

Validated Stability-indicating HPLC and Thin Layer Densitometric Methods for the Determination of Pazufloxacin: Application to Pharmaceutical Formulation and Degradation Kinetics

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Abstract

Two stability indicating methods were developed for the determination of pazufloxacin in presence of its acidic and alkaline degradation products, in pure form and in IV injection. The first method was based on RP-HPLC on X-terra C18 column (25 cm×4.6 mm×5 μm) using methanol and 0.5% phosphoric acid (15:85 (v/v)) with the addition of 1% Triethylamine (TEA) as a mobile phase over a concentration range 2–40 μg ml⁻¹ and a mean percentage recovery of 100.467 ± 0.595. Quantification was achieved with UV detection at 249 nm. The second method was based on TLC separation of pazufloxacin from its degradation products followed by Densitometric measurement of the intact drug spot at 249 nm. The separation was carried out on silica gel 60 F₂₅₄ aluminum sheets using chloroform, methanol and ammonia (10: 8: 2 v/v/v), as the mobile phase over a concentration range 0.1–2 μg per spot and mean percentage recovery of 99.694 ± 0.539. The two methods were simple, precise, and sensitive that could be successfully applied for the determination of pure and intravenous (IV) injection. The proposed HPLC method was used for the determination of acidic and alkaline degradation kinetics of pazufloxacin. The apparent first-order rate constants, half-life times, and activation energies of the degradation processes were calculated.

Keywords: Pazufloxacin; HPLC; TLC; Stability-indicating; kinetics

Introduction

Pazufloxacin(-)-(S)-10-(1-aminocyclopropyl)-9-fluoro-3-methyl-7-oxo-2,3-dihydro-7Hpyrido[2,3-de][1,4]benzoxazine-6-carboxylic acid monomethanesulfonate, figure 1 is a fluoroquinolone synthesized by Toyama Chemical Co. Ltd. This drug has good *in vitro* and *in vivo* activity against a broad range of bacteria, especially Gram-negative bacteria [1,2]. Clinical trials showed its intravenous injection formula was effective in treating respiratory infections [3]. Pazufloxacin is not yet the subject of a monograph in any pharmacopoeia. Reviewing the literature revealed that, few methods have been reported for the determination of pazufloxacin in raw material, pharmaceutical formulation and/or human plasma. These methods include spectrophotometric methods [4-7], spectrophotometric methods [8,9], electrochemical method [10], capillary electrophoresis [11-13], HPLC methods [14-19]. Only one of the previous methods was used as a stability indicating method for the determination of pazufloxacin in pharmaceutical formulation and in presence of its degradation products [19]. This method was of low sensitivity (10 μg ml⁻¹) and lacks either the study of degradation kinetics of pazufloxacin or the identification of the degradation products. Also the method was validated with respect to parameters including linearity, precision and accuracy only. The ICH

guideline Q1A on stability testing of new drug substances and products emphasizes that the testing of those features that are susceptible to change during storage and are unlikely to influence quality, safety and/or efficacy must be done by validated stability-indicating testing methods. It is also mentioned that forced decomposition studies (stress testing) should be performed on the drug substance to establish the inherent stability characteristics and degradation pathways to support the suitability of the proposed analytical procedures [20]. The aim of the present work is to focus on the development of efficient chromatographic methods for the determination of pazufloxacin in IV injection without interference from the excipients used and in the presence of its different degradation products in a short chromatographic run, and to study the results kinetically to prove the stability-indicating property of the method. Although HPLC method is more sensitive and less time consuming, but densitometric TLC method is technically easier and more cost effective. Also the developed densitometric TLC system can further be used in the future to separate the degradation products for their structural elucidation trying to identify the degradation pathway.

Experimental

Materials and chemicals

Pazufloxacin standard and pazufloxacin (IV) injection were purchased from Thonson technology limited, Shanghai, China.

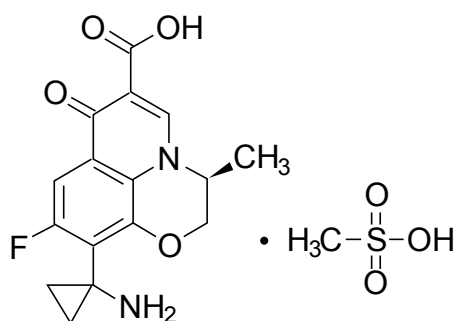


Figure 1: Chemical Structure of Pazufloxacin Mesylate.

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HPLC grade methanol, chloroform, water, phosphoric acid and ammonia were purchased from Sigma Gmbh, Germany.

Triethylamine (TEA) analytical grade was purchased from Sigma Gmbh, Germany.

Instrumentation

For TLC densitometric method, DESAGA CD 60 HPTLC densitometer connected to IBM compatible computer fitted with Proquant evaluation software for Windows was (Sarstedt-Gruppe, Germany) was used. The spots were applied using DESAGA AS30 HPTLC Applicator (Sarstedt-Gruppe, Germany) with 25 μ l Hamilton micro syringe. The used TLC plates were precoated with silica gel 60 F254 (20 \times 20 cm) (E.Merck, Germany). The plates were pre-washed with methanol and activated at 60°C for 5 min prior to chromatography. For development, Chromatographic tank (25 cm \times 25 cm \times 8 cm) was used. UV lamp, Vilber-Lourmat (VL-6-LC, EU) 365-254 nm was used for spot detection. For HPLC, Knauer instrument (Germany) equipped with K-501 pump, Knauer injector and UV-detector was used. Data acquisition was performed on Eurochrom 2000 software. The separation was done using X-terra LC-18-DB, (25 cm \times 4.6 mm \times 5 μ m) column. 0.25 μ m Disposable membrane filters were used for samples filtration. A degasser, Crest Ultrasonics was used. Mettler Toledo MP 225 pH meter 3510 pH/mV was used for pH adjustment.

