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Full length Research Paper

Optimization and simultaneous determination of gemifloxacin and Non-steroidal anti-inflammatory drugs in bulk, pharmaceutical formulations and human serum by RP-HPLC and its applications

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A simple and rapid method using high performance liquid chromatography (HPLC) in isocratic mode has been developed for the simultaneous analysis of gemifloxacin (GFX) and non-steroidal anti-inflammatory drugs (NSAIDs), including ibuprofen (IBU), meloxicam (MEL), mefenamic acid (MEF), flurbiprofen (FLR) and diclofenac sodium (DIC) in bulk, pharmaceutical formulation and human serum. The analytes were analyzed using a Purospher STAR C18 column (250 x 4.6 mm, 5 µm) and a mobile phase consisting of methanol, water and acetonitrile (90 : 5 : 5, v/v/v, pH 2.8 adjusted by phosphoric acid) at a flow rate of 0.8 mL/min. Effluents from the column were monitored at 240 nm. The proposed method is specific, accurate (99.7 - 100.60%) and precise with intra- and inter-run variations within the limits of 0.12 - 1.96. Linearity was within the desired range of 0.625 – 100 µg/mL, with a correlation coefficient of 0.9991 - 0.9997. The detection and quantification limits were 0.043 - 0.015 µg/mL and 0.12 - 0.46 µg/mL, respectively. The results were then correlated using Student's t-test. The proposed method was then applied to study the effect of simulating body environments with respect to pH on *in vitro* interactions with NSAIDs to elucidate the mechanisms of these interactions. The method was validated for selectivity, linearity, accuracy and precision and was found to be applicable for the routine analysis of GFX and NSAIDs in bulk, pharmaceutical formulations, human serum alone, or in combination and in vitro interaction studies.

Keywords: Gemifloxacin, NSAIDs, HPLC, validation, human serum.

INTRODUCTION

Gemifloxacin (GFX, Figure 1) is a fourth generation fluoroquinolone anti-bacterial compound with enhanced affinity for bacterial topoisomerase IV and is being used for the treatment of respiratory and urinary tract infections (Oh et al., 1996; Johnson et al., and Berry et al., 1996). It is particularly active against Gram-positive organisms including penicillin, macrolide and quinolone-resistant Streptococcus *pneumonia* (Hardy et al., 1999; Serkan et al., 2007 and Hannan et al., 2000) and is four-folds more potent than moxifloxacin against *S. pneumonia* (Ann, et al., 2001; Cristian, et al., 2009 and Bridges et al., 1983). Furthermore, the compound has shown potent activity against many organisms that cause urinary tract infections and bronchitis (Ventura et al., and Katzung, et al., 2007). Literature survey revealed the analytical methods for GFX includes high performance liquid chromatography-tandem mass spectrometry (Doyle, et al., 2000 and Ramji et al., 2001), microchip electrophoresis (Seung et al., 2004), chiral high performance liquid chromatography (Hee et al., 2009) and chiral counter-current chromatography (Eun et al., 2004 and Myung et al., 2002). Another simple and sensitive ion-pairing method has been described for the

