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New Method for Optimization and Simultaneous Determination of Sparfloxacin and Non Steroidal Anti-Inflammatory Drugs: Its *In-Vitro* Application

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ABSTRACT

A simple reversed phase HPLC method was developed and validated for the simultaneous determination of sparfloxacin (SPFX), diclofenac sodium, meloxicam, ibuprofen, flurbiprofen, naproxen and mefenemic acid in a relatively short time with high linearity in bulk material, pharmaceutical formulations and human serum. Purospher STAR C_{18} (250 × 4.6 mm, 5 µm) column was utilized with mobile phase, methanol and water (90:10, v/v pH 2.70 adjusted by phosphoric acid), was delivered at a flow rate of 1.5 mL·min^{-1}. Eluent was monitored using UV detector at 240 nm. The proposed method is specific, accurate (98.42% - 102.75%), precise (intra-day and inter-day variation 0.011% - 1.85%) and linear (R^2 > 0.999) with in the desired range 0.15 - 40 µg·mL^{-1} and the detection and quantification limit was 1.19E+08 – 0.150 µg·mL^{-1} and 3.62E+08 – 0.4574 µg·mL^{-1} respectively for SPFX and NSAIDs. The analysis of variance (ANOVA) and student's *t*-test were applied to verify the results. The anticipated method is applicable to routine analysis of SPFX and NSAIDs in pharmaceutical formulations as well as in human serum samples. It has also applied on interaction of SPFX with NSAIDs.

Keywords: Sparfloxacin; NSAIDs; HPLC; Interactions; ANOVA

1. Introduction

Sparfloxacin or cis-5-amino-1-cyclopropyl-7-(3,5-dimethylpiperazin-1-yl)-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (SPFX) (**Figure 1**) is an orally active synthetically broad spectrum third generation quinolone, characterized by good to excellent activity against Gram positive *cocci* (notably *S. pneumoniae*) and in selected agent activity against anaerobes and atypical pathogens. It is also moderately active against some (*B. fragilis group*) L. *mono-cytogenes* resistant [1-9] and has been shown to have excellent activity, not only against *Neisseria gonorrhoeae*, but also against *Chlamydia trachomatis*, M*ycoplasmas* and *Gardnerella vaginalis* [10].

Literature search assembled a number of different methods, which have been used for analysis of fluroquinolones in bulk, human serum and in pharmaceutical preparations. Gonzalez *et al*. [11] discovered the simultaneous determination of cefepime and the quinolones garenoxacin, moxifloxacin and levofloxacin in human urine by HPLC-UV. The method was applied to the determination of the four molecules in spiked samples of human urine. Nguyen *et al*. [12] reported simultaneous determination of levofloxacin, gatifloxacin and moxifloxacin in human serum. Nemutlue *et al*. [13] published simultaneous separation and determination of seven quinolones using HPLC and analysis of levofloxacin. Sultana *et al*. [14-16] provided simultaneous determination of NSAIDs with diltiazem and ceftrioxazone sodium. Koichi Suenami *et al*. [17] developed more sophisticated and sensitive analytical method for the simultaneous determination of NSAID's in human plasma using Oasis HLB solid-phase extraction, followed by reversed-phase high-performance liquid chromatography and quadruple mass spectrometry with electrospray ionization operated in the negative ion mode. Koichi Suenami *et al*., [18] also

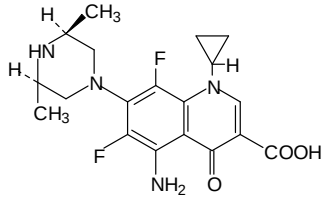

Figure 1. Structure of SPFX.

described a capillary liquid chromatography (LC) and quadruple mass spectrometry with electrospray ionization operated in the negative ion mode for the simultaneous determination of 16 NSAIDs in human plasma. Nagoji and friends [19] developed a simple, selective, rapid, precise and economical reverse phase HPLC method for simultaneous estimation of nimesulide and diclofenac sodium from capsules. There are few methods reported for the simultaneous determination of sparfloxacin with other drugs. Srinivas *et al.* [20] described HPLC method for simultaneous determination of sparfloxacin, moxifloxacin and gatifloxacin using levofloxacin as internal standard in human plasma. Degradation products studies by HPLC have been carried out by Marona *et al.* [21]. Akram *et al.* developed fluorescence probe enhanced spectrofluorimetric method for the determination of sparfloxacin in tablets and biological fluids and spectrophotometric determination method of sparfloxacin in pharmaceutical preparations by ternary complex formation with Pd (II) and eosin [22,23]. Young *et al.* [24] developed an HPLC method for quantitation of SPFX in human serum while. Argekar *et al.*, Marona *et al*. and Nurun *et al.*, developed methods for marketed products and stability testing [25-27]. Srikar [28] and friends developed spectrophotometric methods for quantitative estimation of sparfloxacin in bulk and pharmaceutical dosage forms.