Chromatographic conditions

TLC densitometric method: For TLC with UV Densitometric analysis, solutions of the tested substance were applied to silica gel 60 F₂₅₄ TLC plates 20 \times 20 using a DESAGA AS30 Applicator (Germany). Spots were applied 1.5 cm apart from each other and 2 cm from the bottom edge. The chromatographic chamber was pre-saturated with the developing mobile phase chloroform, methanol and ammonia in the ratio of (10: 8: 2 v/v/v) for 45 minutes. The spots were detected under a UV lamp at 254 nm and the drug was scanned densitometrically at 249 nm. The drug was scanned under the following instrumental conditions, photo Mode: reflection, scan Mode: Linear slit scanning, result Output: densitogram and integrated peak list, slit width: 6mm, slit height: 2 mm.

HPLC method: The mobile phase used was prepared by mixing methanol and 0.5% phosphoric acid in a ratio 15:85 with the addition of 1% (TEA). The mobile phases were filtered by vacuum filtration through 0.45 μ m filter and degassed by ultrasound sonication for 50 minutes just prior to use. The column was equilibrated with the mobile phase. The analysis was done under isocratic conditions at a flow rate 1 ml.min⁻¹ and at ambient temperature using UV detector at 249 nm.

Standard solutions

Standard solutions preparation was conducted at ambient temperature. The solutions were protected from light with aluminum foil wrapping and stored at -20°C.

Stock standard solution: It was prepared by accurately weighing 25 mg of pazufloxacin into 25-ml volumetric flask, dissolved in and diluted to volume with methanol.

Working standard solution: For TLC, pazufloxacin (200 μ g ml⁻¹) was prepared by transferring 5 ml of the previously prepared stock standard solution into 25 ml volumetric flask; the volume was then completed with methanol. For HPLC, pazufloxacin (100 μ g ml⁻¹) was prepared by transferring 2.5 ml of each of the previously prepared stock standard solutions into 25 ml volumetric flasks; the volume was then completed with mobile phase.

Calibration curves

For TLC densitometric method: Accurately measured aliquots (0.5-10.00 μ l) of working standard solution (200 μ g ml⁻¹) were applied separately to the TLC plates, in triplicates. The specified chromatographic conditions were set. After development and scanning, the average peak areas were calculated, the calibration curve, relating the integrated peak area and its corresponding concentration, was constructed and the regression equation was computed.

For HPLC method: Accurately measured aliquots (0.2-4 ml) of working standard solution were transferred into a series of 10 ml-volumetric flasks, diluted to volume with mobile phase to obtain a concentration range of (2-40 μ g ml⁻¹). 20 μ ls volume of each solution was injected, in triplicates, and the drug was analyzed using the chromatographic conditions described above and average peak areas were calculated. The calibration curve, representing the relationship between the average peak area and corresponding concentration was plotted and the regression equation was computed.

Method validation

Linearity: The linearity of response for pazufloxacin mesylate was assessed in the range of 0.1-2 μ g per spot and 2-40 μ g ml⁻¹ for standard drug for TLC and HPLC methods, respectively.

Accuracy and precision: The accuracy of an analytical method is defined as the similarity of the results obtained by this method to the true values. To test the validity of the method it was applied to the determination of pure samples of pazufloxacin over the concentration range of 0.1-2 μ g per spot and 2-40 μ g ml⁻¹ for TLC and HPLC methods, respectively. It was expressed as percent recovery [mean back-calculated concentration/theoretical concentration \times 100].

The intra-day precision was evaluated through replicate analysis of three concentrations of pazufloxacin in pure form on three successive times. The inter-day precision was also evaluated through replicate analysis of three concentrations for a period of three successive days. The precision of the methods was expressed in terms of S.D. and CV%.

Limit of detection and limit of quantitation: The limit of detection (LOD) and limit of quantitation (LOQ) of the studied drugs by the proposed methods were determined using calibration standards close to the expected LOD and LOQ. LOD and LOQ were calculated as 3.3 σ /s and 10 σ /s, respectively, where σ is the standard deviation of y-intercept of regression equation and s is the slope of the calibration curve [20].

Specificity and system suitability tests: Specificity of the method was also confirmed by its ability to measure unequivocally the drug in the presence of degradation products.

For TLC, Specificity of the method was established through the study of the resolution of the drug peak from the nearest resolving peak in stressed degradation samples. System suitability parameters as capacity factor (K), selectivity factor (α) and resolution (R_s) were calculated.

For HPLC, Specificity of the method was studied by determination of peak purity in stress acidic and alkaline degradation samples using PDA detector. The resolution factor of the drug peak from the nearest degradant peak was also calculated. The capacity factor (K), tailing factor (T) and theoretical plates number (N) were also tested.

Robustness: The robustness is a measure of method capacity

to remain unaffected by small but deliberate variations in method parameters.

For TLC, it was studied through testing the influences of small changes in mobile phase composition. Chloroform: methanol: ammonia in varying ratios (10: 6: 2 v/v/v), (11: 6: 2.5 v/v/v), (10: 7: 3 v/v/v) were tried. Changes in mobile phase volume ($\pm 10\%$), duration of saturation (± 10 minutes), time from spotting to chromatography and time from chromatography to scanning (± 10 minutes) were also studied.

For HPLC, the influences of small changes in mobile phase composition (methanol $\pm 3.00\%$), pH (± 0.20), wavelength of detection (± 2.00), flow rate (± 0.1) and column supplier. [Phenomenex-C18 (4.6 \times 250 mm)] column was used.

Application to pazufloxacin IV injection

The proposed methods were applied to the assay of pazufloxacin in IV injection. The average percent recoveries of different concentrations were calculated. It was based on the average of three replicate determinations.