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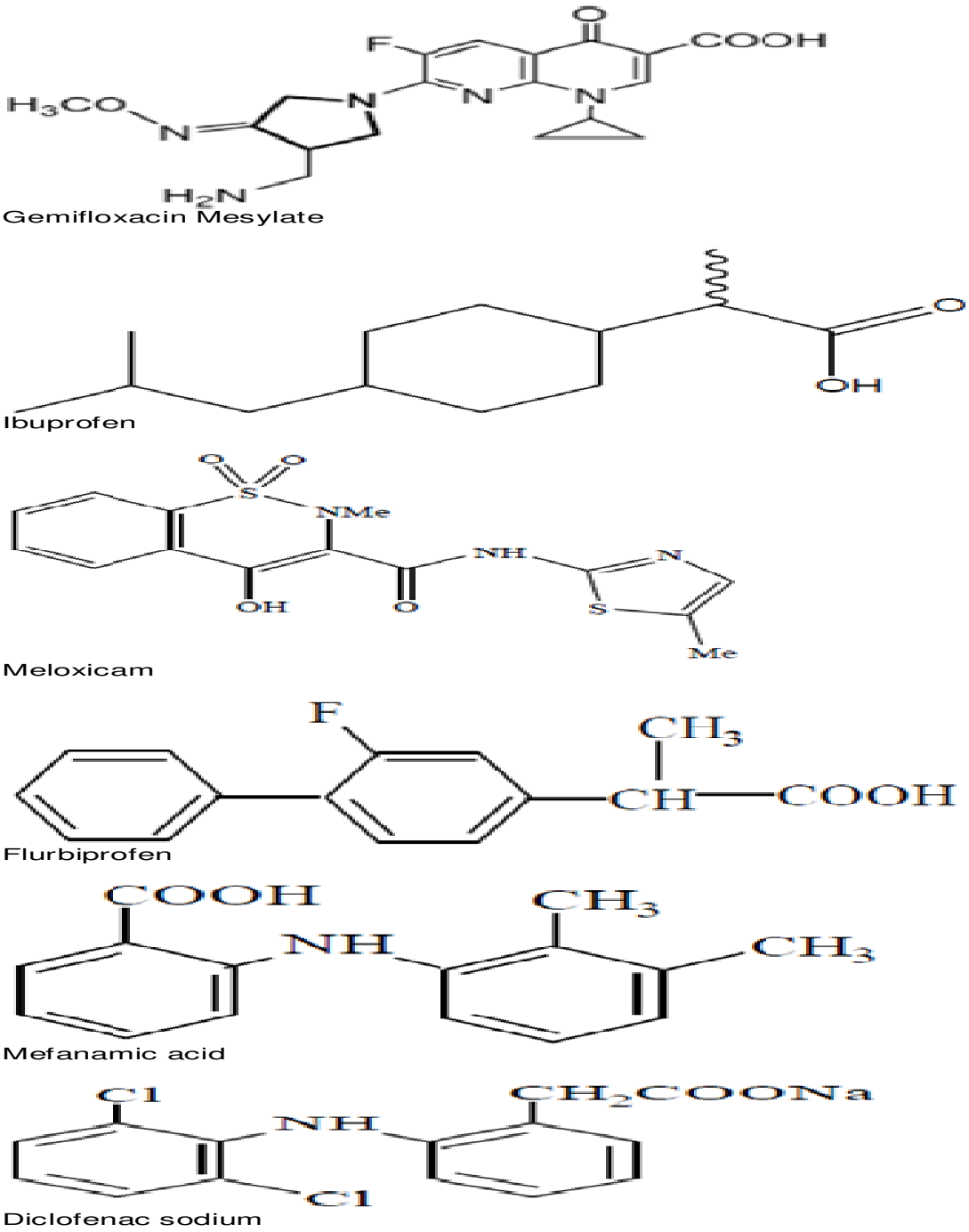

Figure 1. Gemifloxacin mesylate, Ibuprofen, Meloxicam, Flurbiprofen, Mefenamic acid and Diclofenac sodium.

assay of gemifloxacin mesylate by (Marothu et al., 2008 and Barbosa et al., 1997). Survey reveals that concomitant administration of NSAIDs and new quinolones (NQ) can induce a synergistic interaction that results in convulsions. These in vivo and in vitro experiments indicated that the NQ-induced neurotoxic effect was synergistically increased in the presence of NSAIDs (Kawakami et al., 1997; Hori et al., 1989; Hideki et al., 1999 and Tsuji et al., 1988). Therefore, we report a simple and inexpensive isocratic RP-HPLC method for the simultaneous determination of gemifloxacin and NSAIDs with ultraviolet detection at 240 nm in this study. The method is equally valid for determination in bulk materials, pharmaceutical formulations and human serum. (Figure 2).