Here, we report a simple, easy, quick and inexpensive isocratic RP-HPLC method with ultraviolet detection at 240 nm for the determination of SPFX in bulk, dosage form as well as in human serum. Low LOD and LOQ values also worth this method for the determination of sparfloxacin in clinical samples. Moreover, this method is applicable for "*in-vitro*" interaction studies of sparfloxacin with selected NSAIDs in a wide range of pH medium. Usually fluoroquinolones are prescribed for many diseases including respiratory and urinary tract infections. Historical background reveals that parallel administration of NSAIDs and new quinolones (NQ) can induce a synergistic interaction that results in convulsions and NQ-induced neurotoxic effect synergistically increased in the presence of NSAIDs [29-31]. For the same purpose, to determine the effect of simultaneous administration of sparfloxacin (quinolones) and NSAIDs, a new RP-HPLC method has been developed which is simple, fast, cheap and easy to perform. The method is equally valid for the determination in bulk materials, pharmaceutical dosage formulations and human serum and is no where else reported before.

2. Experimental

2.1. Reagents

Standard bulk drug sample of sparfloxacin was gifted by Abbott Laboratories (Pakistan) Ltd. HPLC grade methanol was obtained from Merck Schuchardt OHG, Darmstadt, Germany and Tedia company, INC. (USA). While the NSAIDs used were diclofenac sodium (Fenac 50 mg tablet), flurbiprofen (Vobifen 100 mg tablet), meloxicam (Xobix 7.5 mg tablets), mefenamic acid (Ponstan 250 mg tablet), ibuprofen (Brufen 200 mg tablet) and naproxen (Proxen 250 mg tablet) of Tabros Pharma (Pakistan), Amson Vaccines and Pharma (Pvt) Ltd., Hillton Pharma (Pvt) Ltd., Parke Davis and Co. Ltd., Aventis (Pvt.) Ltd., Abbott Laboratories (Pakistan) Ltd. and Roche Pakistan Ltd., respectively. Each product was labeled and expiry dates were not earlier than two years, at the time of study.

2.2. Instrumentation

Shimadzu HPLC system equipped with LC-20 AT VP Pump was utilized in the method development, SPD-20AV Shimadzu UV visible detectors and Purospher STAR C_{18} (250 × 4.6 mm, 5 μm) column used for separation. The chromatographic and integrated data were recorded using a CBM-102 communication Bus Module Shimadzu to Intel Pentium 4 machine with Shimadzu CLASS-GC 10 software. Mobile phase was sonicated by DGU-14 AM on-line degasser, and filtered through 0.45-micron membrane filter. Rheodyne manual injector fitted with a 20 μL loop. Calibrated Pyrex glassware was used for the solution and mobile phase preparation.

2.3. Preparation of Solutions

10 mg of the SPFX and NSAIDs were diluted to 100 mL with mobile phase to produce a concentration of 100 $\mu g \cdot mL^{-1}$. Working solutions of SPFX and NSAIDs were prepared by diluting the stock solutions of with the same solvent to contain 0.15 - 80 $\mu g \cdot mL^{-1}$.

2.4. Analysis of Formulations

For quality control (QC) samples, twenty tablets of each formulation were accurately weighed, crushed to make a fine powder. Calculated amount of powder was weighed and found to be equivalent to 10 mg and transferred to a separate 100 mL volumetric flask. It was dissolved in the mobile phase. Solutions with high, medium and low concentrations *i.e.*; 80, 100 and 120% were prepared, and then filtered through a 0.45-μm Millipore filter, in order to separate out the insoluble excipients by the same procedure as calibration standards but using different stock solutions. The sample solution was suitably diluted and used for the analysis.

All these solutions, calibration and QC samples were stored at 20˚C. Once prepared, analyzed daily for inter and intra-day variations of the method.

