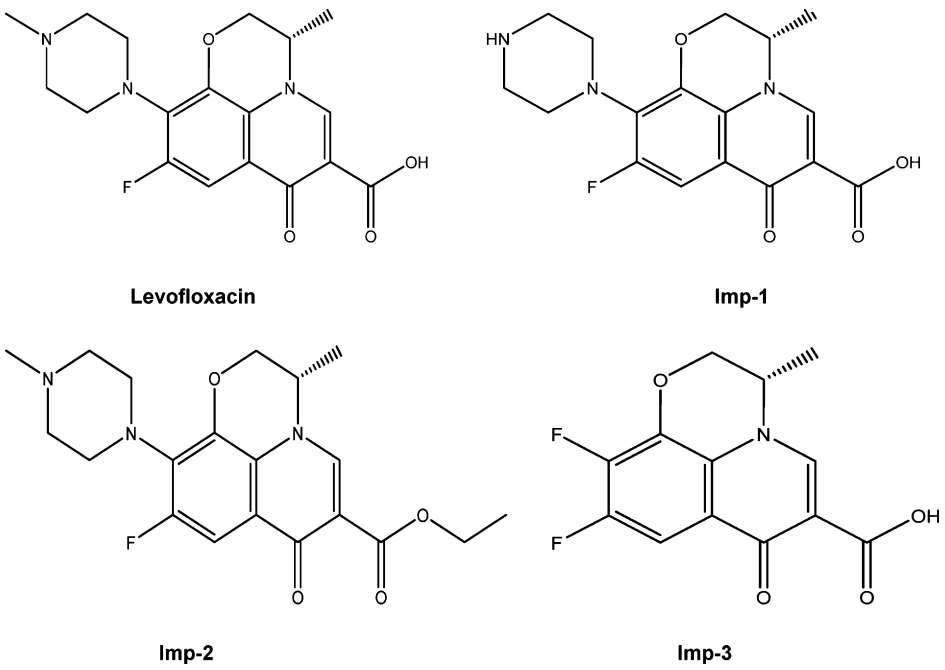

Fig. 1. Chemical structures of levofloxacin and its process related impurities.

detection methods (fluorescence, HPTLC) hence difficult for routine analysis. All these methods were developed for levofloxacin in biological fluids and there were no stability indicating methods published for the determination of levofloxacin in bulk samples in the presence of degradation products and its process related impurities. The present research work was to develop a suitable single stability indicating LC method for the determination of levofloxacin as well as its related substances and the developed LC method was validated with respect to specificity, LOD, LOQ, linearity, precision, accuracy, robustness and forced degradation studies were performed on the drug substance to show the stability indicating nature of the method and also to ensure the compliance in accordance with ICH guidelines [11].

2. Experimental

2.1. Chemicals and reagents

Samples of levofloxacin and its three process impurities (Fig. 1) were received from Bulk Actives, Unit-II of Dr. Reddy's Laboratories, Hyderabad, India.

HPLC grade methanol and acetonitrile was purchased from Rankem, Mumbai, India.

Ortho-phosphoric acid was purchased from Qualigens Fine Chemicals, Mumbai, India.

Sodium dihydrogen orthophosphate dihydrate was purchased from Qualigens Fine Chemicals, Mumbai, India.

Triethyl amine was purchased from Loba Chemie Mumbai, India.

High pure water was prepared by using Millipore Milli Q plus purification system.

2.2. Chemical names for levofloxacin and its impurities

(a) Levofloxacin: (−)-(S)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid (mol. wt.: 361.37)
(b) Imp-1: (−)-(S)-9-Fluoro-2,3-dihydro-3-methyl-10-piperazinyl-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid (mol. wt.: 347.34)
(c) Imp-2: Ethyl(−)-(S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylate (mol. wt.: 389.42)
(d) Imp-3: (−)-(S)-9,10-Difluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid (mol. wt.: 281.21)

2.3. Equipment

The LC method development, validation and forced degradation (stress) studies were done using Agilent 1200 series HPLC system with diode array detector. The data were collected and the peak purity of the levofloxacin peak was checked using Chemistation software. The photolytic degradation was carried out using Binder KBS240 photolytic chamber.