The accuracy of the proposed methods was assessed by applying the standard addition technique. Known amounts of the drug were added to the pharmaceutical product. The procedure stated under linearity was then applied. The concentrations, mean recoveries and the standard deviations were calculated for each added concentration.

Accelerated acidic and alkaline degradation of pazufloxacin mesylate

The accelerated degradation in acidic and basic media was performed in the dark in order to exclude the possible degradative effect of light on the drug.

Preparation of acidic induced degradation product: Ten ml pazufloxacin stock solution were transferred into a conical flask and mixed with 10 ml 1M HCl. It was heated in a thermostatically controlled water bath at 70°C for 2 hours while fitting an air condenser. The solution was cooled and neutralized with 5M NaOH to pH (6-6.5). Then the solution was transferred quantitatively into 25 ml volumetric flask. For TLC, the volume was completed with methanol and 10 and 5 μ l of pazufloxacin were spotted on TLC plates in triplicates and analyzed using the chromatographic conditions described above. For HPLC, the volume was completed with methanol. Further dilution was carried out by transferring 10 ml to 100 ml volumetric flask and the volume was completed with mobile phase to obtain a solution containing 40 μ g ml⁻¹ of intact drug. 20 μ l were injected, in triplicates, and analyzed using the chromatographic conditions described above.

Preparation of alkaline induced degradation product: Ten ml pazufloxacin stock solution were transferred into a conical flask and mixed with 10 ml 1M NaOH. It was heated in a thermostatically controlled water bath at 70°C for 2 hours while fitting an air condenser. The solution was cooled and neutralized with 5M HCl to pH (6-6.5). Then the solution was transferred quantitatively into 25 ml volumetric flask. For TLC, the volume was completed with methanol and 10 and 5 μ l of pazufloxacin were spotted on TLC plates in triplicates and analyzed using the chromatographic conditions described above. For HPLC, the volume was completed with methanol. Further dilution was carried out by transferring 10 ml to 100 ml volumetric flask and the volume was completed with mobile phase to obtain a solution containing 40 μ g ml⁻¹ of intact drug. 20 μ l were injected, in triplicates, and analyzed using the chromatographic conditions described above.

Study of the acidic and alkaline degradation kinetics using the proposed HPLC method

Study the kinetic order of the reactions: Two sets of two different concentrations each was formed of nine replicates were prepared and passed through stressed acidic and alkaline degradation reactions. The proposed HPLC method was used to determine the remaining concentration of the drug in each solution. The degradation reactions were terminated at 15 minutes intervals by adding calculated volume of either 5M HCl or 5M NaOH to adjust the pH in the range 5.5-6.5. The study time was 120 minutes. Triplicate 20 μ L injections were made for each sample at each time interval.

Study the effect of reagent concentration on the reactions rate: The proposed HPLC method was followed using 0.5 and 1.5M HCl and 0.7 and 1.3M NaOH. The reactions were terminated by appropriate volumes of either 3M NaOH or 3M HCl. Triplicate 20 μ L injections were made for each sample at each time interval.

Study the effect of temperature on the reactions rate: The stressed degradation reactions were followed using 1M HCl and 1M NaOH at 60°C and 80°C, separately. The reactions were terminated by appropriate volumes of either 5M NaOH or 5 M HCl.

Results and Discussion

Optimization of chromatographic conditions

TLC Densitometric method: A TLC densitometric technique is suggested for the determination of pazufloxacin in the presence of its acidic and alkaline degradation products based on the difference in R_f values. Several mobile phases were tried to accomplish complete separation of pazufloxacin from its degradation products. Using the mobile phase ethyl acetate: ethanol: ammonia system in varying ratios. Significant tailing was observed in the intact drug spot which caused interference with the degradation products spots. Good resolution and complete separation of the intact drug from the degradation products peaks were achieved by using chloroform: methanol: ammonia (10: 8: 2 v/v/v). It gave a sharp and symmetric peak of pazufloxacin at R_f=0.33 with good separation of the drug peak from the degradants peaks of R=0.54, 0.64 and 0.39 for acidic degradation product (PD1) and alkaline degradation products (PD2 and PD3). A wavelength of 249 nm was used for the quantification of the drug. Representative Densitograms are shown in figure 2 and 3.

HPLC method: The developed HPLC method has been applied for the determination of pazufloxacin in presence of its acidic and alkaline degradation products. To optimize the HPLC parameters, several mobile phases' composition were tried. It is apparent that the retention increases when the pH of the mobile phase is increased. This is because the compounds are less ionized at high pH and thus have more affinity for the stationary phase. By the use of acetonitrile in concentration above 18% at pH 2.5 the retention time decreased to 2.4 minutes but the resolution of pazufloxacin from its alkaline degradation products decreased. The use of methanol instead of acetonitrile as organic modifier led to further increase in the retention of pazufloxacin to 5 minutes.

The chosen mobile phase was methanol: 0.5% phosphoric acid (15:85) with the addition of 1% TEA to the aqueous phase with a flow rate 1ml.min⁻¹.

