Stability indicating RP-HPLC method for simultaneous determination of piroxicam and ofloxacin in binary combination

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Abstract: A simple and precise RP-HPLC method was developed for simultaneous determination of piroxicam and ofloxacin in pharmaceutical formulations and human serum. Optimum separations of piroxicam, ofloxacin and stress-induced degradation products were achieved by use of Hypersil BDS C8 column (250 x 4.6mm, 5µm). The mobile phase was a mixture of acetonitrile: 0.012M K_2HPO_4: 0.008M sodium citrate (both buffers mixed and pH adjusted to 2.8) (50:25:25 v/v/v) delivered at flow rate of 1.5mL min^{-1} using DAD at 254nm. Response was linear function of concentration over the ranges of 70-130mg mL^{-1} for piroxicam and ofloxacin ($r^2 \geq 0.999$). The method efficiently separated the analytical peaks from degradation products with acceptable tailing and resolution. The developed method was successfully used for concurrent analysis of piroxicam and ofloxacin in pharmaceutical formulations, human serum and *in vitro* drug interaction studies.

Keywords: Piroxicam, ofloxacin, degradation products, drug-drug interaction.

INTRODUCTION

Piroxicam is oxicam derivative, commonly used as non steroidal anti-inflammatory drug (NSAID). It is chemically designated as 4 - Hydroxy - 2 - methyl - N - (2 -pyridyl) - 2H - 1, 2 - benzothiazine - 3 - carboxamide 1,1-dioxide (fig. 1A)**.** It is used in joint and musculoskeletal disorders such as osteoarthritis, rheumatoid arthritis including juvenile idiopathic arthritis, ankylosing spondylitis, in soft tissues disorders, in acute gout, and in postoperative pain (Reynolds 2009). Ofloxacin (fig. 1B) is chemically designated as 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**.** It is a fluoroquinolone antibacterial used for the treatment of conjunctivitis and corneal ulcers caused by susceptible strains of bacteria. It is also used for the treatment of otitis externa and otitis media (Reynolds 2009).

Various analytical methods have been reported for piroxicam and ofloxacin either alone or in combination with other drugs. Piroxicam has been determined by high performance liquid chromatography (HPLC) (Kurakula *et al*. 2011; Jiang *et al*. 1991; Bartsch *et al*. 1999; Basan *et al*. 2001) and UV/VIS spectrophotometry (Basan *et al*. 2001; Rele *et al*. 2010). The analytical methods existed for ofloxacin includes determination by spectrophotometry (Abbasi *et al*. 2010), HPLC (Maraschiello *et al*. 2001; Ohkubo *et al*. 1992; Fabre *et al*. 1994), microbiological assay (Ev and Schapoval 2002), and flow-injection analysis (Rao *et al*., 2002).

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The combination of piroxicam and ofloxacin has not been adopted by any official pharmacopoeia. An extensive review of the literature also resulted in finding no stability indicating HPLC method for simultaneous determination of both drugs in pharmaceutical formulations, human serum and drug interaction studies. So attempts were made to develop and validate a precise and accurate reverse phase stability indicating HPLC method for simultaneous determination of both drugs in pharmaceutical formulations, human serum and drug interaction studies. We are currently engaged in binary combination analysis of different classes of drugs in pharmaceutical formulations and human plasma (Ashfaq *et al*., 2007; Ashfaq *et al*., 2008; Ashfaq *et al*., 2013; Ashfaq *et al*., 2014; Khan *et al*., 2008; Khan *et al*., 2010a,b; Khan *et al*., 2012; Khan *et al*., 2013; Khan *et al*., 2014; Qutab *et al*., 2007a, b; Qutab *et al*., 2009; Razzaq *et al*., 2012a, b, c, d; Razzaq *et al*., 2013; Sharif *et al*., 2010). The present work is the continuation of the work we have already reported.

MATERIALS AND METHODS

Chemicals and reagents

Reference standards of piroxicam, ofloxacin, moxifloxacin and sparfloxacin with stated purity of 99.69%, 99.48%, 99.72% and 99.89% respectively, were kindly gifted by Lahore Chemical & Pharmaceutical Works (Pvt.) Ltd., (Lahore, Pakistan). Acetonitrile (HPLC grade), sodium hydroxide, dipotassium hydrogen phosphate, sodium citrate, hydrochloric acid and potassium permanganate (analytical reagent grade) were procured from Central Chemicals Lahore, Pakistan (Fluka origin). Double distilled water was used during method

development and validation. Mobile phase was filtered through 0.45µm nylon filters by Millipore (USA).