2.4. Chromatographic conditions

The chromatographic separations were achieved on ACE C18 column 250 mm length × 4.6 mm ID with 5 μm particle size using the solvent A as 25 mM sodium dihydrogen orthophosphate dihydrate with 0.5% triethyl amine pH adjusted to 6.0 with ortho-phosphoric acid in water and solvent B as methanol with a gradient programme: time (t)/% solvent B: 0/30, 20/50, 25/80, 30/80 with a post-run time of 5 min. The column temperature was maintained at 40 °C and the detection was carried out at 294 nm. The test concentration was about 300 μg mL^{-1} and the injection volume was 20 μL for related substances and 10 μL for assay determination. A degassed mixture of water and acetonitrile in the ratio of 60:40 (v/v) was used as diluent during the standard and test samples preparations.

2.5. Preparation of standard solutions

A working solution of 300 μg mL^{-1} of levofloxacin was prepared for the determination of assay and related substances analysis. Separate stock solutions of impurities (imp-1, imp-2 and imp-3) at 300 μg mL^{-1} were also prepared in diluent.

Table 1
Results of stress degradation studies.

Stress condition	Period of study	% Assay of levofloxacin	Mass balance (% assay + % impurities + % degradants)	Remarks
Acid hydrolysis (0.5N HCl at 70 °C)	7 days	96.7	99.4	Slight degradation was observed
Base hydrolysis (0.5N NaOH at 70 °C)	7 days	99.1	99.5	No degradation products formed
Oxidation (0.01% H_2O_2 at ambient temperature)	12 h	87.3	99.3	One major degradation product was formed
Water hydrolysis at 70 °C	7 days	99.5	99.8	Mild degradation observed
Thermal degradation at 100 °C	5 days	99.2	99.6	No degradation products formed
Photolytic degradation	11 days	99.3	99.7	No degradation products formed

3. Method validation

3.1. Stress studies/specificity

Stress testing of the drug substance can help to identify the likely degradation products, which can in turn help to establish the degradation pathways and the intrinsic stability of the molecule. Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities [12]. All stress degradation studies were performed at an initial drug concentration of 300 μg mL^{-1}. Acid hydrolysis was performed in 0.5N HCl at 70 °C for 7 days. The study in basic solution was carried out in 0.5N NaOH at 70 °C for 7 days. For study in neutral solution, the drug dissolved in water and was kept at 70 °C for 7 days. Oxidation studies were carried out at ambient temperature in 0.01% hydrogen peroxide for 12 h. Photo degradation studies were carried out according to Option 2 of Q1B in ICH guidelines [13]. The drug sample was exposed to light for a overall illumination of 1.2 million lux hours and an integrated near ultraviolet energy of 200 W h m^2. The drug sample was exposed to dry heat at 100 °C for 5 days. Samples were withdrawn at appropriate times and subjected to LC analysis after suitable dilution (300 μg mL^{-1}) to evaluate the ability of the proposed method to separate levofloxacin from its degradation products. Photodiode array detector was employed to check and to ensure the homogeneity and purity of levofloxacin peak in all the stressed sample solutions. Assessment of mass balance in the degraded samples was carried out to confirm the amount of impurities detected in stressed samples matches with the amount present before the stress was applied. Quantitative determination of levofloxacin was carried out in all the stressed samples against qualified working standard and the mass balance (% assay + % sum of all impurities + % sum of all degradation products) was tabulated in Table 1.

3.2. Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ for imp-1, imp-2 and imp-3 were estimated at a signal-to-noise ratio of 3:1 and 10:1, respectively [14,15], by injecting a series of diluted solutions with known concentration. Precision study was also carried at the LOQ level by injecting six individual preparations of imp-1, imp-2 and imp-3 and calculated the percentage RSD of the area.

3.3. Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of the analyte in the sample [16]. Linearity test solutions for the assay method were prepared from 50 to 150% with respect to analyte concentration 300 μg mL^{-1} (i.e. 150, 225, 300, 375 and 450 μg mL^{-1}) respectively. The peak area versus concentration data was performed by least-squares linear regression analysis. Linearity test solutions for related substance method were prepared by diluting the impurity stock solution to the required concentrations. The solutions were prepared at six concentration levels from LOQ to 200% with respect to the impurities specification level of 0.10% (i.e. LOQ, 0.05, 0.075, 0.10, 0.125, 0.15 and 0.2%). The calibration curve was drawn by plotting the peak areas of imp-1, imp-2 and imp-3 versus its corresponding concentration. Linearity test was performed for two consecutive days in the same concentration range for both assay and related substance method. The correlation coefficient of the calibration curve was calculated.

3.4. Precision

Assay method precision was evaluated by carrying out six independent assays of test sample of levofloxacin against qualified working standard and calculated the percentage of RSD. The precision of the related substance method was checked by injecting six individual preparations of levofloxacin spiked with 0.10% level of imp-1, imp-2 and imp-3 with respect to target analyte concentration (i.e. 300 μg mL^{-1}). RSD percentage of area for each imp-1, imp-2 and imp-3 was calculated. The intermediate precision of the method was also verified using different analyst, different day and different make instrument in the same laboratory.