2.5. Procedure for Human Serum

Plasma sample, obtained from healthy volunteers, was collected and stored at −20˚C. To an aliquot of 1.0 mL plasma, 10 mL of acetonitrile was added and the mixture was vortexed for one minute centrifuged at 10,000 rpm for 10 minutes. It was then alienated supernatant by filtration (0.45 µ pore size membrane filter). An aliquot serum sample was fortified with sparfloxacin and NSAIDs to get the final concentrations of 0.15 - 80 $\mu g \cdot mL^{-1}$.

2.6. Procedure of Interaction Studies

Sparfloxacin and interacting drugs (NSAIDs solutions (100 $\mu g \cdot mL^{-1}$) were prepared in buffers of pH 4, 7.4 and 9 individually. These solutions were mixed in 1:1 ratio in Erlenmeyer flasks individually to get 50 $\mu g \cdot mL^{-1}$ concentrations; heated on a water bath at 37˚C ± 5˚C with constant stirring at 100 rpm speed. Aliquots of 5 mL were with-drawn at an interval 30 min for 180 minutes. The sample was diluted in suitable dilution then filtered through 0.45 µ filter membrane. Three replicates of filtered samples were injected to HPLC system.

The concentration of each drug was determined using linear equation and % availability was calculated. Effect of pH on the availability of sparfloxacin in presence of interacting drugs, was also monitored.

2.7. Statistical Analysis

Standard regression curve analysis was performed by use of STATISTICA version 7.0 (USA), without intersecting through zero. Linearity graphs were obtained by 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.

3. Results and Discussion

3.1 Method Development and Optimization

The aim of the present study was to develop a simple, isocratic, accurate and sensitive HPLC method for the simultaneous determination of SPFX and NSAIDs. Different C_{18} columns were used but SPFX and NSAIDs could not separate properly. Finally *Purospher* STAR C_{18} (25 × 4.6 mm, 5 µm) column provided the best peak shapes and efficiencies. To investigate the appropriate wavelength for simultaneous determination of sparfloxacin and all the 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 (**Figure 2**), it was observed there was no interference from the mobile phase or baseline disturbance at 240 nm. It was, therefore, concluded that 240 nm is the most appropriate wavelength for analysis with suitable sensitivity.

Chromatographic conditions, especially the composition of the mobile phase, were optimized through several trials to achieve symmetric peak shapes for SPFX 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 symmetric peak shapes for all drugs. A mobile phase containing high proportion of methanol gives shorter run time. Therefore the final mobile phase of methanol and water in the ratio of 90:10 (v/v), provided good resolution. These solvents are easily available, non-toxic, cheap and commonly used solvents for RP-HPLC. To select the optimum mobile phase pH range, 2.5 to 4.0 were investigated, excellent performance was achieved at pH 2.7 adjusted with phosphoric acid. Flow rate was 1.5 $mL \cdot min^{-1}$ with isocratic elution.

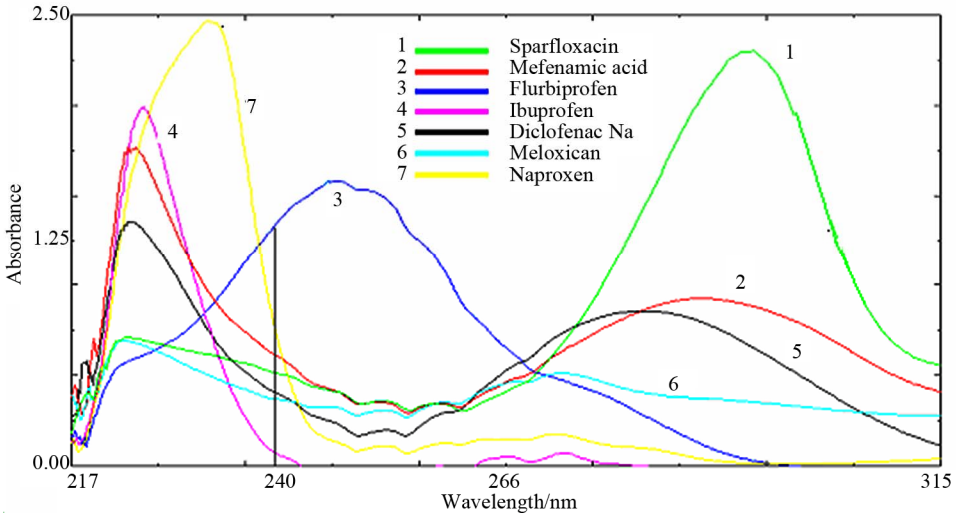

Figure 2. Uv-visible spectra of sparfloxacin and NSAIDs.