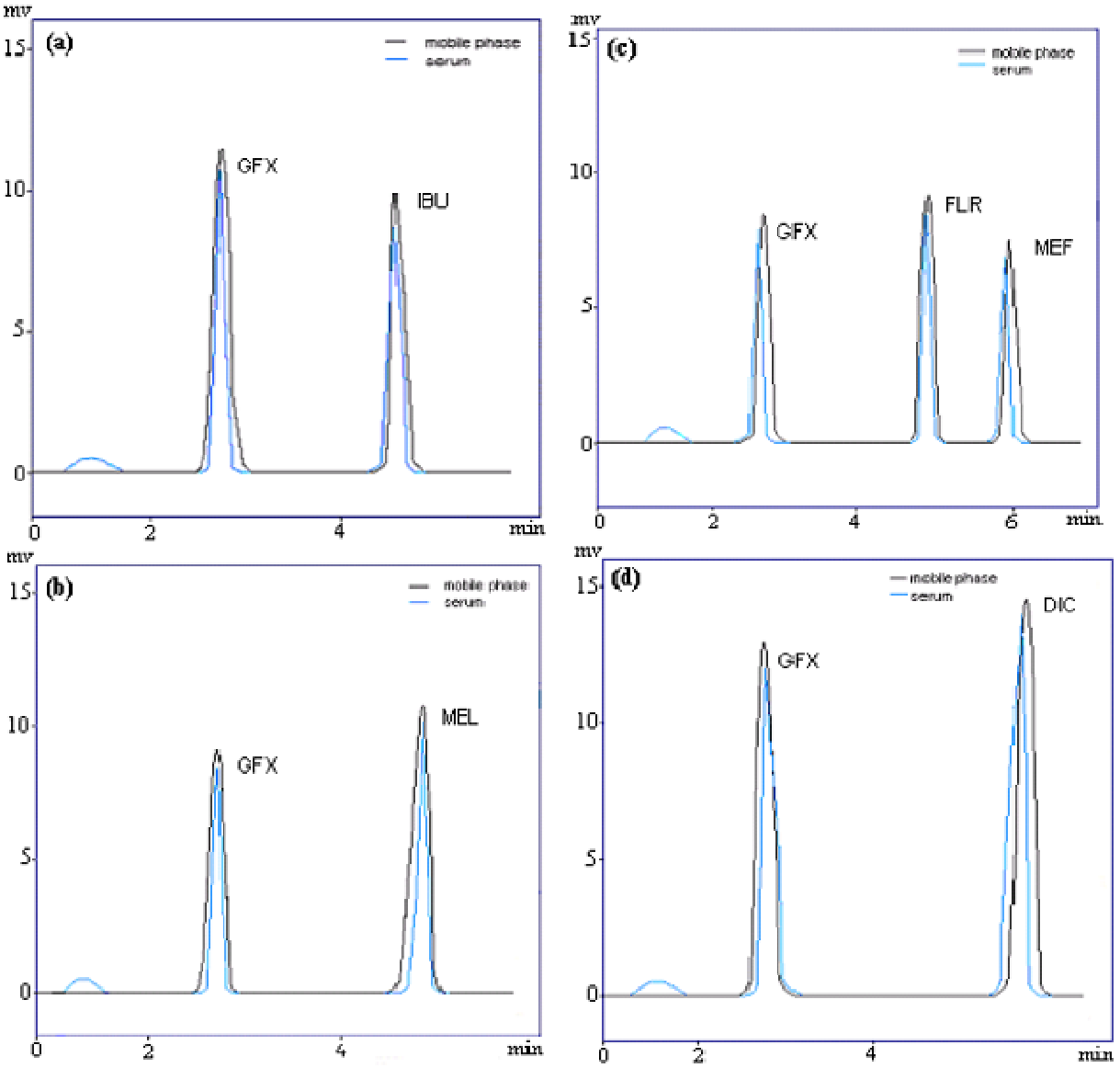

Figure 2. a. Representative chromatograms of GFX (2.5 µg/mL) and IBU (10 µg/mL), **b.** Representative chromatograms of GFX (2.5 µg/mL) and MEL (7.5 µg/mL), **c.** Representative chromatograms of GFX (2.5 µg/mL), FLR (2.5 µg/mL) and MEF (5 µg/mL) and **d.** Representative chromatograms of GFX (2.5 µg/mL) and DIC (7.5 µg/mL), in both mobile phase and human serum at 240 nm.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents were of analytical grade. Gemifloxacin was gift from PharmEvo (Pvt) Ltd, Pakistan. HPLC grade CH3OH was obtained from Merck (Schuchardt OHG, Darmstadt, Germany). NSAIDs used were diclofenac sodium (Fenac 50 mg tablet), flurbiprofen (Vobifen 100 mg tablet), meloxicam (xobix 7.5 mg tablets), mefenamic acid (Ponstoan 250 mg tablet) and ibuprofen (Brufen 200 mg tablet) from Tabros Pharma (Pakistan), Amson Vaccines and Pharma (Pvt) Ltd., Hillton Pharma (Pvt) Ltd., Parke Davis and Co. Ltd. and Aventis (Pvt) Ltd., respectively. Each product was labeled and expiry dates were not earlier than two years at the time of study. Mobile phase consisting of methanol, water and acetonitrile (90: 5: 5, v/v/v, pH 2.8 adjusted by phosphoric acid) was used.

Statistical Study

Standard regression curve analysis was performed on STATISTICA version 7.0 (USA), without forcing through zero. Linearity graphs were obtained on Microsoft Excel

2007 software. SPSS software version 10.0 (Carry, NC, USA) was used for the calculation of means, standard deviations, homoscedasticity of the calibration plots and Student's t-test.