Figure 4 and 5 show the chromatograms of pazufloxacin with PD1, PD2 and PD3. The average retention times \pm SD, for 3 replicate

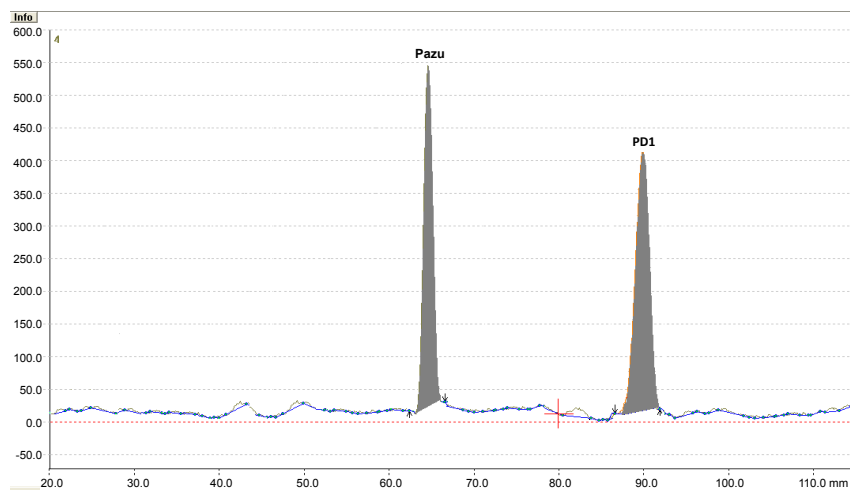


Figure 2: TLC densitogram of pazufloxacin stressed acidic degradation sample.

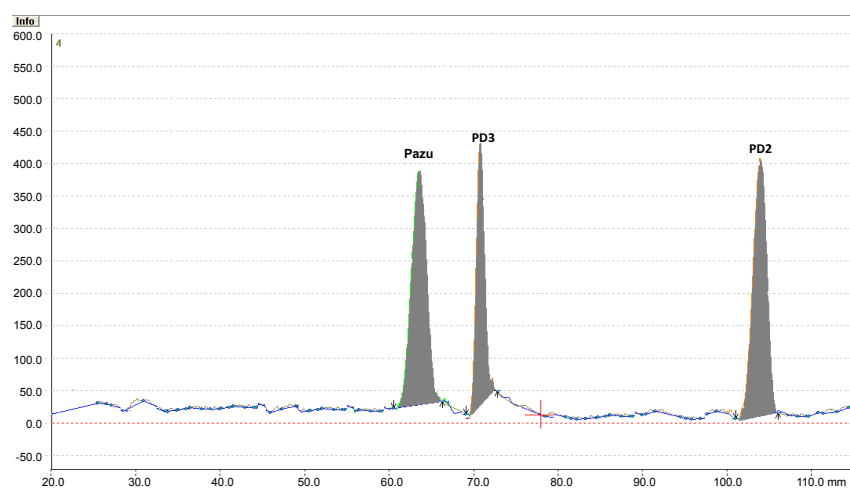


Figure 3: TLC densitogram of pazufloxacin stressed alkaline degradation sample.

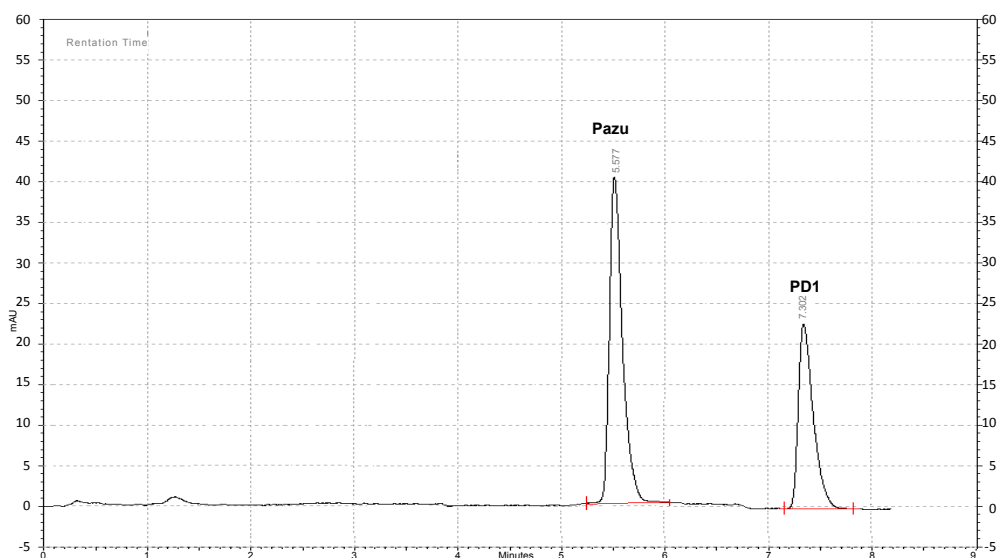


Figure 4: HPLC chromatogram of pazufloxacin stressed acidic degradation sample.

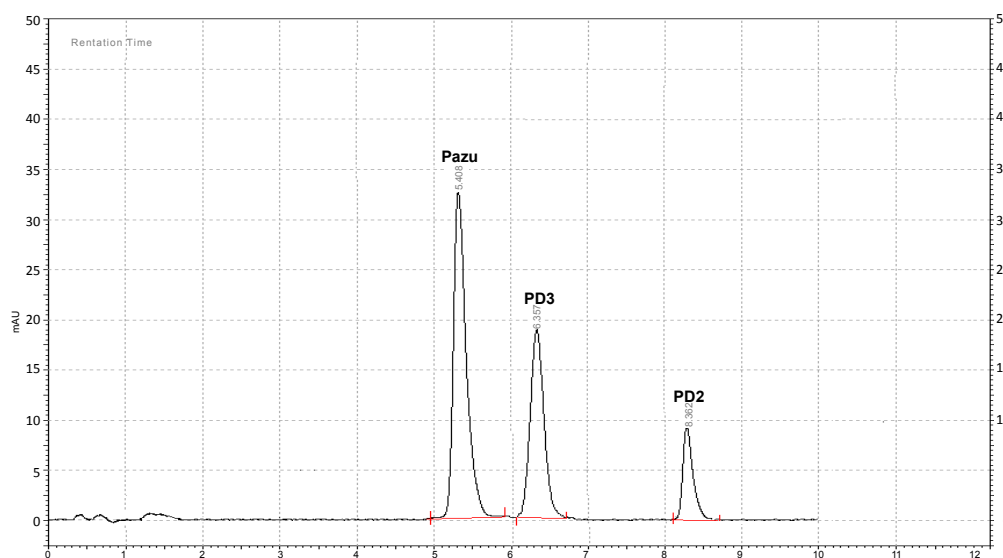


Figure 5: HPLC chromatogram of pazufloxacin stressed alkaline degradation sample.