3.5. Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found [17]. The accuracy of the assay method was evaluated in triplicate at three concentration levels, i.e. 150, 300 and 450 μg mL^{-1} in bulk drug samples. The percentage recoveries were calculated from the slope and *Y*-intercept of the calibration curve. The bulk samples available for development work do not show the presence of imp-1, imp-2 and imp-3. Standard addition and recovery experiments were conducted to determine accuracy of the related substance method for the quantification of all three impurities in bulk drug samples. The study was carried out in triplicate by spiking each impurity at 0.05, 0.10 and 0.15% in bulk drug sample solution (300 μg mL^{-1}). The percentage recoveries for imp-1, imp-2 and imp-3 were calculated from the slope and *Y*-intercept of the calibration curve.

3.6. Selectivity

The selectivity of the method was established from the resolution of the drug peak from the nearest peak and also among all the other peaks. All the degradants and impurities were separated amongst as well as from analyte with a resolution greater than 4.2 shows the selectivity of the method.

3.7. Solution stability and mobile phase stability

The solution stability of levofloxacin was carried out by leaving the test solution in a tightly capped volumetric flask at room tem-

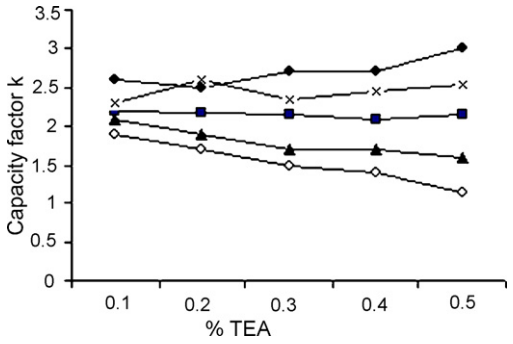

Fig. 2. Effect of triethyl amine in mobile phase on the resolution between (■) levofloxacin; (◊) imp-1; (▲) oxidative degradant; (×) imp-2; (♦) imp-3.

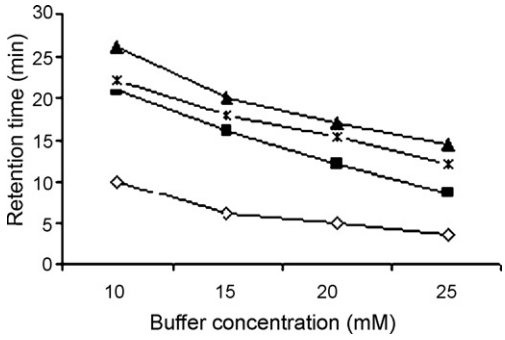

Fig. 4. Effect of buffer concentration on the retention of (■) levofloxacin; (◊) imp-1; (*) imp-2; (▲) imp-3.

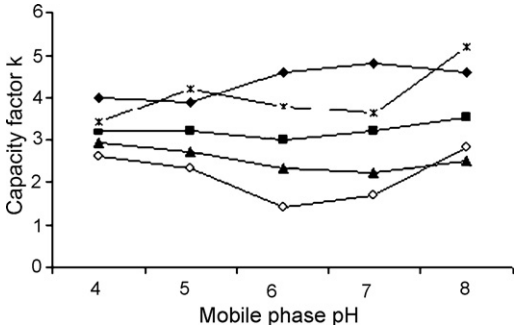

Fig. 3. Effect of mobile phase pH on the resolution between (■) levofloxacin; (◊) imp-1; (▲) oxidative degradant; (×) imp-2; (♦) imp-3.

perature for 48 h. The solution was assayed at 6 h intervals to the end of the study period, using a freshly prepared standard solution of levofloxacin for comparison each time. The mobile phase stability was also investigated by assaying the freshly prepared sample solutions against freshly prepared standard solutions at 6 h intervals up to 24 h. Mobile phase composition and preparation was kept constant during the study period. The % RSD of the assay of levofloxacin was calculated during the duration of the mobile phase and solution stability experiments.

3.8. Robustness

To evaluate the robustness of the developed method, the chromatographic conditions were deliberately altered and the resolution between closely eluting impurity, i.e. imp-2 and levofloxacin was evaluated. To study the effect of flow rate on the resolution, the flow rate was altered by 0.2 units, i.e. 0.8 and 1.2 mL min^{-1} from the actual flow 1.0 mL min^{-1}. The effect of column temperature on resolution was studied at 35 and 45 °C instead of 40 °C. The effect of pH was studied by changing pH by 0.5 units from the value 6.0 keeping remaining method conditions were constant.