HPLC System

UV/VIS spectrophotometer (Schimadzu 1601), integrated with a Pantium IV computer loaded with UVPC version 3.91 was used to optimize the wavelength. HPLC system consisted of an LC-10 AT VP Shimadzu pump and SPD-10AV VP Shimadzu UV-VIS detector. Separation was achieved on a Hiber, RT, Purospher STAR C18 column (25 cm x 54.6 mm, 5 µm; Merck, Germany). The chromatographic and integrated data were recorded using a CBM-102 communication Bus Module (Shimadzu, Japan). Mobile phase (CH_3OH, H_2O and ACN, 90: 5: 5, v/v/v) was sonicated using DGU-14 AM online degasser (Shimadzu, Japan) and filtered through a 0.45 µm millipore filter.

Calibration Standards and Quality Control Samples

Standard Preparation

Calibration standard solutions of GFX and NSAIDs were prepared by dissolving 1 mg/mL of drug using mobile phase as solvent and kept in 100 mL volumetric flasks. Working solutions were prepared separately by making serial dilutions from the standard solution to obtain concentration range of 0.625 - 25, 2.5 - 100, 1.875 - 75, 1.25 - 50, 0.625 - 25 and 1.875 - 75 µg/mL for GFX, IBU, MEL, MEF, FLR and DIC, respectively. All the solutions were filtered through a 0.45 µm millipore filter before being chromatographed.

Procedure for Tablets

For quality control samples, twenty tablets of each pharmaceutical formulation were powdered finely and an amount equivalent to 10 mg of GFX and NSAIDs were weighed and then dissolved in the mobile phase. Solutions with high, medium and low concentrations (80, 100 and 120%) were prepared and filtered through a 0.45 µm Millipore filter in order to separate out the insoluble excipients by the same procedure as the calibration standards but using different stock solutions. All solutions were stored at 20ºC, analyzed for both inter and intra-day variations of the method. Twenty µL of solution were injected into system.

Procedure for Human Serum

Serum sample obtained from healthy volunteers were collected and stored at -20˚C. To a 1.0 mL aliquot of human serum, 10 mL of acetonitrile was added and the mixture was vortexed for 1 min, followed by centrifugation at 10,000 rpm for 10 min. Supernatant was filtered through a 0.45-µm membrane filter. Portion of human serum sample was fortified with gemifloxacin and NSAIDs to get the final concentrations of 0.625 - 100 105 µg/mL.

Procedure for In Vitro Interaction Studies

Stock solutions of GFX and NSAIDs were prepared by dissolving 10 mg of each drug in 100 mL of buffers (pH 4.0, 7.4 and 9.0) followed by sonication. Gemifloxacin solution was mixed with solution of diclofenac sodium in a flask to give final concentration of 50 µg/mL. Mixture was then kept in water bath at 37 ± 5oC for 3 h. Aliquots of 5 mL were withdrawn at an interval of 30 min for 180 min, followed by filtration through normal filter paper then 0.45 m filter paper to avoid any hindrance and subjected to assay by RP-HPLC. Same procedure was repeated with every NSAID.

RESULTS AND DISCUSSION

Optimization of Wavelength

To investigate appropriate wavelength for simultaneous determination of gemifloxacin and NSAIDs, solutions of these compounds in the mobile phase were scanned by UV- visible spectrophotometer in the range 200 - 400 nm. From the overlaid UV spectra, it was observed there was no interference from the mobile phase or baseline disturbance at 240 nm. Therefore, it was concluded that 240 nm is the most appropriate wavelength for analysis with suitable sensitivity.

Method Development

The aim of the present study was to develop a simple and accurate HPLC method in the isocratic mode for the simultaneous determination of GFX and NSAIDs. Purospher STAR C18 column (250 x 4.6 mm, 5 µm) provides the best peak shapes and efficiencies. The chromatographic conditions, especially the composition of the mobile phase, were optimized through several trials to achieve symmetrical peak shapes for GFX and NSAIDs as well as shorter run time. Initially, various mobile phases were tested to obtain the best separation and resolution. It was found that a mobile phase containing a certain proportion of methanol and water gave symmetrical peak shapes for all drugs. A mobile phase containing high proportion of methanol gave shorter run time. Inclusion of acetonitrile in the mobile

Table 1. System suitability parameters.

Drugs	Retention time (tr)	Capacity factor (K')	Tailing factor (T)	Resolution (Rs)	Theoretical plates (N)	Separation factor (α)
GFX	2.37	2.33	1.79	2.35	2314	0.00
IBU	4.91	2.12	1.99	1.93	6131	4.21
MEL	4.47	5.08	1.83	1.34	5065	2.26
MEF	5.98	7.41	1.95	1.63	7886	3.59
FLR	4.56	5.41	1.88	1.94	5589	4.23
DIC	4.94	2.20	1.91	1.61	4559	3.32

Table 2. Regression characteristics.