Parameters	TLC densitometric method [n=11]	HPLC method [n=10]
Linearity range	0.100-2.00 µg per spot	2-40 µg.ml ⁻¹
Correlation coefficient	0.9996	0.9998
Detection limit (LOD)	0.033	0.593
Quantitation limit (LOQ)	0.100	1.790
Slope ± SD	1902.5 ± 13.555	3.4813 ± 0.0275
Confidence limit of slope ^a	1900.3-1905.7	3.461-3.500
S.E. of slope	4.087	0.0087
Intercept ± SD	86.803 ± 19.025	0.2747 ± 0.626
Confidence limit of intercept ^a	85.765-87.435	-0.183-0.732
S.E. of intercept	5.736	0.198

a: 95% confidence limit

Table 1: Linear regression data for the calibration curves.

injections of Pazufloxacin and µµ (PD1) were found to be 5.553 ± 0.131 and 7.266 ± 0.083 ; respectively.

The average retention times ± SD, for 3 replicate injections of Pazufloxacin and its alkaline degradation products (PD2, PD3) were found to be 5.452 ± 0.086 , 6.231 ± 0.056 and 8.311 ± 0.097 ; respectively.

Method validation

Linearity: The linear regression data for the calibration curves (n=6) showed good linear relationship over the concentration range of 0.1-2 µg per spot and 2-40 µg.ml⁻¹ for standard drug for TLC and HPLC methods, respectively.

Characteristic parameters for regression equations and correlation coefficients were given in table 1. The linearity of the calibration graphs were validated by the high value of correlation coefficients of the regression.

Accuracy and precision: The accuracy and precision of the developed methods were expressed in terms of recovery% and %RSD, respectively. Table 2 summarizes the accuracy, intra and inter-day precision of pazufloxacin. The low values of SD and % RSD demonstrate excellent precision of the methods.

Limit of detection and limit of quantitation: For calculation

of LOD and LOQ, the standard deviation of response method based on the standard deviation of intercept was used. They were found to be 0.033 and 0.100 µg per spot, respectively, for TLC densitometric method and 0.593 and 1.790 µg ml⁻¹, respectively for HPLC method which indicates the adequate sensitivity of the methods.

Specificity and system suitability tests: For TLC, the densitograms of stressed samples of pazufloxacin showed separated spots of the drug from the nearest degradants. This indicates that the method is sufficiently specific to the drug.

For HPLC, Peak purity test results determined by PDA detector under the optimized chromatographic conditions, confirmed that no additional peaks were co-eluted with the drug in stressed degradation samples and evidencing the ability of the method to assess the drugs of interest in the presence of stressed samples.

The results of the system suitability tests in table 3 assured the adequacy of the proposed TLC and HPLC methods for the routine analysis of pazufloxacin and the methods capacity remains unaffected by small variations in methods parameters.

Robustness: The S.D, %RSD and S.E. of the peak areas for each parameter at a concentration level 1 µg per spot and 20 µg ml⁻¹ for TLC and HPLC, respectively, are summarized in table 4. The low values

	TLC densitometric method ^a					HPLC method ^a				
	Concentration $\mu\text{g per spot}$	Recovery%	SD	%R.S.D.	Concentration $\mu\text{g.ml}^{-1}$	Recovery%	SD	%R.S.D.		
Intra-day	0.300	100.667	0.004	1.325	6.000	100.500	0.065	1.078		
	1.200	99.667	0.031	2.592	20.000	99.450	0.211	1.061		
	2.000	99.250	0.042	2.116	35.000	100.886	0.432	1.223		
	0.300	1 st day	99.000	0.005	1.684	6.000	1 st day	99.670	0.068	1.137
		2 nd day	101.333	0.004	1.316		2 nd day	98.500	0.072	1.218
		3 rd day	99.667	0.006	2.007		3 rd day	98.000	0.075	1.276
	1.200	1 st day	100.917	0.032	2.642	20.000	1 st day	99.550	0.266	1.336
		2 nd day	100.500	0.027	2.239		2 nd day	100.550	0.301	1.497
		3 rd day	99.083	0.029	2.439		3 rd day	99.850	0.289	1.447
	2.000	1 st day	97.700	0.042	2.149	35.000	1 st day	98.770	0.511	1.478
		2 nd day	101.050	0.038	1.880		2 nd day	100.340	0.587	1.671
		3 rd day	102.250	0.045	2.200		3 rd day	98.91	0.603	1.742

^aEach result is an average of three determinations.

Table 2: Accuracy and precision of TLC densitometric and HPLC methods.

TLC densitometric method			HPLC method		
Parameters	Acidic degradation	Alkaline degradation	Parameters	Acidic degradation	Alkaline degradation
Capacity factor [K']	2.211	2.015	Capacity factor [K']	4.567	4.411
Selectivity factor [α]	2.388	1.301	Selectivity factor [α]	1.377	1.215
Resolution [R]	4.714	1.667	Resolution [R]	3.833	1.898
			Theoretical plates [N]	3110.29	2924.65
			Tailing factor [t]	1.25	1.25
			Peak purity	0.996	0.997

Table 3: System Suitability results of TLC densitometric and HPLC methods.