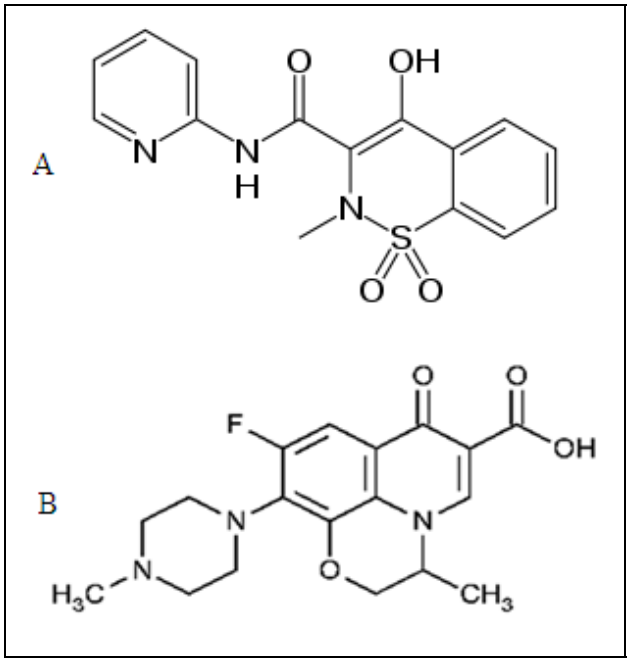

Fig. 1: Chemical structure of (A) Ofloxacin and (B) Piroxicam

Equipment and chromatographic conditions

For method development Hitachi (Tokyo, Japan) HPLC system was utilized. The system was equipped with the auto sampler L-7200 with 100µL loop, UV detector L-7400 and pump L-7100. Separations were optimized on Hypersil BDS C8 column (250 x 4.6mm, 5 µm). Mobile phase consisted of acetonitrile: 0.012M K_2HPO_4 : 0.008M sodium citrate (mixed both buffers and adjusted to pH 2.8 with dilute phosphoric acid) (50:25:25 v/v/v). Mobile phase was degassed using Elma-E30H elma-sonic (Germany) prior to analysis. Mobile phase flow rate was optimized 1.5mL min^{-1} and all the experiments were performed at room temperature.

Preparation of standard stock solution (A)

Standard stock solution (A) of piroxicam and ofloxacin was prepared by taking 100 mg each of piroxicam and ofloxacin in 100mL volumetric flask diluted to the mark with mobile phase. The final solution was sonicated for 8-10 minutes to obtain final concentration of solution equivalent to 1000µg mL^{-1} each of piroxicam and ofloxacin.

Preparation of working standard solution (B)

5 mL of standard stock solution (A) was diluted to 50mL with mobile phase to prepare working standard solution (B) 100 mg mL^{-1} each of piroxicam and ofloxacin. The solution was filtered through 0.45µm nylon filter before analysis.

Preparation of piroxicam and ofloxacin tablets

Piroxicam and ofloxacin tablets were prepared by mixing 100 g each of piroxicam and ofloxacin with 100 g micro crystalline cellulose, 100 g lactose, 5 g talcum and 5g magnesium stearate. The final powder was compacted using roller compactor GK 100 (China). The final granules were compressed into tablets using rotary compression machine ZP 29 (China). The compression weight of each tablet was 410 mg. Each tablet contained ofloxacin 100 mg and piroxicam 10 0mg.

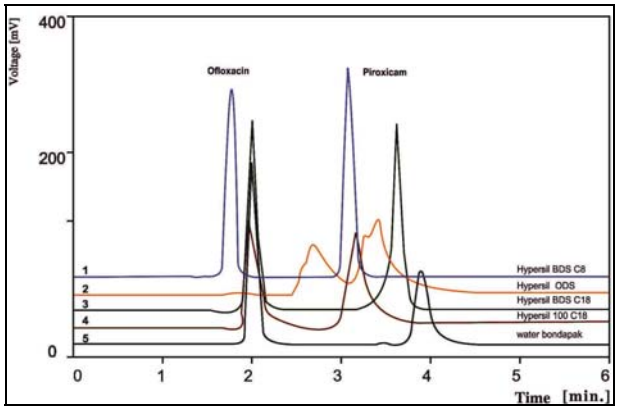

Fig. 2: Chromatograms of ofloxacin and piroxicam on various stationary phases.

(1) Chromatogram of ofloxacin and piroxicam on Hypersil BDS C8 column, (2) chromatogram of ofloxacin and piroxicam on Hypersil ODS column, (3) chromatogram of ofloxacin and piroxicam on Hypersil BDS C18 column, (4) chromatogram of ofloxacin and piroxicam on Hypersil 100 C18 column, (5) chromatogram of ofloxacin and piroxicam on Water bondapak column.

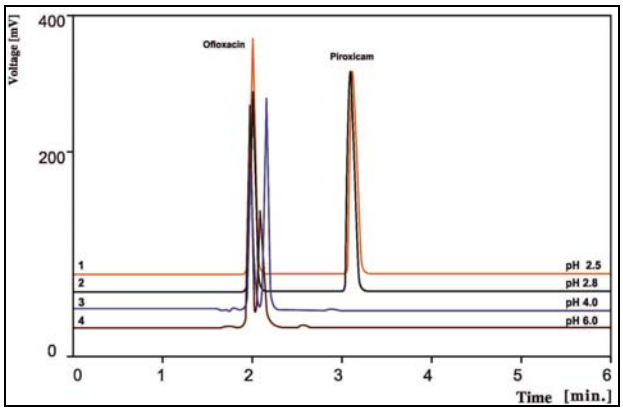

Fig. 3: Chromatograms of ofloxacin and piroxicam at different pH.