Drugs	Conc. (µg/mL)	r^2	S.E.E.	S.E.	Intercept	Regression equation	LOD (µg/mL)	LOQ (µg/mL)
GFX	0.625 - 25	0.9991	1.27	0.73	1.75	y = 5553x + 9915	0.040	0.120
IBU	2.5 - 100	0.9997	0.73	0.42	1.62	y = 1837x + 2995	0.102	0.308
MEL	1.875 - 75	0.9991	1.35	0.78	2.59	y = 5824x + 15318	0.152	0.460
MEF	1.25 - 50	0.9997	0.74	0.42	1.23	y = 6250x + 77455	0.047	0.143
FLR	0.625 - 25	0.9994	1.01	0.66	10.54	y = 6754x + 71399	0.065	0.197
DIC	1.875 - 75	0.9992	1.21	0.69	1.46	y = 6065x + 9010	0.106	0.321

phase was crucial in obtaining high signal intensity. Therefore the final mobile phase that provides good resolution was composed of methanol, water and acetonitrile in ratio of 90: 5: 5 (v/v/v). In order to keep pH of the mobile phase constant, 85% phosphoric acid was used to achieve the desire pH. Mobile phase pH had little impact on resolution and the best separations were observed at pH 2.8. A flow rate of 0.8 mL/min gave a short chromatographic run time. The chromatographic conditions were optimized to achieve best resolution between analytes and to optimize chromatographic parameters such as resolution, tailing factor and retention time. Peaks were identified by comparing the retention times with those of standards. Retention times were 2.3, 5, 4.5, 6.1, 4.6 and 4.9 min for GFX, IBU, MLX, MEF, FLR and DIC, respectively. For validation, the guidelines of the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use ICH and USP 2002 United States Pharmacopeia were followed for the tests on accuracy, precision, specificity, linearity, work strip and robustness of the method.

Method Validation

System Suitability

It is an imperative module of method validation to make certain that the operational system is running appropriately throughout the analysis. The system was equilibrated with the initial mobile phase composition, followed by 10 injections of the same standard (Table 1). These 10 consecutive injections were used to evaluate the system suitability on each day of method validation.

Calibration Curves

Calibration curves were characterized by different linear segments for all the drugs. These curves were obtained using the linear least squares regression procedure (Table 2) over concentration range of 0.625 - 100 µg/mL. The results revealed good linear correlations with all the drugs having correlation coefficient (r2) value > 0.999 and LOD (Limit of Detection is the lowest analyte concentration which can be detected) and LOQ (Limit of Qantitation is the lowest quantity of an analyte which can be quantified) values ranging from 0.043 - 0.015 µg/mL and 0.12 - 0.46 µg/mL, respectively.

Accuracy

The accuracy of the method was evaluated from the recovery results of spiked placebo samples. Blank placebo matrix was spiked to produce concentrations of 80, 100 and 120% (4, 5 and 6 µg/mL) (Shabir, et al., 2003; Ermer, et al., 2001 and United States Pharmacopeia (2007)). Mean recovery of spiked samples were in the ranges of 99.70 - 100.60% without human serum and 98.30 - 100.50% in human serum. Recovery tests were performed by adding known amounts of standard solutions to sample, followed by analysis using proposed method. Three runs were performed for every concentration and the peak area was calculated (Table 3).

Table 3. Accuracy of gemifloxacin and NSAIDs.

Parameters	Conc. (%) spiked	Assay (spiking method)						Assay in human serum					
Conc. found		GFX	IBU	MEL	MEF	FLR	DIC	GFX	IBU	MEL	MEF	FLR	DIC
	80	79.98	79.00	79.94	79.34	79.70	79.89	79.92	78.92	97.90	79.25	79.62	79.80
	100	99.85	99.90	99.79	99.70	99.60	99.34	99.82	99.90	99.69	99.59	99.55	99.30
	120	120.35	120.10	120.33	120.11	120.10	120.05	120.30	119.94	120.27	120.08	120.02	120.0
% Recovery	80	99.96	98.40	99.85	98.34	99.30	99.72	99.91	98.10	99.79	98.30	99.10	99.60
	100	99.70	99.90	99.58	99.40	99.20	98.67	99.50	99.70	99.49	99.31	99.14	98.60
	120	100.60	100.10	100.60	100.20	100.10	100.10	100.50	100.13	100.50	100.10	100.10	100.0

The average recovery for each level was calculated as indicated by Association of Official Analytical Chemists International (United States Pharmacopeia, Leite, et al., 2002; Harris, et al., and Official Methods of Analysis).