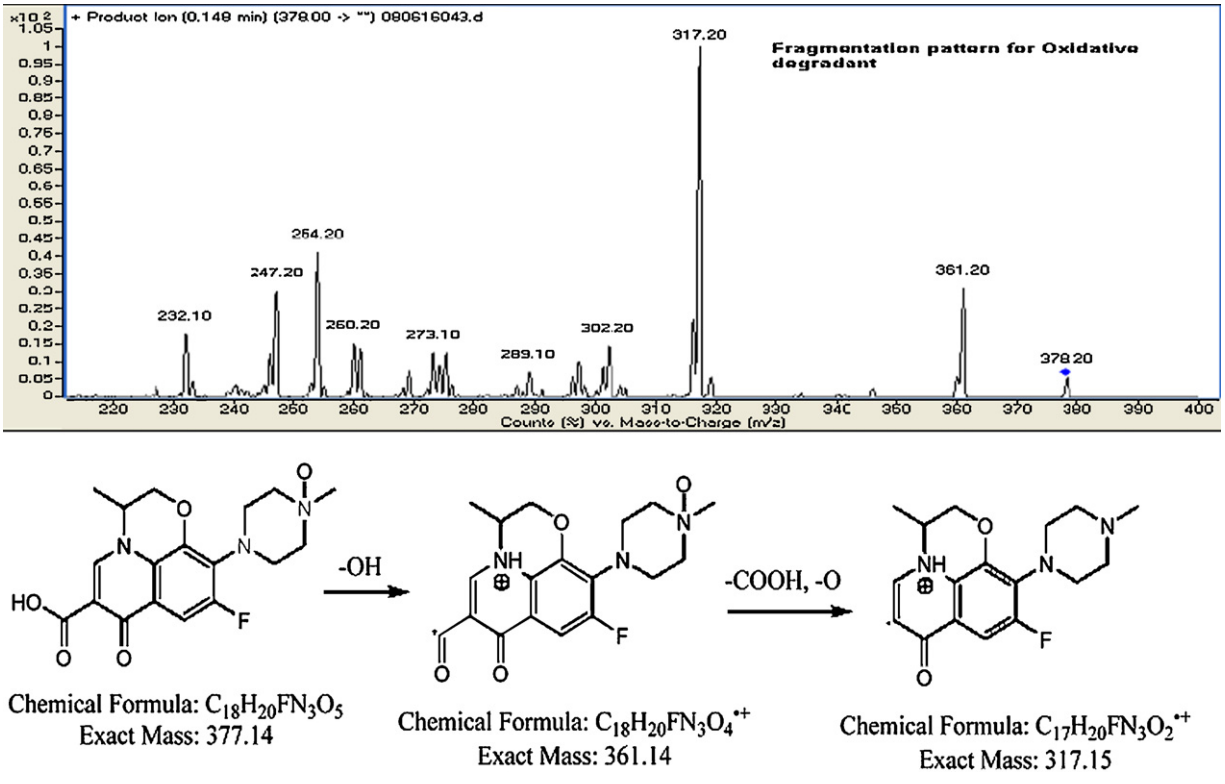

Fig. 5. Fragmentation mass spectrum of 0.50 RRT degradation product formed in oxidative degradation of levofloxacin.

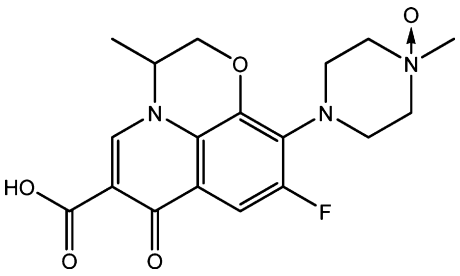

Fig. 6. Structure of 0.50 RRT degradation product (mol. wt.: 377.37) formed in oxidative degradation of levofloxacin.