(1) Chromatogram of ofloxacin and piroxicam at pH 2.5, (2) chromatogram of ofloxacin and piroxicam at pH 2.8, (3) chromatogram of ofloxacin and piroxicam at pH 4.0, (4) chromatogram of ofloxacin and piroxicam at pH 6.0.

Preparation of sample solution

Accurately weighed 20 tablets were finally grounded in mortar and pestle. Took tablets powder equivalent to 100

mg each of piroxicam and ofloxacin in 100 ml volumetric flask, dissolved in mobile phase and then completed up to the mark with mobile phase. Then 5 mL of this solution was diluted to 50 mL with mobile phase to obtain 100 mg mL^{-1} each of piroxicam and ofloxacin. The solution was filtered through 0.45 μm nylon filter before analysis.

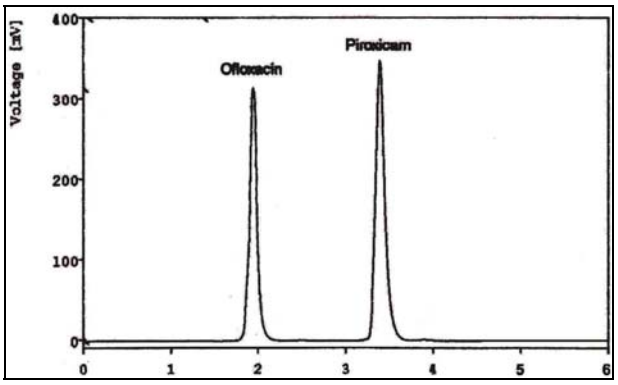

Fig. 4: Chromatogram of ofloxacin and piroxicam in human serum.

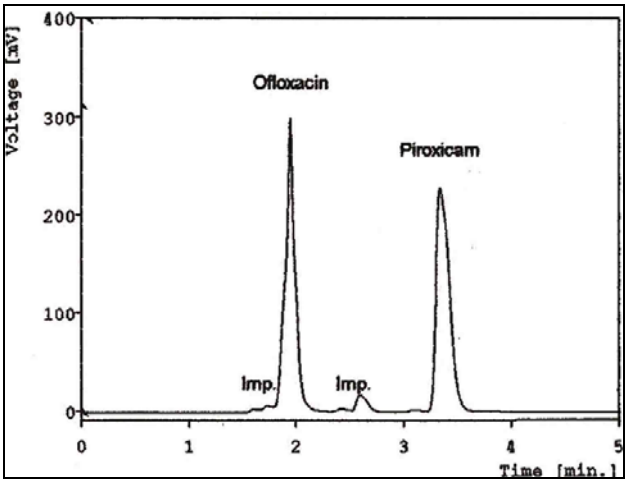

Fig. 5: Chromatogram of oxidative stress induced degradation of ofloxacin and piroxicam.

Preparation of human serum samples

The objective of the method development was to develop a simple and precise method without laborious liquid-liquid extraction or expensive solid phase extraction. Serum samples were injected directly after proteins precipitation. For preparation of serum sample 100 μL of human serum was spiked with 100 μL of stock solution (A). Then 800 μL of acetonitrile was added and centrifuged the final solution at 10,000 rpm for 10 minutes to precipitate proteins. We obtained final concentration of solution equivalent to 100 μg mL^{-1} each of piroxicam and ofloxacin. The supernatant was collected in polypropylene tube and filtered through 0.45μm nylon filter before analysis.

Linearity

Linear calibration plots of the proposed method were obtained over concentration ranges of 70-130 μg mL^{-1} (70, 80, 90, 100, 110, 120 and 130 μg mL^{-1}) each of piroxicam and ofloxacin. Each solution was prepared in triplicate.

Accuracy

Accuracy of the developed method was determined by analysis of synthetic mixtures of piroxicam and ofloxacin in pharmaceutical formulations and human serum. Declared amounts (50, 100 and 150%) of piroxicam and ofloxacin of documented purity were spiked to placebo components (microcrystalline cellulose, lactopress, talcum powder, magnesium stearate), and human serum. Synthetic mixture (100% nominal analytical concentration) of piroxicam (100 μg mL^{-1}) and ofloxacin (100 μg mL^{-1}) was prepared by mixing piroxicam (100 mg), ofloxacin (100 mg), microcrystalline cellulose (100 mg), lactopress (100 mg), talcum (5 mg) and magnesium stearate (5 mg) in mortar and pestle. Three levels of synthetic mixtures were prepared corresponding to 50, 100 and 150% of nominal analytical concentration (100 μg mL^{-1} each of piroxicam and ofloxacin) and analysed by the developed method.

Precision

Repeatability of the developed method was studied by determination of intra-day and inter-day precision. Intra-day precision was calculated by injecting five standard solutions of three dissimilar concentrations on the single day and inter-day precision was calculated by injecting the same solutions for three uninterrupted days. Relative standard deviation of the injecting samples was then calculated to represent precision.

Specificity (Stress testing)

Stress testing was carried out according to ICH stated stress conditions such as acidic, basic, oxidative, thermal and photolytic stresses.