3.2. Method Validation

The developed method was validated according to ICH guidelines [32] and USP 2002 [33]. It includes various parameters for example system suitability, selectivity, specificity, linearity, accuracy test, precision, robustness, ruggedness, sensitivity, limit of detection and quantification.

3.2.1. System Suitability

It is a vital section 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. These 10 consecutive injections were used to evaluate the system suitability on each day of method validation. Parameters of system suitability are peaks symmetry (symmetry factor), theoretical plates of the column, resolution, mass distribution ratio (capacity factor) and relative retention [32,34]. They are summarized in **Table 1**.

3.2.2 Linearity

The reason of the check for linearity was to demonstrate that the entire analytical system (including detector and data acquisition) presents a linear response and it is directly proportional over the relevant concentration range of analytes [34]. These curves were obtained using the linear least squares regression procedure as shown in **Table 2** over the concentrations ranging from 0.15 - 80 $\mu g \cdot mL^{-1}$. These analysis results reveals good linear correlations between all the drugs having correlation coefficient (r^2) value >0.999. The homoscedasticity of the calibration plots, tested by Friedman's tests were found to be significantly linear over the tested ranges (**Figures 3(a)** and **(b)**).

3.2.3. Accuracy

The accuracy of the method was determined from the recovery results of spiked placebo samples. Stock solutions of drugs in appropriate portions were spiked into blank placebo matrix to produce concentrations of 80%, 100% and 120% of the theoretical concentration. Mean recovery of spiked samples were in the ranges of 98.42% - 102.75%, Recovery tests were performed by adding known amounts of standard solutions to sample followed by analysis using proposed method. Five runs were performed for every concentration and then peak area was calculated (**Table 3**, **Figure 3(c)**). The average recovery for each level was calculated as indicated by Association of Official Analytical Chemists International [35-37]. Thus, it can be concluded that used excepients (normally present in tablets) did not interfere with drug present in tablets and that filtration medium did not absorb the drug

Table 1. System suitability parameters.

Drugs	Retention time(t_r)	Capacity factor (K')	Tailing factor (T)	Resolution (R_s)	Theoretical plates (N)	Separation factor (α)
SPFX	3.189	0.6	1.28	2.72	2761	1.7
NAPRO	5.825	1.92	1.32	0.31	6792	1.1
FLUR	6.479	2.25	1.32	2.25	7548	1.17
IBU	7.25	2.64	1.21	2.48	8040	1.17
MEF	9.507	3.77	1.33	3.85	8562	1.26
DIC	6.754	4.02	1.27	-	8435	1.07
MEL	5.531	3.11	1.4	2	5866	1.15

Table 2. Regression characteristics.

Drugs	Conc. ($\mu g \cdot mL^{-1}$)	r^2	S.E.E	S.E	Intercept	Regression equation	LOD ($\mu g \cdot mL^{-1}$)	LOQ ($\mu g \cdot mL^{-1}$)
SPFX	0.625 - 25	0.9998	2197.161	1489.741	2224.318	2224.316 + 18299.757X	0.023775	0.072047
NAPRO	0.156 - 2.5	0.9994	3127.509	2117.334	6090.947	6090.946 + 0.000012X	$1.19E^{-08}$	$3.62E^{-08}$
FLUR	0.3125 - 5	0.999	4724.287	3198.358	4461.622	4461.624 + 67858.17X	0.020024	0.06068
IBU	5.00 - 80	0.999	3214.137	2175.981	1537.784	1537.784 + 3028.923X	0.15095	0.45742
MEF	0.3125 - 5	0.9997	1277.189	864.661	5682.952	5682.952 + 37506.593X	0.023931	0.072517
DIC	0.625 - 25	0.9999	1486.7632	1008.07	2388.392	2388.392 + 17380.8X	0.078429	0.237663
MEL	0.625 - 25	0.997	10908.815	7396.501	8684.988	8684.984 + 18330.97X	0.036996	0.112108

Table 3. Accuracy of sparfloxacin and NSAIDs.