4. Results and discussion

4.1. Optimization of chromatographic conditions

The main target of the chromatographic method is to get the separation of imp-1, imp-2, imp-3 and the degradation products generated during stress studies from the analyte peak. Impurities were co-eluted by using different stationary phases like C8, Cyno, XTerra and phenyl and different mobile phases containing buffers like phosphate, sulphate and acetate with different pH (4–10) and using organic modifiers like acetonitrile, methanol and ethanol in the mobile phase. Apart from the co-elution of impurities, poor peak shapes for some impurities and degradation products were also noticed. Sodium dihydrogen orthophosphate buffer with pH 6.0 and methanol at 1.0 mL min^{-1} flow was chosen for initial trail with a 250 mm length × 4.6 mm ID column and 5 μm particle size C18 stationary phase. When impurity spiked sample was injected the resolution between impurities and analyte was poor. To get the good resolution of impurities from analyte, in the phosphate buffer triethyl amine added from 0.1 to 0.5% (v/v) then pH adjusted to 6.0 at each level of triethyl amine and injected impurity spiked sample solution, from Fig. 2 at 0.5% triethyl amine level the resolution was good among impurities and analyte. At low concentrations of triethyl amine the resolution between oxidative degradant, imp-1 and also the resolution between imp-2, imp-3 was poor although they were well separated from analyte. At 0.5% (v/v) level of triethyl amine at pH 6.0 all the impurities and degradation products were well separated amongst as well as from analyte.

The effect of buffer pH (Fig. 3) was also studied under the above conditions and it was found that at higher and lower pH the tailing of the levofloxacin peak was more and also resolution was poor between impurities and degradants and also from the analyte.

The effect of buffer concentration on the retention of amsacrine and its impurities was also studied (represented in Fig. 4). At low concentration of buffer the retention time of analyte as well as impurities was very high to decrease the retention time of analyte and impurities buffer concentration was increased to 25 mM with out changing other conditions.

At these chromatographic conditions all the impurities and degradants were well separated amongst and also from levofloxacin. The effect of solvent B was also studied, when acetonitrile used instead of methanol the resolution between imp-1, oxidative degradant and resolution between imp-2, imp-3 was very poor when 100% methanol used as solvent B all the impurities were well separated. The results clearly indicated that on ACE C18 column 250 mm length × 4.6 mm ID with 5 μm particle size and solvent A as 0.5% triethyl amine in sodium dihydrogen orthophosphate dihydrate (25 mM; pH 6.0), solvent B as methanol with a gradient programme: time (*t*)/% solvent B: 0/30, 20/50, 25/80, 30/80 with a post-run time of 5 min at detection wavelength 294 nm was successful in separation of drug from its impurities and degrada-

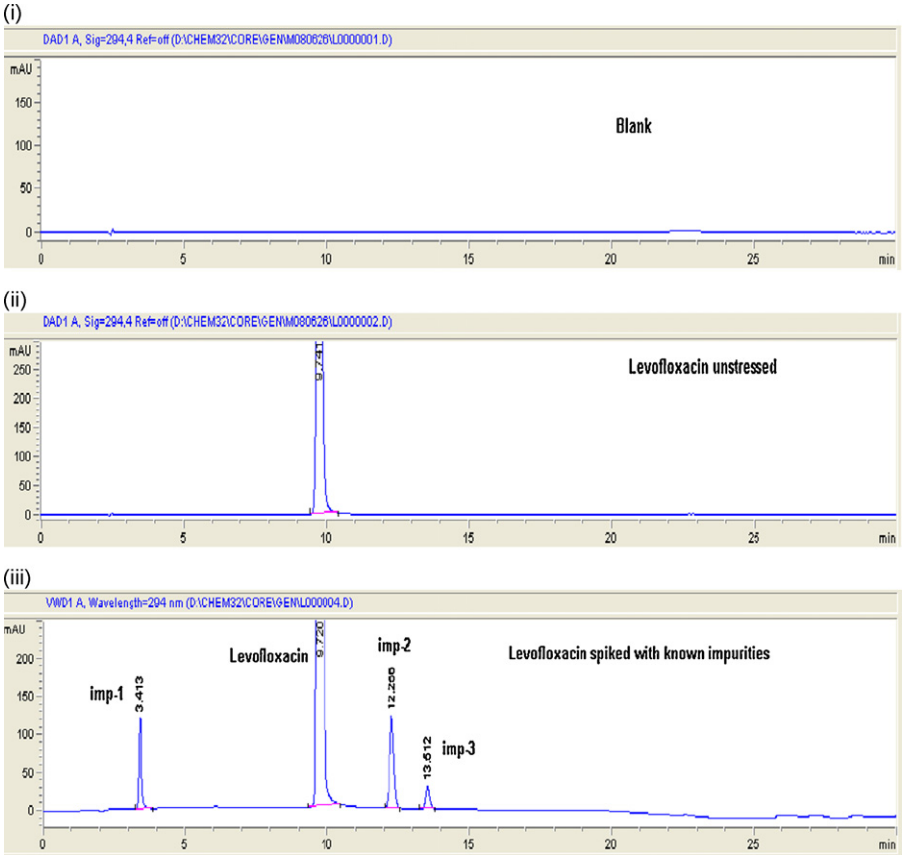

Fig. 7. Typical HPLC chromatograms of (i) blank, (ii) levofloxacin unstressed and (iii) levofloxacin spiked with known impurities.