	Parameters	S.D. [peak area]	% R.S.D.	S.E.
TLC densitometric method	Mobile phase composition	0.415	0.021	0.169
	Mobile phase volume ($\pm 10\%$)	0.799	0.040	0.326
	Time of saturation (± 10 minutes)	0.225	0.011	0.092
	Time from spotting to development (± 10 min)	0.309	0.015	0.126
	Time from chromatography to scanning (± 10 min)	0.319	0.016	0.130
HPLC method	Change in organic phase percent in mobile phase ($\pm 3.00\%$)	0.383	0.542	0.156
	Change in mobile phase pH (± 0.20)	0.187	0.265	0.076
	Change in wavelength of detection (± 2.00)	0.137	0.194	0.056
	Change in flow rate (± 0.1)	0.375	0.531	0.153
	Change column supplier	0.467	0.661	0.191

Table 4: Robustness of TLC densitometric and HPLC methods [n=6].

obtained after introducing small deliberate changes in the developed methods.

Application to pazufloxacin IV injection

The proposed methods were successfully applied for the determination of pazufloxacin in its pharmaceutical formulation. No interaction was observed between the drug and excipients present in the formulation. A single spot at $R_f=0.33$ was observed in the densitogram of pazufloxacin samples extracted from the pharmaceutical formulation.

TLC densitometric method		HPLC method	
Added standard ($\mu\text{g per spot}$)	Recovery %	Added standard ($\mu\text{g/ml}$)	Recovery %
0.2	100.450	5.000	101.740
0.6	98.717	10.000	99.210
1	99.650	20.000	99.490
1.4	99.114	30.000	100.040
1.8	99.500	40.000	99.160

Table 5: Application of standard addition technique on pazufloxacin IV injection using TLC densitometric and HPLC methods [n=3].

	TLC densitometric method	HPLC method	Reported method [5]
Mean	98.654	100.125	99.651
SD	1.0221	0.8765	1.0881
n	6	6	6
Variance	1.0447	0.7683	1.1839
t [2.228]*	1.64	0.83	
F [5.05]*	1.1332	1.541	

Table 6: Statistical comparison between the results obtained by the proposed TLC densitometric and HPLC methods with the reported method [5] for the analysis of pazufloxacin in IV injection.

The standard addition technique was applied to assess the accuracy of the proposed methods as shown in table 5. Statistical comparison showed that there was no significant difference between the results obtained from the proposed methods and those obtained from the reported method [5] as shown in table 6, since the calculated t and F values are less than the tabulated ones.

Study of stressed acidic and alkaline degradation of pazufloxacin

Kinetic studies of pazufloxacin have not been previously investigated. It was investigated in 1M HCl and 1M NaOH using the proposed HPLC method for a period of 2 hours at 15 minutes interval. A decrease in concentration of drug with increasing time was observed. To study the effect of the reagent concentration on the reaction rate, experiments were performed using 3 different concentrations of HCl and NaOH that are always in large excess with respect to the drug. (Figure 6 and Figure 7) and table 7 show that the reaction rate was increased by uplifting the reagent concentration. For each experiment k_{obs} , $t_{1/2}$ and t_{90} (time where 90% of original concentration of the drug is left) were determined. The influence of temperature on the acid and alkaline degradation process of pazufloxacin was investigated at 60,

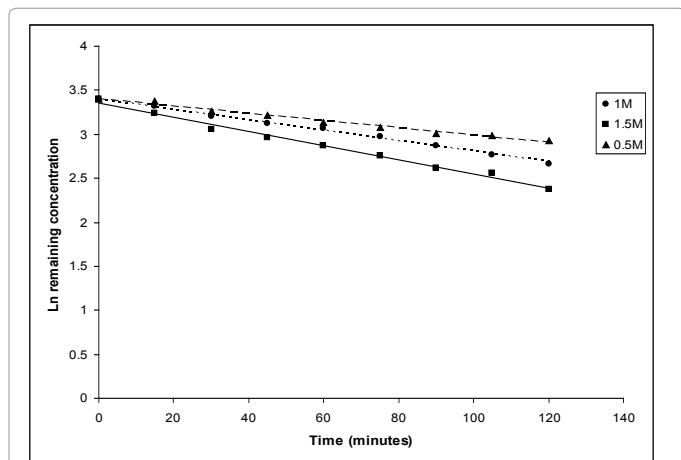


Figure 6: Effect of HCl concentration on the degradation of Pazufloxacin using 0.5, 1 and 1.5M HCl.

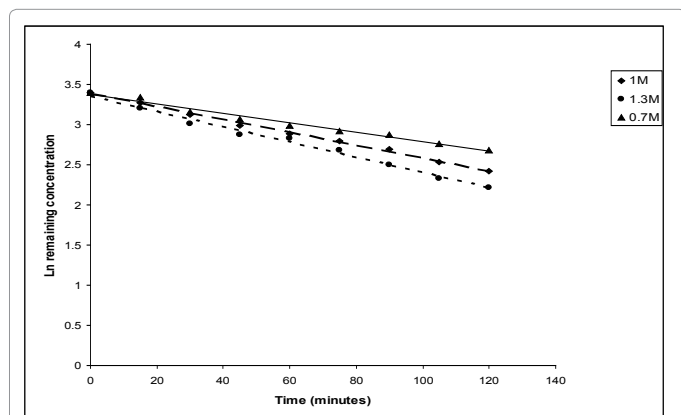


Figure 7: Effect of NaOH concentration on the degradation of Pazufloxacin using 0.7, 1 and 1.3M NaOH.