Precision

Precision of the method was determined by repetitive analysis of standard solution (n = 6). Method precision or intra-assay precision was performed by preparing six different samples involving different weightings. Each solution was injected in triplicate under the same conditions and the mean values of peak area responses for each solution were taken. The precision of the method was analyzed as % RSD throughout the linear range of concentrations (Table 4). Student's *t*-test was applied between two groups, day 1 (D1) and day 2 (D2). All the results were correlated and found insignificant. Student's *t*-test indicates no remarkable difference in inter-day precision (Kaul et al., 2005; Sultana et al., 2011 and Sultana et al., 2010).

Specificity/Selectivity

Figures no 3a-d shows the typical chromatograms of drugs alone and with spiked human serum samples. The retention times were 2.3, 5, 4.5, 6.1, 4.6 and 4.9 min for GFX, IBU, MLX, MEF, FLR and DIC respectively. No significant interference was observed from endogenous substances in drug free human serum at the retention time of all drugs.

Ruggedness

The ruggedness of the method was established by determining GFX and NSAIDs in bulk, pharmaceutical formulation and human serum in two different laboratories. First lab was Research Institute of Pharmaceutical Sciences, University of Karachi while the other lab was Department of Chemistry, University of Karachi. Different instruments (LC 20 and LC 10) were used on different days by different analysts. All the results were in good limits.

Robustness

Robustness of the method was achieved by designed modifications to method parameters such as composition, flow rate, pH of the mobile phase, detection wavelength, injection volume and column temperature (Table 5). It was found that the RSD values did not exceed more than 1.5 % (Shabir et al., 2003; Ermer et al., 2001 and United States Pharmacopeia (2007).

Application of the proposed method for *in vitro* interaction study

Simultaneous determination of gemifloxacin, diclofenac sodium, mefenamic acid, flurbiprofen, ibuprofen and meloxicam was achieved as above. The applicability of the proposed method was demonstrated for *in vitro* interaction studies of gemifloxacin with these drugs. Gemifloxacin was analyzed by measuring the area under curve (AUC), % recovery and considerable drift in retention time (Table 6). When gemifloxacin was studied for interaction with meloxicam in buffers of pH 4, 7.4 and 9, recoveries of gemifloxacin and meloxicam after 180 min were 66.12 - 75.36% and 61.92 - 101.6%, respectively. However, no change was found when gemifloxacin alone was subjected to the tested conditions. Results indicate slight interactions, which need

Table 4. Precision of gemifloxacin and NSAIDs (n = 6).

Drugs	Conc. (µg/mL)	Pharmaceutical Formulation (% RSD)		Human Serum (% RSD)	Drugs	Conc. (µg/mL)	Pharmaceutical Formulation (% RSD)		Human Serum (% RSD)
		D1	D2	D1			D1	D2	D1
GFX	0.625	0.029	0.03	0.25	MEF	1.25	0.33	0.34	0.39
	1.25	1.55	1.57	1.59		2.5	0.39	0.35	0.34
	2.5	1.90	1.93	1.96		5	0.34	0.32	0.36
	6.25	1.79	1.81	1.82		12.5	0.56	0.54	0.59
	12.5	1.07	1.09	1.10		25	1.35	1.39	1.36
	25	0.25	0.28	0.29		50	0.40	0.42	0.41
IBU	2.5	0.49	0.53	0.52	FLR	0.625	0.75	0.77	0.79
	5	0.33	0.38	0.34		1.25	0.19	0.14	0.12
	10	0.92	0.98	0.95		2.5	0.35	0.38	0.39
	25	0.95	0.93	0.92		6.25	0.62	0.67	0.65
	50	0.29	0.26	0.25		12.5	0.55	0.57	0.60
	100	0.55	0.57	0.59		25	0.14	0.16	0.15
MEL	1.875	0.85	0.83	0.81	DIC	1.875	0.65	0.70	0.75
	3.75	0.55	0.58	0.56		3.75	1.12	1.14	1.15
	7.5	0.94	0.91	0.90		7.50	1.65	1.68	1.67
	18.75	0.22	0.24	0.27		18.75	0.32	0.30	0.29
	37.5	0.35	0.38	0.40		37.50	1.05	1.03	1.00
	75	0.89	0.86	0.85		75.00	0.99	0.92	0.94

t-Test: paired two sample for precision

Drugs	S.D	t stat	P (T>t) two-tail
GFX	0.075	-2.300	0.070
IBU	0.037	1.309	0.247
MEL	0.296	1.432	1.00
MEF	0.030	0.136	0.897
FLR	0.040	-1.080	0.328
DIC	0.044	0.094	0.929

GFX, Gemifloxacin: MEL, Meloxicam; MEF, Mefenamic acid; DIC, Diclofenac sodium; FLR, Flurbiprofen; IBU, Ibuprofen; S.D, Standard Deviation; D1 , Intra-day and D2, Inter-day variations.