tion products. Under the above conditions, results were as follows: retention time of levofloxacin was around 9.7 min, with a tailing factor of 1.1, number of theoretical plates (N) for the levofloxacin peak was 72659 and % RSD for five replicate injections was 0.1% the typical retention times imp-1, imp-2, imp-3 were about 3.4, 12.3, 13.5 min respectively (Fig. 7iii). Peak purity of stressed samples of levofloxacin was checked by using a photodiode array detector of Agilent 1200 series, the purity factor is with in the threshold limit in all the stress samples, demonstrating the homogeneity of analyte peak. Accelerated and long term stability study results as per ICH Q1A (R2) for levofloxacin were generated for 12 months by using the developed LC method and the results were well within the limits, this further confirms the stability indicating of the developed LC method.

4.2. Results of forced degradation

Levofloxacin was stable under stress conditions such as photolytic stress, basic hydrolysis and thermal conditions. Slight degradation was observed in acid hydrolysis, while significant degradation of the drug substance was observed under oxidative stress leads to the formation of one major unknown degradation product at 0.50 RRT (Fig. 8vi). Peak purity test results obtained from PDA confirm that the levofloxacin peak is homogeneous and pure in all the stress samples analyzed. The mass balance is a process of adding together the assay value and the levels of degradation products to see how closely these add up to 100% of initial value with due consideration of the margin of analytical error [18]. The mass balance of stressed samples was in between 99.4 and 99.8% (Table 1). The assay of levofloxacin was unaffected by the presence of imp-1, imp-2, imp-3 and degradation products thus confirms the stability indicating of the developed LC method.

4.2.1. Identification of major degradation product (at 0.50 RRT) formed in oxidative stress condition

LCMS/MS analysis was carried out for the oxidative stress sample of levofloxacin using Agilent 6410 QQQ mass spectrometer with suitable volatile buffer ammonium acetate (10 mM, pH 6.0) as mobile phase. The degradation product formed at 0.50 RRT shows the mass of 377 which is 16 higher mass than levofloxacin mass 361. The fragmentation for the degradant was also carried out for degradation product and levofloxacin using product ion scan by LCMS/MS with optimum collision energy about 25. The fragmentation pattern (Fig. 5) clearly indicates that formed degradant was N-oxide of levofloxacin which was supported by chemical properties of levofloxacin. The fragment 361.20 formed from the cleavage of CO–OH bond in acid group and the fragment 317.2 results from the cleavage of N-oxide (mass of 16) followed by loss of carbonyl group (mass of 28). Due to steric hindrances and localization of lone pair on nitrogen the N-oxide will form in piperazine ring at N-methyl position. So the probable structure as shown in Fig. 6. The N-oxide was formed due to oxidation so this impurity was reduced by adding antioxidant during purification of levofloxacin.

5. Results of method validation

5.1. Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD of imp-1, imp-2 and imp-3 were 0.027, 0.018 and 0.064 $\mu g\,mL^{-1}$ respectively (of analyte concentration, i.e. 300 $\mu g\,mL^{-1}$). The LOQ of imp-1, imp-2 and imp-3 were 0.09, 0.06 and 0.21 $\mu g\,mL^{-1}$ respectively (of analyte concentration 300 $\mu g\,mL^{-1}$).

Table 2
Results of accuracy for related substances and assay.

Compound	Level %	Spiked impurity quantity ($\mu g\,mL^{-1}$)	Recovered quantity ($\mu g\,mL^{-1}$)	% Recovery
Imp-1	0.05	0.151	0.146	96.7
	0.10	0.302	0.303	100.3
	0.15	0.453	0.436	96.2
Imp-2	0.05	0.148	0.143	96.6
	0.10	0.296	0.291	98.3
	0.15	0.444	0.449	101.1
Imp-3	0.05	0.152	0.144	94.8
	0.10	0.304	0.299	98.4
	0.15	0.456	0.451	98.9
Levofloxacin	50	149	150	100.6
	100	298	297	99.7
	150	447	445	99.1

5.2. Linearity

Linear calibration plot for the assay method was obtained over the calibration ranges tested, i.e. 50–150% of assay analyte concentration and the correlation coefficient obtained was greater than 0.9998. Linearity was checked for the assay method over the same concentration range for two consecutive days. The results show that an excellent correlation existed between the peak area and concentration of the analyte. Linear calibration plot for the related substance method was obtained over the calibration ranges tested, i.e. LOQ to 0.20% for imp-1, imp-2 and imp-3. The correlation coefficient obtained was greater than 0.9988. Linearity was checked for the related substance method over the same concentration range for two consecutive days. The results demonstrate that an excellent correlation existed between the peak area and concentration of imp-1, imp-2 and imp-3.