Acid degradation studies

Acid degradation studies were carried out by using 5M hydrochloric acid. For these studies 5 mL of the standard stock solution was taken in the 50 mL volumetric flask. 2 mL of 5M HCl was added and the flask was kept at 40°C for 168 hours. The solution was neutralized using 5M NaOH after completion of the stress studies. Volume was made upto the mark with the mobile phase.

Base degradation studies.

Base degradations studies were carried out using 5M sodium hydroxide. For this purpose 5 mL of standard stock solution was taken in 50ml volumetric flask. 2 mL of 5M NaOH was added and flask was kept at 40°C for 72 hours. The solution was neutralized using 5M HCl after the completion of the stress studies. Volume was made upto the mark with mobile phase.

Table 1: Optimization of pH of buffer

Mobile phase	Theoretical plates (N)	Tailing factor (T)	Resolution (R)	Peak shape
Acetonitrile: K_2HPO_4: Sod. Citrate				
pH 2.5 (50:25:25)				
Ofloxacin	4760	1.00		+++
Piroxicam	3634	1.62	8.595	---
Acetonitrile: K_2HPO_4: Sod. Citrate				
pH 2.8 (50:25:25)				
Ofloxacin	3261	1.25		+++
Piroxicam	4027	1.43	8.556	+++
Acetonitrile: K_2HPO_4: Sod. Citrate				
pH 4.0 (50:25:25)				
Ofloxacin	3012	1.25		---
Piroxicam	3860	1.09	1.81	---
Acetonitrile: K_2HPO_4: Sod. Citrate				
pH 6.0 (50:25:25)				
Ofloxacin	3113	1.00		---
Piroxicam	2166	1.62	1.09	---

+++ is peak acceptable and --- is peak not acceptable

Table 2: Accuracy of the proposed HPLC method

Nature of sample	Spiked concentration ($\mu g\ mL^{-1}$)	Measured concentration ± SD; RSD (%) ($\mu g\ mL^{-1}$)	Recovery (%)
Pharmaceutical tablets			
Ofloxacin	80	80.11 ± 0.08; 0.09	100.14
	100	99.52 ± 0.06; 0.06	99.52
	120	119.73 ± 0.18; 0.19	99.77
Piroxicam	80	79.70 ± 0.09; 0.10	99.62
	100	98.87 ± 0.69; 0.71	98.87
	120	120.51 ± 0.49; 0.51	100.42
Spiked Serum			
Ofloxacin	80	81.13 ± 0.29; 0.30	101.41
	100	100.0 ± 0.14; 0.14	100.00
	120	119.44 ± 1.09; 1.10	99.53
Piroxicam	80	79.54 ± 1.39; 1.40	99.42
	100	97.87 ± 0.59; 0.61	98.87
	120	118.72 ± 0.82; 0.88	98.93

Oxidative degradation studies

Oxidative degradation studies were conducted at room temperature using 0.005% $KMnO_4$ solution. 5 mL of standard stock solution was taken in the 50mL volumetric flask and 2mL of 0.005% $KMnO_4$ was added and kept the flask at room temperature for 20 minuts. After completion of the stress, volume of the flask was made upto the mark with mobile phase.

Photolytic degradation studies

For photolytic degradation studies 5mL of the standard stock solution was taken in 50mL volumetric flask and kept in the direct sunlight for 20 minuts.

UV degradation studies

For the UV degradation studies 5mL of the standard stock solution was taken in 50mL volumetric flask and kept under the UV light for 30minuts.

Thermal degradation studies

Thermal degradation studies were carried out at 160^oC on hot plate. 5 mL of the standard stock solution was taken in the 50mL volumetric flask and kept at hot plat for 2 hours. After completion of the stress volume of the flask was made upto the mark with mobile phase.

Robustness

Robustness of the developed method was determined by slight varying the experimental conditions. For this

purpose, slight changes were made in mobile phase composition, flow rate and pH of buffer solution and the effects of these changes on chromatographic parameters such as retention time, tailing factor and number of theoretical plates were noted.

Limit of detection and limit of quantitation

For LOD and LOQ values a signal-to-noise (S/N) ratio method was used. For this purpose, solutions of decreasing concentrations were prepared by spiking known amounts of piroxicam and ofloxacin into drug excipients (microcrystalline cellulose, lactopress, talcum and magnesium stearate) and human serum. The solutions were prepared according to the predefined procedure and analysed repeatedly to determine the S/N ratio. The average S/N ratio at each concentration level was used to calculate the LOD and LOQ. The lowest concentration of piroxicam and ofloxacin that gives a S/N ratio of about 10:1 at which piroxicam and ofloxacin can be readily quantified with accuracy and precision was reported as LOQ and the lowest concentration level that gives a S/N ratio of about 3:1 at which piroxicam and ofloxacin can be readily detected was reported as the limit of detection.