Table 5. Robustness of the proposed method (n = 6).

Drugs	Retention time (tr)	Capacity factor (K')	Tailing factor (T)	Resolution (Rs)	Theoretical plates (N)	Separation factor (α)
A: pH of mobile phase, 2.8 ± 0.2						
GFX	2.37 ± 0.21	2.33 ± 0.15	1.79 ± 0.12	2.35 ± 0.31	2314 ± 41	0 ± 0.5
IBU	4.91 ± 0.41	2.1 ± 0.9	1.99 ± 0.4	1.93 ± 0.74	6131 ± 32	1.24 ± 0.11
MEL	4.47 ± 0.5	5.08 ± 0.21	1.83 ± 0.14	1.341± .29	5065 ± 25	2.26 ± 0.09
MEF	5.98 ± 0.82	7.41 ± 0.2	1.95 ± 0.15	1.63 ± 0.53	7886 ± 43	1.2 ± 0.23
FLR	4.56 ± 0.21	5.41 ± 0.25	1.88 ± 0.07	1.94 ± 0.35	5589 ± 36	2.32 ± 0.21
DIC	4.94 ± 0.3	2.2 ± 0.19	1.91 ± 0.5	1.61 ± 0.2	4559 ± 15	2.3 ± 0.3
B: Flow rate, 0.8 ± 0.2 (mL/min)						
GFX	2.37± 0.22	2.33± 0.19	1.79± 0.18	2.35± 0.35	2314 ± 45	0 ± 0.47
IBU	4.91± 0.45	2.1 ± 0.6	1.99 ± 0.5	1.93± 0.79	6131 ± 39	1.24 ± 0.13
MEL	4.47 ± 0.7	5.08± 0.25	1.83± 0.19	1.34± 0.24	5065 ± 24	2.26 ± 0.08
MEF	5.98 ± 0.85	7.41 ± 0.3	1.95 ± 0.17	1.63 ± 0.51	7886 ± 47	1.2 ± 0.19
FLR	4.56 ± 0.25	5.41 ± 0.29	1.88 ± 0.17	1.94 ± 0.32	5589 ± 32	2.32 ± 0.25
DIC	4.94 ± 0.33	2.2 ± 0.15	1.91 ± 0.47	1.61 ± 0.27	4559 ± 19	2.3 ± 0.31
C: Percentage of methanol in mobile phase, 90 ± 5 (v/v/v)						
GFX	2.37 ± 0.19	2.33 ± 0.17	1.79 ± 0.17	2.35 ± 0.34	2314 ± 39	0 ± 0.43
IBU	4.91 ± 0.39	2.1 ± 0.9	1.99 ± 0.49	1.93 ± 0.71	6131 ± 28	1.24 ± 0.14
MEL	4.47 ± 0.4	5.08 ± 0.22	1.83 ± 0.15	1.34 ± 0.22	5065 ± 29	2.26 ± 0.07

Table 5 Cont.

MEF	5.98 ± 0.81	7.41 ± 0.29	1.95 ± 0.14	1.63 ± 0.47	7886 ± 42	1.2 ± 0.17
FLR	4.56 ± 0.21	5.41 ± 0.31	1.88 ± 0.13	1.94 ± 0.28	5589 ± 35	2.32 ± 0.22
DIC	4.94 ± 0.29	2.2 ± 0.18	1.91 ± 0.42	1.61 ± 0.27	4559 ± 21	2.3 ± 0.35

tr= Retention time, K' = Capacity factor, N=Theoratical plates, T= Tailing factor, Rs = Resolution

Table 6. Recovery (%) of Gemifloxacin with NSAIDs at pH 4.