Parameters	Conc. (%) spiked	Assay (spiking method)						
		SPFX	NAPRO	FLUR	IBU	MEF	DICLO	MEL
Conc. found	12	12.19	11.662	12.296	12.308	12.034	11.696	11.923
	15	14.707	15.126	14.759	15.431	14.848	14.678	14.991
	18	18.223	17.596	18.46	18.34	18.446	18.403	18.323
% Recovery	80	101.584	97.19	102.47	102.57	100.29	97.466	99.36
	100	98.05	100.841	98.395	102.88	98.991	97.853	99.944
	120	101.244	97.758	102.56	101.89	102.48	102.24	101.179
Parameters	Conc. (%) spiked	Assay in serum						
		SPFX	NAPRO	FLUR	IBU	MEF	DICLO	MEL
Conc. found	12	12.08	11.658	12.103	12.229	12.014	11.675	11.723
	15	14.617	15.029	14.623	15.371	14.654	14.568	14.791
	18	18.125	17.526	18.37	18.194	18.242	18.113	18.123
% Recovery	80	100.66	97.15	100.86	101.91	100.12	97.291	97.691
	100	97.446	100.193	97.486	102.47	97.693	97.12	98.606
	120	100.694	97.366	102.06	101.08	101.34	100.63	100.683

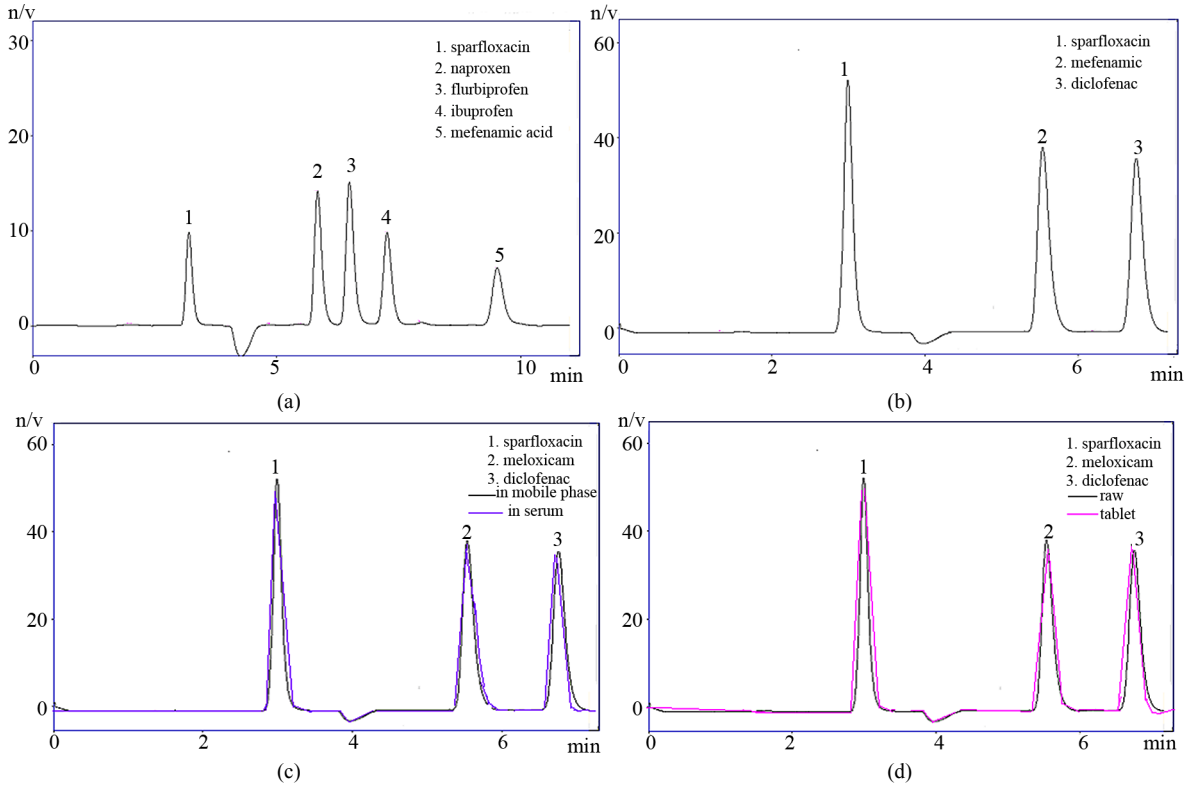

Figure 3. (a) Representative chromatogram of SPFX and NSAIDs at 240 nm; (b) Representative chromatogram of SPFX and NSAIDs at 240 nm; (c) Representative chromatogram of SPFX and NSAIDs at 240 nm in both mobile phase and human serum; (d) Representative chromatogram of SPFX and NSAIDs at 240 nm with and without excepients.

to any extent. High recovery indicated that the method has a high degree of accuracy for the simultaneous determination of sparfloxacin with selected NSAIDs.