Table 3: Intra-Day and Inter-Day precision of the proposed HPLC method

Drugs	Actual concentration ($\mu g\ mL^{-1}$)	Intra-Day precision measured conc. $\mu g\ mL^{-1}$ ± SD; RSD (%)	Inter-Day precision measured conc. $\mu g\ mL^{-1}$ ± SD; RSD (%)
Pharmaceutical tablets			
Ofloxacin	80	79.23 ± 0.40; 0.41	81.01 ± 1.40; 1.41
	100	100.04 ± 0.64; 0.67	101.98 ± 0.47; 0.49
	120	120.73 ± 0.47; 0.47	119.94 ± 0.59; 0.61
piroxicam	80	80.41 ± 1.04; 1.07	80.99 ± 0.18; 1.19
	100	99.55 ± 0.80; 0.81	100.02 ± 1.10; 1.10
	120	121.01 ± 1.09; 1.10	120.97 ± 0.91; 0.98
Spiked Serum			
Ofloxacin	80	81.20 ± 1.15; 1.16	80.41 ± 1.08; 1.08
	100	100.41 ± 1.88; 2.01	100.66 ± 1.88; 1.94
	120	122.33 ± 1.00; 1.05	121.70 ± 1.09; 1.10
Piroxicam	80	80.01 ± 1.19; 1.25	80.04 ± 1.12; 1.14
	100	101.03 ± 1.02; 1.07	99.98 ± 2.00; 2.03
	120	119.01 ± 1.12; 1.12	120.96 ± 1.00; 1.10

Table 4a: Robustness study of ofloxacin

Chromatographic conditions	Assay (%)	t_R (min)	Theoretical plates	Tailing
Acetonitrile: mixed buffers (52:48)	98.92	1.88	1420	1.231
Acetonitrile: mixed buffers (50:50)	100.11	1.94	3261	1.250
Acetonitrile: mixed buffers (48:52)	101.97	2.08	4760	1.385
Flow rate (1.3 mL min^{-1})	101.08	2.56	3156	1.385
Flow rate (1.5 mL min^{-1})	99.92	1.94	3261	1.250
Flow rate (1.7 mL min^{-1})	99.83	1.38	3662	1.182
Buffer (pH 2.6)	96.71	1.95	4760	1.320
Buffer (pH 2.8)	99.82	1.94	3261	1.250
Buffer (pH 3.0)	98.47	1.95	3977	1.320

Table 4b: Robustness study of piroxicam

Chromatographic conditions	Assay (%)	t_R (min)	Theoretical plates	Tailing
Acetonitrile: mixed buffers (52:48)	98.50	3.20	2849	1.681
Acetonitrile: mixed buffers (50:50)	100.28	3.41	4027	1.429
Acetonitrile: mixed buffers (48:52)	98.04	3.58	4011	1.701
Flow rate (1.3 mL min^{-1})	100.93	3.81	4347	1.375
Flow rate (1.5 mL min^{-1})	100.28	3.41	4027	1.429
Flow rate (1.7 mL min^{-1})	99.94	3.05	3885	1.727
Buffer (pH 2.6)	95.04	3.54	3634	1.615
Buffer (pH 2.8)	100.28	3.41	4027	1.429
Buffer (pH 3.0)	97.04	3.31	3677	1.500

Table 5: Stress testing results of ofloxacin and piroxicam

Nature of stress	Storage conditions	Time (h)	Amount of ofloxacin remaining (%)	Amount of piroxicam remaining (%)	Extent of decomposition
5M HCl	(40°C)	72	84.03	95.24	Substantial
5M NaOH	(40°C)	168	89.33	92.88	Substantial
0.005 % $KMnO_4$	(25°C)	0.33	93.70	76.09	Substantial
Photolytic	Sunlight	0.33	94.50	74.71	Substantial
Ultra violet	UV lamp	0.50	88.87	84.65	Substantial
Thermal (hot plate)	(160°C)	2.00	95.76	69.53	Substantial

Table 6: Assay results of ofloxacin and piroxicam in pharmaceutical tablets

Product	Ingredients	Label value (mg per tablet)	Recovery (%), ± RSD (%)
Synthetic tablets	ofloxacin	100	101.2 ± 0.3
	piroxicam	100	99.81 ± 0.7

Table 7: Drug-Interaction of piroxicam with quinolones

pH of buffers	Percentage recovery after interaction (180 min)					
	Piroxicam	Ofloxacin	Piroxicam	Moxifloxacin	Piroxicam	Sparfloxacin
1.2	75.82	99.57	59.16	99.47	66.26	100.26
4.0	37.53	99.39	24.93	101.51	30.29	103.40
7.4	97.81	97.90	101.13	104.67	103.44	98.88
9.0	95.66	98.45	98.43	97.96	98.11	96.47

Procedure for drug interaction studies in different buffers

Standard solutions of piroxicam and quinolones (ofloxacin, moxifloxacin and sparfloxacin) were prepared in mobile phase separately. These solutions were mixed (1:1) in four different dissolution beakers containing buffer solutions of pH 1.2, 4.0, 7.4 and 9.0 respectively. Temperature of the dissolution medium was maintained at 37°C ± 1°C with constant stirring at 100 rpm. 5 mL aliquots were withdrawn from each beaker separately at an interval of half hour to three hours. The aliquots were diluted in mobile phase to get the concentrations of 100 µg mL^{-1}. Solutions were filtered through 0.45 µm Millipore filter and injected into the HPLC system.