Time (min)	GFX + MLX		GFX + MEF		GFX + FLR		GFX + DIC		GFX + IBU	
0	100.11	100.45	100.59	100.98	102.78	103.12	104.54	100.07	100.24	101.06
30	95.07	87.75	83.37	103.35	93.81	84.96	79.64	100.19	82.04	97.94
60	96.63	87.45	79.70	106.18	81.23	82.20	78.67	97.06	89.69	85.93
90	92.74	77.04	74.62	101.64	78.97	82.16	81.13	96.35	89.40	85.23
120	89.89	76.96	73.43	95.23	77.44	89.03	82.10	92.98	78.27	74.12
150	70.17	74.41	69.99	89.74	74.82	85.26	78.63	90.14	76.25	74.06
180	66.12	72.07	68.89	87.24	75.71	85.39	77.35	73.54	69.04	72.10
Recovery (%) of Gemifloxacin with NSAIDs at pH 7.4										
Time (min)	**GFX + MLX**		**GFX + MEF**		**GFX + FLR**		**GFX + DIC**		**GFX + IBU**	
0	100	100.2	100.5	100.3	100.1	100.8	100.5	100.21	100.1	100.1
30	93.25	81.81	92.67	97.14	97.48	98.88	104.5	81.76	105	88.4
60	93.33	85.11	95.82	84.57	84.95	95.76	90.24	80.28	105.3	83.19
90	85.18	79.24	83.81	73.97	84.53	85.12	85.98	78.14	102.5	84.81
120	82.36	78.61	72.83	69.78	80.37	85.85	81.39	76.11	87.32	86.91
150	71.35	68.18	70.26	53.84	73.15	79.97	72.38	60.07	76.59	73.56
180	68.83	61.92	61.5	54.22	61.01	70.28	71.44	59.24	68.5	73.02
Recovery (%) of Gemifloxacin with NSAIDs at pH 9.0										
Time (min)	**GFX + MLX**		**GFX + MEF**		**GFX + FLR**		**GFX + DIC**		**GFX + IBU**	
0	100	101.6	100.5	100.5	100.7	102.1	100.1	102.5	100.2	101.8
30	90.88	85.93	98.54	104.9	63.16	129.6	69.94	109.87	55.56	85.14
60	89.6	85.09	96.61	100	62.34	126.1	58.33	109.65	53.28	83.63
90	87.34	82.1	94.48	99.02	60.99	122.5	57.28	107.03	52.94	75.47
120	88.95	81.25	94.4	96.52	59.37	118.8	57.12	106.28	51.97	73.22
150	79.71	80.87	92.57	95.38	59.27	113.5	51.92	102.44	51.77	62.53
180	75.36	78.16	90.88	86.87	54.06	104.9	51.76	78.13	35.1	60.87

to be confirmed after *in vivo* interaction studies. Recovery studies showed that 68.89 and 90.88% of gemifloxacin was recovered at pH 4 and, respectively indicative of interaction with mefenamic acid. In buffers of pH 4, 7.4 and gemifloxacin significantly decrease up to 54.06% in presence of flurbiprofen at 37^{o}C after 180 min. Recovery of gemifloxacin is also indicative of formation of some gemifloxacin-flurbiprofen complex, which cannot be eluted by this method. Interaction studies of gemifloxacin were also studied with diclofenac sodium in buffers of pH 4, 7.4 and 9.0. The recoveries of gemifloxacin were found to be 77.35 - 51.76% after 180 min, indicative of significant interaction which may be pH dependent or temperature mediated. There was also some evidence of gemifloxacin interaction with ibuprofen on the basis of recovery of gemifloxacin and ibuprofen in buffers of pH 4, 7.4 and 9, and after 180 min, 69 - 55% and 72.01 - 62.80% for gemifloxacin and ibuprofen, respectively. It can be inferred that these interactions were pH dependent and complex formation, if any, would be favored by temperature. Significant changes in availability of drugs might be due to the reason that drug had undergone some changes at its chromophoric group, resulting in deviation of molar absorptivity value which itself is an evidence of drug interaction with NSAIDs. Moreover, variation in availability had occurred due to an addition of functional group to the pharmacophore of gemifloxacin and in case of reduction there might be a loss of axuochromes. Results need to be evaluated or confirmed by *in vivo* interaction studies.

CONCLUSION

The proposed HPLC method is simple, rapid, specific, accurate and precise for simultaneous determination of

GFX and NSAIDs in bulk, pharmaceutical formulation and human serum and has been developed for the first time. It can be recommended for the routine quality control and evaluation of clinical data of these drugs. It was apparent that gemifloxacin may interact with commonly used NSAIDs like ibuprofen, diclofenac sodium, flurbiprofen, mefenamic acid and meloxicam which may result in convulsions. Therefore, above mentioned method is applicable in investigating the *in vivo* interactions of gemifloxacin with commonly used NSAIDs.

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