3.2.4. Precision

Precision of the anticipated method was determined by repeatability (intra-day precision) and intermediate precision (intra-day precision). It is expressed as relative standard deviation (RSD). Every sample was injected five times. Both intra- and inter-day RSD values were in the range of 0.112% to 2.77% confirming good precision (**Table 4**). Student *t*-test was applied to evaluate the difference in results by variations. The *t*-stat value was lower than the *t*-two-tailed value indicating no significant difference between intra-day and inter-day precision (df = 4). The results were insignificant indicating no remarkable difference in intra-day and inter-day precision.

Table 4. Precision of sparfloxacin and NSAIDs (n = 5).

Drugs	[a]Conc. (μg·mL^{-1})	Formulation (%RSD)		Serum (%RSD)	Drugs	Conc. (μg·mL^{-1})	Formulation (%RSD)		Serum (%RSD)
		D_1	D_2	D_1			D_1	D_2	D_1
	25	2.6	2.64	2.63		2.5	0.586	0.589	0.587
	12.5	0.848	0.852	0.849		1.25	0.96	0.98	0.97
SPFX	6.25	2.249	2.256	2.252	NAPRO	0.625	0.38	0.42	0.39
	3.125	0.881	0.889	0.884		0.3125	0.862	0.869	0.864
	1.625	0.852	0.858	0.854		0.15625	1.495	1.497	1.492
	5	0.984	0.989	0.987		80	0.612	0.619	0.617
	2.5	0.772	0.779	0.775		40	1.418	1.429	1.424
FLUR	1.25	1.099	1.21	1.17	IBU	20	1.009	1.027	1.019
	0.625	0.862	0.867	0.865		10	2.19	2.26	2.24
	0.3125	1.321	1.37	1.34		5	1.07	1.13	1.11
	5	0.814	0.819	0.816		25	1.138	1.142	1.139
	2.5	1.163	1.21	1.168		12.5	0.908	1.05	1.04
MEF	1.25	0.742	0.749	0.744	DIC	6.25	1.353	1.359	1.357
	0.625	1.614	1.619	1.615		3.125	0.412	0.419	0.417
	0.3125	1.7	1.76	1.73		1.625	0.112	0.119	0.116
	25	0.519	0.523	0.521					
	12.5	2.68	2.73	2.69					
MEL	6.25	2.756	2.77	2.761					
	3.125	1.971	2.01	1.991					
	1.625	0.237	0.242	0.239					

***t*-Test: paired two sample for precision**

Drugs	S.D	*t* stat	P (T > *t*) two-tail
SPFX	0.0151	–1.917	0.128
NAPRO	0.016	–2.011	0.115
FLUR	0.0462	–1.712	0.162
IBU	0.0295	–2.516	0.066
MEF	0.0266	–2.084	0.106
DIC	0.0608	–1.22	0.289
MEL	0.0209	–2.395	0.075

[a]Conc. = concentrations, df = 4; SPFX, Sparfloxacin; MEL, Meloxicam; MEF, Mefenamic acid; DIC, Diclofenac sodium; FLUR, Flurbiprofen; IBU, Ibuprofen; S.D, Standard Deviation; D_1: Intra-day and D_2: Inter-day variations.

3.2.5. Limit of Detection (LOD) and Quantification (LOQ)

The LOD and LOQ are calculated as: LOD = 3.3 μ/S and LOQ =10 μ/S; where μ is the standard deviation of the lowest standard concentration and S is the slope of the standard curve. The limits of detection (LOD) and quantification (LOQ) were determined from the calibration curve. The LOD and LOQ were shown in **Table 2**.

3.2.6. Specificity and Selectivity

The selectivity and specificity of the method was documented by studying resolution factor of the peak of sparfloxacin from that of NSAID's. The method confirmed good resolutions ≥2 (**Table 1**) and was found to be free of hindrance from the excepients used in pharmaceutical formulation, which indicated the specificity of the system (**Figure 3(d)**).