RESULTS AND DISCUSSION

Please separated this section

A simple, fast and accurate stability indicating RP-HPLC method was developed for simultaneous determination of ofloxacin and piroxicam in combination form. In order to obtain symmetrical peaks with good resolution, different chromatographic conditions like mobile phase composition, mobile phases having different pH, and columns with different packing materials (Hypersil BDS C18, Hypersil ODS, Hypersil BDS C8, Hypersil 100 C18 and µBondapak C18) were used.

Optimization of mobile phase, stationary phase and pH

Composition of the mobile phase was optimized through series of experiments to obtain symmetrical peaks of piroxicam and ofloxacin. Initially it was tried to use only one buffer along with methanol to separate both the components, but success was not achieved after a reasonable number of experiments. The reason of that might be differential interactions of both the analytes with different buffering components. It was therefore decided to use both the citrate and phosphate buffers along with methanol. Various ratios such as (80:10:10, 60:20:20:, 40:30:30, 20:40:40) of methanol: sodium citrate: dipotassium hydrogen phosphate were tested to obtain best resolution and separations but ofloxacin showed peak broadening and tailing on all stationary phases. In order to overcome the peak broadening effects, organic modifier was changed from methanol to acetonitrile. Use of acetonitrile changed the symmetry of the ofloxacin and very sharp peaks were observed using it. The final mobile phase was then found to be acetonitrile, 0.012M dipotassium hydrogen phosphate buffer and 0.008M sodium citrate buffer (both buffers mixed and pH adjusted to 2.8) (50:25:25 v/v/v) which gave good resolution and reasonable number of theoretical plates on Hypersil BDS C8 column as compared to other columns (fig. 2).

To optimize the suitable pH of the buffer solution, series of experiments were carried out at four different pH (2.5, 2.8, 4.0 and 6.0) values of buffer solutions. Hypersil BDS C8 column was used to optimize the suitable pH. The effect of pH of buffer solution on the elution of both the components is shown in (fig. 3). During this study retention time of ofloxacin was unaffected by buffer pH.

This is due to overall neutral charge on ofloxacin (containing both acidic -COOH and basic -NH group). However, the retention time of piroxicam showed a considerable decrease, with increasing pH. This is because piroxicam tends to be protonated as pH decreases, thus exhibiting an increased preference for bonding to the stationary phase. In addition as the pH of the buffer increased from pH 2.5 to pH 6.0, piroxicam structure changed and the resolution between piroxicam and ofloxacin was compromised and considerable overlapping of peaks was observed at pH 6.0, making quantitation difficult. In contrast, when a buffer of pH 2.8 was used, peaks were well resolved, sharp and symmetrical. Sharp and symmetrical peaks of the piroxicam and ofloxacin were attained at pH 2.8 using acetonitrile: 0.012M dipotassium hydrogen phosphate buffer: 0.008M sodium citrate buffer (50:25:25 v/v/v), with reasonable number of theoretical plates, good resolution and acceptable tailing (table 1). Finally acetonitrile: 0.012M dipotassium hydrogen phosphate buffer: 0.008M sodium citrate buffer (50:25:25 v/v/v) was adopted that showed symmetrical peaks of piroxicam and ofloxacin using Hypersil BDS C8 column at retention times of 1.94 and 3.4 of ofloxacin and piroxicam respectively.

Analytical method validation

The developed method was validated according to ICH guidelines. For determination of linearity, different concentrations of ofloxacin and piroxicam (70, 80, 90, 100, 110, 120 and 130 µg mL^{-1}) were prepared and calibration curve was drawn using peak areas. Linear regression for ofloxacin was Y=0.0518X-0.0385 and for piroxicam linear regression was Y=0.0376X +1.3688 with value of r greater than 0.999 for both analytes.

For calculation of the limit of detection (LOD) and limit of quantitation (LOQ) sequential dilutions were prepared. LOD was found to be 0.20 µg mL^{-1} for ofloxacin and piroxicam (S/N ratio 3:1). LOQ was found to be 0.66µgmL^{-1} for ofloxacin and piroxicam (S/N ratio 10:1).

To explain the accuracy, known amounts of ofloxacin and piroxicam were added in the sample solution containing known amounts of analytes. Results were then compared with the true values. Three concentrations ranging from 80-120% were made and each concentration was prepared in triplicate. The results of the percentage recoveries are shown in the (table 2). The results revealed that the method is highly accurate.

Precision of the proposed method was estimated by both intra-day precision and inter-day precision to validate the proposed method. It was expressed in term of relative standard deviation (RSD %). Results of intra-day and inter-day precision are shown in the (table 3).

Robustness of the method was accomplished by the premeditated modifications made to the method parameters, such as mobile phase composition, flow rate and pH of the buffer solution. The effect of these changes on the chromatographic parameters such as theoretical plates, resolution and tailing factor was negligible and show that method is robust for intended use. Results of the robustness are shown in (tables 4a and 4b).

Specificity of the developed method was evaluated by applying various stress conditions like (acid, base, oxidative, thermal, UV and photolytic) to ofloxacin and piroxicam in combination form. A typical chromatogram of oxidative degradation is shown in the (fig. 5).