5.3. Precision

The RSD percentage of assay of levofloxacin during assay method precision study was well within 1.0% and the RSD percentage of area of imp-1, imp-2 and imp-3 in related substance method precision study was within 8.5%. The RSD percentage of assay results obtained in the intermediate precision study was within 1.0% and the RSD percentage of area of imp-1, imp-2 and imp-3 were within 11.3%, confirming the good precision of the developed LC method. The method precision for imp-1, imp-2 and imp-3 at LOQ level was below 11% RSD.

5.4. Accuracy

The percentage recovery of levofloxacin in bulk drug samples ranged from 99.1 to 100.6. The percentage recovery of imp-1, imp-2 and imp-3 in bulk drugs samples ranged from 94.8 to 101.1 (Table 2).

5.5. Robustness

In all the deliberate varied chromatographic conditions (flow rate, column temperature, pH variation) the resolution between levofloxacin and closely eluting impurity, i.e. imp-2 was greater than 4 and also the resolution between remaining impurities from analyte was not significantly effected hence the developed LC method was robust for the determination of levofloxacin in bulk samples.

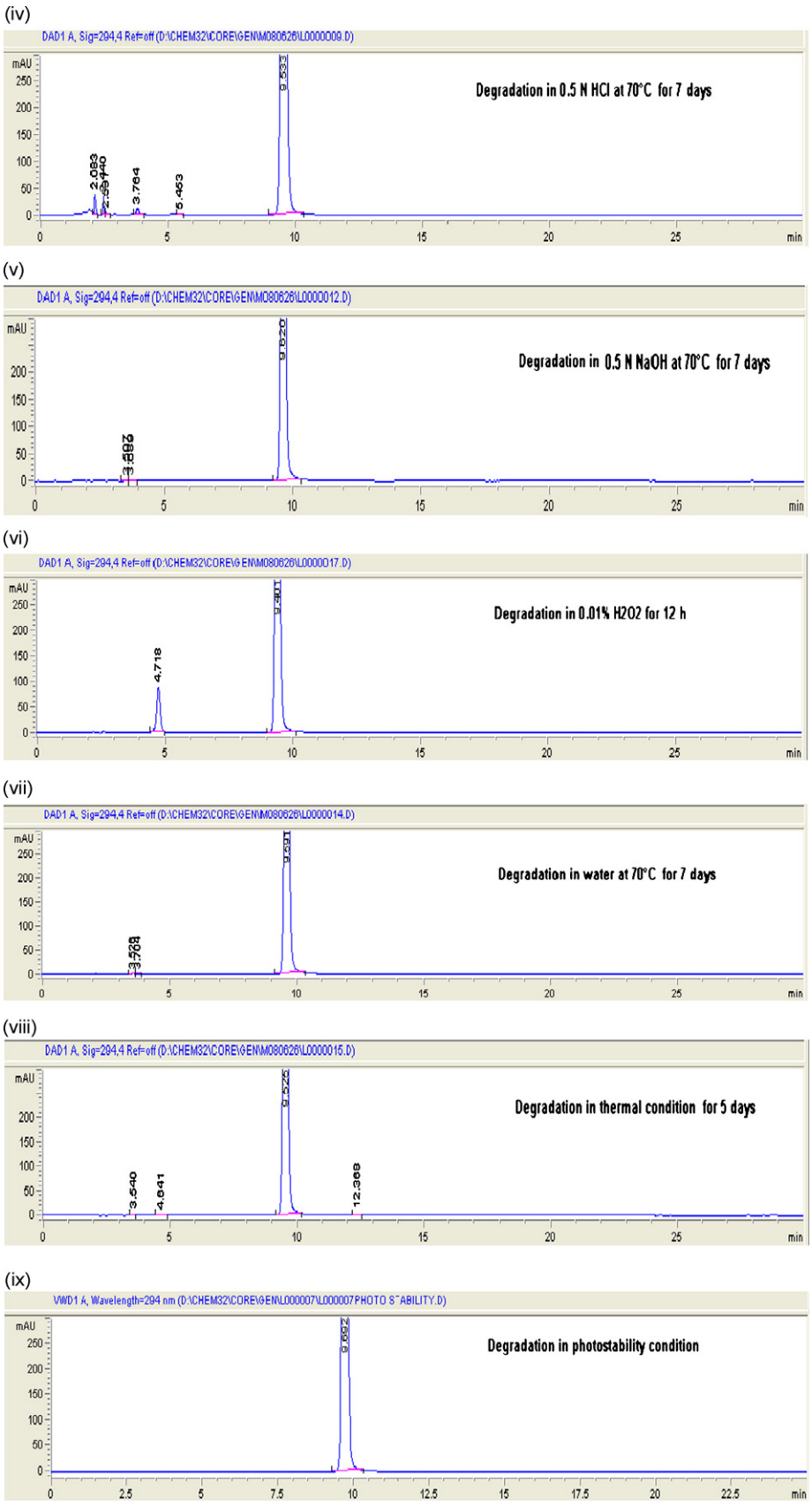

Fig. 8. Typical HPLC chromatograms of levofloxacin recorded under various stress conditions, i.e.: (iv) acidic, (v) basic, (vi) oxidative, (vii) water, (viii) thermal, (ix) photolytic stress.

Table 3
Results of stability samples (long term and accelerated) of levofloxacin (B. No.: LP001F07).

Storage condition	Period	Description	Water content by KF	Specific optical rotation (°)	Related substances by HPLC			Any unknown impurity	Total impurities	Assay on anhydrous basis
					Imp-1	Imp-2	Imp-3			
Long term condition										
Temperature	Initial	Yellowish white crystalline powder	2.3	−78	ND	ND	0.02	0.01	0.03	99.5
30 ± 2 °C	1st month	Yellowish white crystalline powder	2.4	−79	ND	ND	0.01	0.01	0.03	99.5
Relative	2nd month	Yellowish white crystalline powder	2.4	−78	ND	ND	0.02	ND	0.02	99.9
Humidity	3rd month	Yellowish white crystalline powder	2.3	−78	ND	ND	0.02	ND	0.02	99.4
65 ± 5%	6th month	Yellowish white crystalline powder	2.5	−78	ND	ND	0.01	ND	0.02	99.7
	9th month	Yellowish white crystalline powder	2.6	−79	ND	ND	0.02	ND	0.02	99.3
	12th month	Yellowish white crystalline powder	2.4	−79	ND	ND	0.02	ND	0.02	99.7