3.2.7. Ruggedness

The ruggedness of the method was established by determining SPFX and NSAIDs, in bulk materials, dosage formulation and in human serum in two different labs. Lab 1 was Research Institute of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Karachi while other lab was lab 9, Department of Chemistry, Faculty of Science, University of Karachi. Two different instruments of same configuretion *i.e.* LC 20, were used on different days by different analytes (**Table 4**). All the results were in good limits.

3.2.8. Robustness

Robustness of the method was accomplished by intended modifications made to the method parameters such as composition, flow rate, pH of the mobile phase, injection volume and column temperature and was found quite stable indicated better robustness of the developed method.

Results are shown in **Table 5**.

4. Application of the Proposed Method for *In-Vitro* Interaction Study

Simultaneous determination of sparfloxacin, naproxen, flurbiprofen, ibuprofen, mefenamic acid, diclofenac sodium, and meloxicam was achieved as above. The developed method was also used on spiking the analytes in human serum. The applicability of the proposed method was demonstrated for "*in-vitro*" interaction studies of sparfloxacin with these selected NSAIDs in simulated gastric juice and buffers of pH 4, 7.4 and 9. Sparfloxacin and the reacting drugs were analyzed by measuring the area under curve (AUC), % recovery and considerable drift in retention time. The results of possible interactions are given in **Table 6**.

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

Drugs	Retention time(t_R)	Capacity factor (K')	Tailing factor (T)	Resolution (R_s)	Theoretical plates (N)	Separation factor(α)
A: pH of mobile phase 2.7 ± 0.2						
SPFX	3.18 ± 0.21	0.6 ± 0.15	1.28 ± 0.12	2.72 ± 0.31	2761 ± 41	1.7 ± 0.5
NAPRO	5.825 ± 0.41	1.92 ± 0.9	1.32 ± 0.4	0.31 ± 0.74	6792 ± 32	1.1 ± 0.11
FLUR	6.479 ± 0.51	2.25 ± 0.21	1.32 ± 0.14	2.25 ± 0.29	7548 ± 25	1.17 ± 0.09
IBU	7.25 ± 0.82	2.64 ± 0.2	1.21 ± 0.15	2.48 ± 0.53	8040 ± 43	1.17 ± 0.23
MEF	9.507 ± 0.21	3.77 ± 0.25	1.33 ± 0.07	3.85 ± 0.35	8562 ± 36	1.26 ± 0.21
DIC	6.754 ± 0.26	4.02 ± 0.19	1.27 ± 0.5	0 ± 0.51	8435 ± 15	1.07 ± 0.3
MEL	5.531 ± 0.23	3.11 ± 0.27	1.4 ± 0.19	2 ± 0.17	5866 ± 27	1.15 ± 0.19
B: Flow rate 1.5 ± 0.2 ($mL \cdot min^{-1}$)						
SPFX	3.189 ± 0.19	0.6 ± 0.17	1.28 ± 0.17	2.72 ± 0.34	2761 ± 39	1.7 ± 0.43
NAPRO	5.825 ± 0.39	1.92 ± 0.9	1.32 ± 0.49	0.31 ± 0.71	6792 ± 28	1.1 ± 0.14
FLUR	6.479 ± 0.4	2.25 ± 0.22	1.32 ± 0.15	2.25 ± 0.22	7548 ± 29	1.17 ± 0.07
IBU	7.25 ± 0.81	2.64 ± 0.29	1.21 ± 0.14	2.48 ± 0.47	8040 ± 42	1.17 ± 0.17
MEF	9.507 ± 0.21	3.77 ± 0.31	1.33 ± 0.13	3.85 ± 0.28	8562 ± 35	1.26 ± 0.22
DIC	6.754 ± 0.29	4.02 ± 0.18	1.27 ± 0.42	0 ± 0.27	8435 ± 21	1.07 ± 0.35
MEL	5.531 ± 0.17	3.11 ± 0.29	1.4 ± 0.23	2 ± 0.32	5866 ± 41	1.15 ± 0.27
C: Percentage of methanol in mobile phase 90 ± 5 (V/V/V)						
SPFX	3.189 ± 0.22	0.6 ± 0.19	1.28 ± 0.18	2.72 ± 0.35	2761 ± 45	1.7 ± 0.47
NAPRO	5.825 ± 0.45	1.92 ± 0.6	1.32 ± 0.5	0.31 ± 0.79	6792 ± 39	1.1 ± 0.13
FLUR	6.479 ± 0.7	2.25 ± 0.25	1.32 ± 0.19	2.25 ± 0.24	7548 ± 24	1.17 ± 0.08
IBU	7.25 ± 0.85	2.64 ± 0.3	1.21 ± 0.17	2.48 ± 0.51	8040 ± 47	1.17 ± 0.19
MEF	9.507 ± 0.25	3.77 ± 0.29	1.33 ± 0.17	3.85 ± 0.32	8562 ± 32	1.26 ± 0.25
DIC	6.754 ± 0.33	4.02 ± 0.15	1.271 ± 0.47	0 ± 0.27	8435 ± 19	1.07 ± 0.31
MEL	5.531 ± 0.82	3.11 ± 0.63	1.4 ± 0.42	2 ± 0.57	5866 ± 51	1.15 ± 0.12