Under photolytic stress ofloxacin and piroxicam were degraded upto 5.5% and 25.29% respectively within 20 min. Under acidic stress ofloxacin and piroxicam were degraded upto 10.67% and 7.12% respectively. Under basic stress ofloxacin and piroxicam were degraded upto 15.97% and 4.76% respectively. Under thermal stress, ofloxacin and piroxicam were degraded upto 4.24% and 30.47% respectively. In UV stress, ofloxacin and piroxicam were degraded upto 11.13% and 15.35% respectively. In oxidative stress studies ofloxacin and piroxicam degraded up to 6.3% and 23.91% respectively. The greatest degradation of ofloxacin (15.97%) occurred in basic stress conditions and piroxicam (30.47%) under thermal stress conditions. Results of degradation studies are reported in (table 5). Comparison of the two drugs revealed that ofloxacin is more stable as compared to piroxicam. In addition to percentage degradation of each drug, different numbers of degradation products (impurities) were developed under oxidative, basic, acidic, photolytic and thermal conditions.

The developed chromatographic method was used for the estimation of piroxicam and ofloxacin in synthetic tablets, human serum (fig. 4) and drug interaction studies in buffer solutions of different pH. Application of the developed method was checked by analyzing ofloxacin and piroxicam (table 6) in pharmaceutical tablets, and spiked human serum. The drug interaction results of piroxicam with different quinolones are shown in table 7. Strong drug interactions between piroxicam and quinolones were observed at pH 1.2 and 4.0. Compared to other quinolones piroxicam showed greatest drug interactions with moxifloxacin at pH 4.0 which reduced the *in vitro* bioavailability of piroxicam upto 75.07% within 180 minutes. Similarly piroxicam showed less drug interactions with ofloxacin (pH 4.0) as compared to other quinolones. Piroxicam showed no drug interactions with quinolones at pH 7.4 and 9.0. The above discussion revealed that drug interactions of piroxicam with different quinolones are pH dependent.

CONCLUSION

A new method for the simultaneous estimation of piroxicam and ofloxacin in pharmaceutical formulation,

and human serum is presented. The described reversed phase HPLC method is very simple, fast, specific and isocratic. The method is suitable for routine quality control analysis, stability studies, serum analysis and recommended for the drug interactions studies. The developed method is validated using ICH guidelines. The method is very fast and separation is achieved within 5 minutes. Sample preparation is very simple and is without the use of expensive solid phase extraction.

REFERENCES

Abbasi MA, Ali SH, Ashfaq M, Rehman AU, Jahangir M, Qureshi MZ, Siddiqui SZ and Ahmad VU (2010). 2,7-dicholorofluorescein: A suitable complexing agent for the spectrophotometric determination of ofloxacin in pure and pharmaceutical dosage forms. *J. Chem. Soc. Pak*., **32**: 172-175.

Ashfaq M, Akhtar T, Mustafa G, Danish M, Razzaq SN and Nazar MF (2014). Simultaneous estimation of rosuvastatin and amlodipine in pharmaceutical formulations using stability indicating HPLC method. *Braz. J. pharm. Sci.*, In Press.

Ashfaq M, Khan IU and Asghar MN (2008). Development and validation of liquid chromatographic method for gemfibrozil and simvastatin in binary combination. *J. Chil. Chem. Soc.*, **53**: 1617-1619.

Ashfaq M, Khan IU, Qutab SS and Razzaq SN (2007). HPLC determination of ezetimibe and simvastatin in pharmaceutical formulations. *J. Chil. Chem. Soc*., **52**: 1220-1223.

Ashfaq M, Khan, IU, Ahmad H and Mustafa G (2013). LC determination of rosuvastatin and ezetimibe in human plasma. *J. Chil. Chem. Soc*., **58**: 2177-2181.

Bartsch H, Eiper A and Kopelent-Frank H (1999). Stability indicating assays for the determination of piroxicam-comparison of methods. *J. Pharm. Biomed. Anal*., **20**: 531-541.

Basan H, Göğer NG, Ertaş N and Orbey MT (2001). Quantitative determination of piroxicam in a new formulation (piroxicam-beta-cyclodextrin) by derivative UV spectrophotometric method and HPLC. *J. Pharm. Biomed. Anal*., **25**: 171-178.

Ev LD and Schapoval EES (2002). Microbiological assay for determination of ofloxacin injection. *J. Pharm. Biomed. Anal*., **27**: 91-96.

Fabre D, Bressolle F, Kinowski JM, Bouvet O, Paganin F and Galtier M (1994). A reproducible, simple and sensitive HPLC assay for determination of ofloxacin in plasma and lung tissue: Application in pharmacokinetic studies. *J. Pharm. Biomed. Anal*., **12**: 1463-1469.

ICH (Q2B) (1996). Note for guidance on validation of analytical procedures: Methodology. International conference on Harmonization, IFPMA, Geneva.

Jiang XG, Ge SD, Wanga XJ and Xi NZ (1991). A HPLC method for determining piroxicam in body fluids. *Zhongguo. Yao. Li. Xue. Bao*., **12**(4): 381-384.