Accelerated condition										
Temperature	Initial	Yellowish white crystalline powder	2.3	−78	ND	ND	0.02	0.01	0.03	99.5
40 ± 2 °C	1st month	Yellowish white crystalline powder	2.5	−78	ND	ND	0.01	ND	0.02	99.9
Relative	2nd month	Yellowish white crystalline powder	2.6	−79	ND	ND	0.01	ND	0.02	99.8
Humidity	3rd month	Yellowish white crystalline powder	2.4	−79	ND	ND	0.02	ND	0.02	99.9
75 ± 5%	6th month	Yellowish white crystalline powder	2.4	−79	ND	ND	0.02	ND	0.02	99.7

Note: ND - not detected.

6. Application of the developed LC method to stability samples and quality monitoring of levofloxacin

Accelerated and long term stability studies are carried out to establish retest period or a shelf life of drug product, to know the effect of storage conditions at different atmospheric conditions and to show the stability indicating of the method [19]. Levofloxacin samples stored at long term condition (temperature: 30 ± 2 °C, relative humidity 65 ± 5%) accelerated (temperature: 40 ± 2 °C, relative humidity 75 ± 5%) were analyzed by using the developed LC method for period of 1 year at different intervals, i.e. initial, 1, 2, 3, 6, 9 and12 months (Table 3). And also by using the developed LC method quality of the levofloxacin was monitored during production of three batches. The results clearly indicates that the drug was stable under long term and accelerated conditions and there were no interference of the impurities for levofloxacin which demonstrates that developed LC method was stability indicating and well applied for drug stability study as well as to quality monitoring of levofloxacin.

7. Conclusions

In this paper a sensitive specific, accurate, validated and well-defined stability indicating LC method for the determination of levofloxacin in the presence of degradation products, its process related impurities was described. The behavior of levofloxacin under various stress conditions was studied, the oxidative degradant was identified by LCMS/MS and presented. All the degradation products and process impurities were well separated from the drug substance demonstrates the stability indicating of the method. The information presented here in could be very useful for quality monitoring of bulk samples and as well employed to check the quality of drug during stability studies.

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