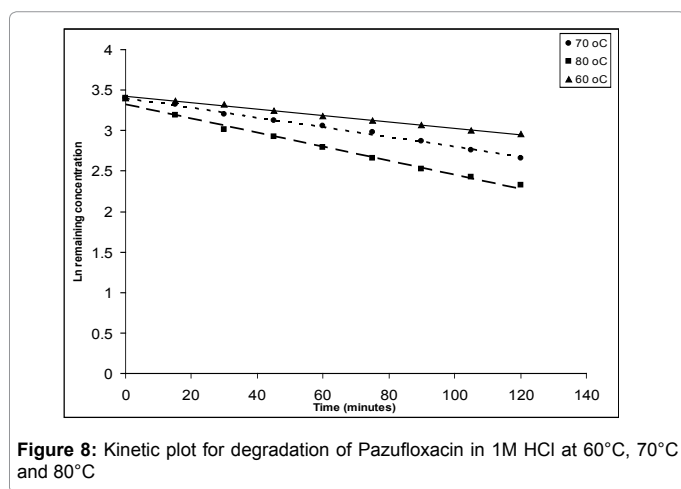


Figure 8: Kinetic plot for degradation of Pazufloxacin in 1M HCl at 60°C, 70°C and 80°C

70, 80°C below 60°C the reaction is too slow to be monitored. At the selected temperatures the degradation process followed pseudo first order kinetics (Figures 8 and 9). Data obtained from first order kinetics treatment were further subjected to fitting in Arrhenius equation

$$\ln K = \ln A - E_a/RT$$

Where, K specific reaction rate (min^{-1}), A constant known as Arrhenius factor or frequency factor (min^{-1}), E_a energy of activation (Kcal/mol), R gas constant (1.987 cal/deg.mole) and T absolute temperature

The plot of $\ln k_{\text{obs}}$ versus $1/T$ gave the Arrhenius plots (Figures 10 and 11) which were found to be linear in the temperature range 60-80°C. From the regression equations, the activation energy and the frequency factor were calculated for the acidic and alkaline degradation of the drug. An extrapolation to Arrhenius plot is used to calculate the degradation rate constants at room temperature ($25^\circ\text{C} \pm 2$). The half-life and t_{90} were also calculated at room temperature as shown in tables 8 and 9. The obtained data suggests that the drug is susceptible to acidic and alkaline degradation.

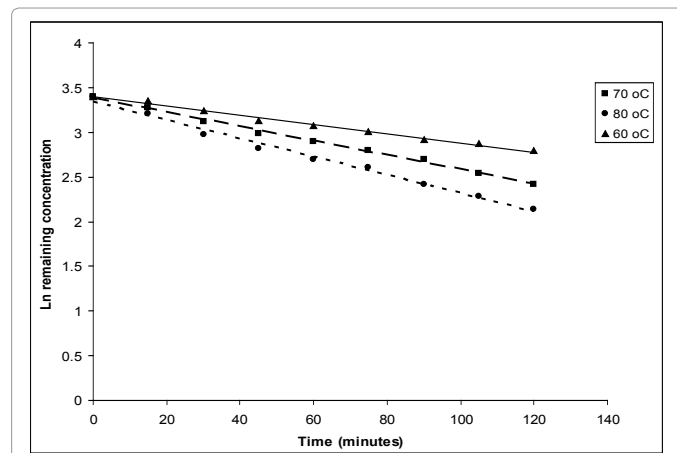


Figure 9: Kinetic plot for degradation of Pazufloxacin in 1M NaOH at 60°C, 70°C and 80°C

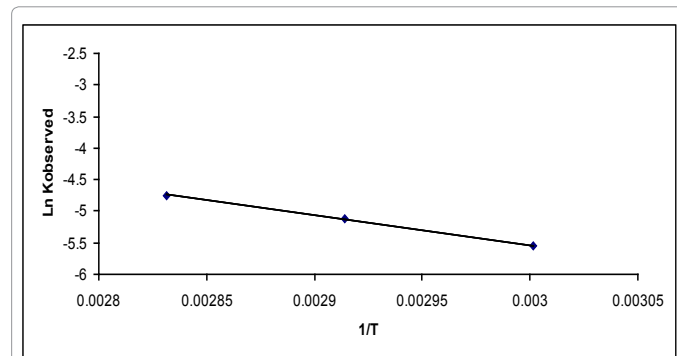


Figure 10: Arrhenius plot for acidic degradation of Pazufloxacin in 1M HCl.

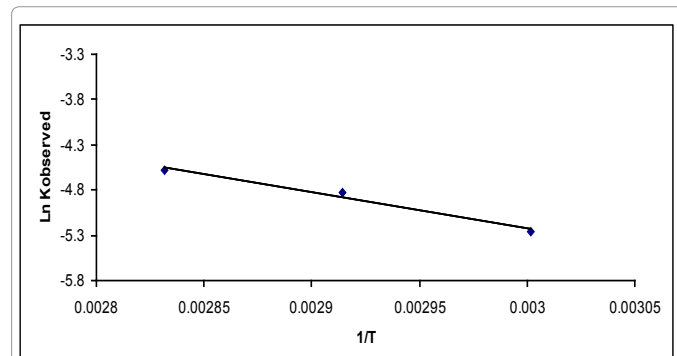


Figure 11: Arrhenius plot for alkaline degradation of Pazufloxacin in 1M NaOH.

Parameter		K_{observed} (min ⁻¹)	$t_{1/2}$ (min)	t_{90} (min)
Acid-induced degradation	0.5 M	0.0041	169.024	25.609
	1 M	0.006	115.500	17.500
	1.5 M	0.0081	85.556	12.963
Alkaline-induced degradation	0.7 M	0.0059	117.458	17.797
	1 M	0.008	86.625	13.125
	1.3 M	0.0095	72.947	11.053

Table 7: Kinetic data of pazufloxacin acidic and alkaline degradation at 70°C.

Parameter		K_{observed} (min ⁻¹)	$t_{1/2}$ (min)	t_{90} (min)
Acid-induced degradation	60°C	0.0039	177.692	26.923
	70°C	0.006	115.500	17.500
	80°C	0.0087	79.655	12.069
Alkaline-induced degradation	60°C	0.0052	133.269	20.192
	70°C	0.008	86.625	13.125
	80°C	0.0102	67.941	10.294

Table 8: Kinetic data of Pazufloxacin acidic and alkaline degradation in the presence of 1M HCl and 1M NaOH at different temperatures.