Khan IU, Ashfaq M, Razzaq SN and Mariam I (2013). Simultaneous determination of piroxicam and Paracetamol in Pharmaceutical formulations using stability indicating HPLC Method. *J. Liq. Chrom. Rel. Technol*., **36**:1437-1450.

Khan IU, Jillani SMS and Ashfaq M (2010a). Determination of atorvastatin and gemfibrozil in human plasma by reversed-phase liquid chromatography. *Lat. Am. J. Pharm*., **29**: 1383-1388.

Khan IU, Kausar T, Ashfaq M and Sharif S (2010b). Development and validation of liquid chromatographic method for the simultaneous estimation of ezetimibe and lovastatin in human plasma. *J. Chil. Chem. Soc*., **55**: 461-464.

Khan IU, Razzaq SN, Mariam I, Ashfaq M and Razzaq SS (2014). Stability indicating RP-HPLC method for simultaneous determination of gatifloxacin and flurbiprofen in binary combination. *Quim. Nova*, **37**(2): 349-354.

Khan IU, Sharif S, Ashfaq M and Asghar MN (2008). Simultaneous determination of potassium clavulanate and cefixime in synthetic mixtures by high performance liquid chromatography. *J. AOAC Int*., **91**: 744-749.

Kurakula M, Mohd AB, Rao AP and Diwan PV (2011). Estimation of piroxicam in proliposomal formulation using RP HPLC method. *Int. J. Chem. Anal. Sci*., **2(9)**: 1193-1196.

Maraschiello C, Cusidó E, Abellán M and Vilageliu J (2001). Validation of an analytical procedure for the determination of the fluoroquinolone ofloxacin in chicken tissues. *J. Chromatogr. B Biomed. Sci. App.*, **754**: 311-318.

Ohkubo T, Kudo M and Sugawara K (1992). Determination of ofloxacin in human serum by high-performance liquid chromatography with column switching. *J. Chromatogr*., **573**: 289-293.

Qutab SS, Razzaq SN, Ashfaq M, Khan IU and Mumtaz AM (2009). Simultaneous quantitation of olmesartan medoxomil and amlodipine besylate in combined tablets using HPLC. *J. Chil. Chem. Soc.*, **54**: 234-237.

Qutab SS, Razzaq SN, Ashfaq M, Shuja ZA and Khan IU (2007a) Simple and sensitive LC-UV method for simultaneous analysis of hydrochlorothiazide and candesartan cilexetil in pharmaceutical formulations. *Acta. Chromatographica.*, **19**: 119-129.

Qutab SS, Razzaq SN, Khan IU, Ashfaq M and Shuja ZA (2007b). Simultaneous determination of atorvastatin calcium and ezetimibe in pharmaceutical formulations by liquid chromatography. *J. Food Drug Anal*., **15**: 139-144.

Rao Y, Tong Y, Zhang XR, Luo G and Baeyens WRG (2000). Determination of ofloxacin using a

chemiluminescence flow-injection method. *Analytica. Chimica. Acta.*, **416**: 227-230.

Razzaq SN, Ashfaq M, Khan IU and Mariam I (2012b). Stability indicating HPLC method for the simultaneous determination of ofloxacin and ketorolac tromethamine in Pharmaceutical formulations. *Analytical Methods*, **4**: 2121-2126.

Razzaq SN, Ashfaq M, Khan IU and Mariam I (2012c). Development and validation of liquid chromatographic method for moxifloxacin and ketorolac tromethamine in combined dosage form. *Quim. Nova*, **35**(6): 1216-1221.

Razzaq SN, Ashfaq M, Khan IU and Mariam I (2012d). Development and validation of liquid chromatographic method for naproxen and esomeprazole in binary combination. *J. Chil. Chem. Soc*., **54**: 1456-1459.

Razzaq SN, Ashfaq M, Mariam I, Khan IU and Razzaq SS (2013). Simultaneous RP-HPLC determination of sparfloxacin and dexamethasone in pharmaceutical formulations. *Braz. J. pharm. Sci.*, **49**: 301-309.

Razzaq SN, Mariam I, Khan IU and Ashfaq M (2012a). Development and validation of liquid chromatographic method for gatifloxacin and ketorolac tromethamine in combined dosage form. *J. Liq. Chrom. Rel. Technol*., **35**(5): 651-661.

Rele RV, Sawant SA and Warkar CB (2010). Simple spectrophotometric methods for determination of piroxicam in Pharmaceutical formulation. *Int. J. Chem. Tech. Res*., **2**: 2173-2176.

Reynolds JEF (2009). Royal Pharmaceutical Society of Great Britain. Martindale, The Extra Pharmacopoeia, 36th ed., Pharmaceutical Press: London. p.117 and p.310.

Sharif S, Khan IU, Ashfaq M, Iqbal MS and Ahmad S (2010). Development and validation of a high performance liquid chromatographic method for the simultaneous determination of potassium clavulanate and cefadroxil in synthetically prepared tablets. *J. Anal. Chem.,* **65**: 1029-1034.