Parameters	1M HCl	1M NaOH
Activation energy, E_a (Kcal mol ⁻¹)	9.382	7.894
Degradation rate constant k_{25} (min ⁻¹)	0.00074	0.00132
Half life, $t_{1/2}$ (min)	936.487	525.000
Shelf life, t_{90} (min)	141.892	79.545
Arrhenius frequency factor, A (min ⁻¹)	5611.649	806.981

Table 9: Kinetic data of Pazufloxacin degradation at 25°C.

Conclusion

The goal of this work was achieved by separating and quantitation the new fluoroquinolone pazufloxacin in presence of its acidic and alkaline degradation products in bulk powder and in IV infusion. HPLC and TLC densitometric methods have been developed and validated for the determination of the drug without any interference from excipients and in presence of degradation products. They provide significant sensitivity as well as significant decrease in sample preparation, instrument run time over other separation methods.

References

- Muratani T, Inoue M, Mitsunashi S (1992) In vitro activity of T-3761, a new fluoroquinolone. *Antimicrob Agents Chemother* 36: 2293-2303.
- Fukuoka Y, Ikeda Y, Yamashiro Y, Takahata M, Todo Y, et al. (1993) In vitro and in vivo antibacterial activities of T-3761, a new quinolone derivative. *Antimicrob Agents Chemother* 37: 384-392.
- Higa F, Akamine M, Haranaga S, Tohyama M, Shinzato T, et al. (2005) In vitro activity of pazufloxacin, tosufloxacin and other quinolones against Legionella species. *J Antimicrob Chemother* 56: 1053-1057.
- Rewatkar NS, Dinesh R, Mahavir C, Ghante H, Umkar AR (2011) Development of UV spectrophotometric methods for estimation of Pazufloxacin in infusion form using absorbance ratio method Research. *Journal of Pharmaceutical, Biological and Chemical Sciences* 2: 456-463.
- Rewatkar NS, Umkar AR, Chaple D, Ghante H (2011) Validated spectrophotometric determination of pazufloxacin in formulation. *J Pharm Res* 4: 1394-1395.
- Wang X, Chen S, Zhao H, Jin L (2005) Europium sensitized chemiluminescence determination of pazufloxacin mesylate in urine and serum. *Anal Lett* 38: 971-979.
- Yang C, Zhang Z, Chen S, Yang F (2007) Molecularly imprinted on-line solid-phase extraction combined with chemiluminescence for the determination of pazufloxacin mesylate *Mikrochim Acta* 159: 299-304.
- Jin J, Zhang X (2008) Spectrophotometric studies on the interaction between pazufloxacin mesilate and human serum albumin or lysozyme. *J Lumin* 128: 81-86.
- Chen S, Ma H, Zhao H, Feng R, Jin L (2004) Terbium-sensitized fluorescence method for the determination of pazufloxacin mesilate and its application. *Anal Sci* 20: 1075-1078.
- Lu Z, Rong YG, Ruo Y, YaQin C, SuMing C. (2007) A capacitive sensor based on molecularly imprinted polymers and poly(p-aminobenzene sulfonic acid) film for detection of pazufloxacin mesylate *Sci China Ser B-Chem*. 50: 547-553.
- Zu LZ, Jian JL, Ling BQ, Ran Y (2008) Determination of Pazufloxacin Mesylates by Capillary Electrophoresis with Electrochemiluminescence Detection. *Chinese J Anal Chem* 36: 941-946.
- Zhou S, Ouyang J, Baeyens WR, Zhao H, Yang Y (2006) Chiral separation of four fluoroquinolone compounds using capillary electrophoresis with hydroxypropyl-beta-cyclodextrin as chiral selector. *J Chromatogr A* 1130: 296-301.
- Wang Y, Baeyens WR, Huang C, Fei G, He L, et al. (2009) Enhanced separation of seven quinolones by capillary electrophoresis with silica nanoparticles as additive. *Talanta* 77: 1667-1674.
- Li Q, Wang R, Pei F (2004) Determination of pazufloxacin mesylate in human plasma and urine by high performance liquid chromatography. *Asian Journal of Drug Metabolism and Pharmacokinetics Asian Journal of Drug Metabolism and Pharmacokinetics* 4: 289-294.
- Zhang Z, Yang G, Wang D, Liang G, Chen Y (2004) Chiral Separation and Enantiomeric Purity Determination of Pazufloxacin Mesilate by HPLC Using Chiral Mobile Phase Additives. *J Liq Chromatogr Relat Technol* 27: 813-827.
- Zhang H, Ren Y, Bao X (2009) Simultaneous determination of (fluoro) quinolones antibacterials residues in bovine milk using ultra performance liquid chromatography-tandem mass spectrometry. *J Pharm Biomed Anal* 49: 367-374.
- Watabe S, Yokoyama Y, Nakazawa K, Shinzaki K, Hiraoka R, et al. (2010) Simultaneous measurement of pazufloxacin, ciprofloxacin, and levofloxacin in human serum by high-performance liquid chromatography with fluorescence detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 878: 1555-1561.
- Phapale PB, Lee HW, Kim SD, Lim MS, Kale DD, et al. (2010) Analysis of Pazufloxacin Mesilate in Human Plasma and Urine by LC with Fluorescence and UV Detection, and Its Application to Pharmacokinetic Study *Chromatographia* 71: 101-106.
- Rewatkar NS, Umkar AR, Chaple D, Ghante H (2011) A validated stability-indicating RP-HPLC method for the estimation of pazufloxacin in presence of its degradation products. *J Pharm Res* 4: 3060-3062.
- http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6_R1/Step4/E6_R1_Guideline.pdf