t_R = Retention time, K' = Capacity factors, N = Theoretical plates, T = Tailing factor, R_s = Resolution.

Table 6. % Recovery of sparfloxacin with NSAID's.

At pH 1.0												
Time (min)	Spar + NAPRO		Spar + FLUR		Spar + IBU		Spar + MEF		Spar + DICLO		Spar + MEL	
0	101	87.16	101	8.86	106	47.4	78.2	45.7	66.8	17.9	85.68	96.02
30	92	57.08	88.69	7.87	92.6	44.3	119	26	74.3	22.1	77.32	90.55
60	91	57.46	83.83	8.17	89.3	31.2	111	26.7	67.3	23.7	74.98	87.73
90	105	61.55	97.83	8.08	89.3	30.9	104	17.8	68.8	27.7	77.84	86.79
120	105	62.68	86.13	8.08	96	39.6	99.5	24.9	70.1	36.7	76.16	85.68
150	106	60.02	91.96	13.2	90.3	32.5	69.3	21.7	73.6	43.5	83.59	71.7
180	113	57.72	87.39	12.6	94.9	36.3	118	20.7	77.2	47.1	76.51	59.31
At pH 4.0												
Time (min)	Spar + NAPRO		Spar + FLUR		Spar + IBU		Spar + MEF		Spar + DICLO		Spar + MEL	
0	97.7	105.9	64.64	63.7	123	107	81.6	56	70.2	125	95.18	92.85
30	88.5	98.84	55.53	41.6	90.8	95.1	83.3	57.3	61.5	60.9	79.27	83.37
60	66	95.8	52.44	52.2	99.1	40.2	88.5	59.2	61.6	81.3	74.12	62.31
90	65.3	94.38	55.74	49	78.7	34.3	94.1	71.1	60.6	66.6	67.46	78.01
120	64.1	93.6	46.59	71.4	81.1	37.4	96	76.4	58.7	70.6	65.07	72.72
150	62.7	16.58	41.1	78.2	89.9	30.7	82.8	78.2	64.9	69.4	66.8	89.14
180	65.2	15.09	66.83	47.9	92.8	87.8	80.8	82.4	70.2	103	52.72	104.6
At pH 7.4												
Time (min)	Spar + NAPRO		Spar + FLUR		Spar + IBU		Spar + MEF		Spar + DICLO		Spar + MEL	
0	29	49.66	69.89	76.2	60.3	52.9	112	81.4	82.8	31.1	93.55	71.4
30	30.2	54.54	74.84	73	58.3	49.7	122	80.4	87.6	28.7	89.21	74.94
60	31.6	56	75.33	70.6	58.3	47	119	87.2	69.8	24.1	88.1	80.29
90	32.8	58.52	76.62	72.8	56.9	45	114	91.2	77.1	23	86.91	84.07
120	34.5	61.03	72.87	70.2	45.3	45	113	90.4	85.1	19.5	85.59	89.21
150	36.4	62.48	70.21	67.1	47.4	44.8	110	89.6	77.9	22.1	80.85	92.63
180	37	66.22	67.57	64.8	55.9	31.2	75.7	87.9	66.4	14.5	65.19	94.13
At pH 9.0												
Time(min)	Spar + NAPRO		Spar + FLUR		Spar + IBU		Spar + MEF		Spar + DICLO		Spar + MEL	
0	59	50.64	103.2	92.2	138	118	74.7	84.9	94.1	103	48.37	83.99
30	92	48.24	90.1	67.8	101	66.5	70.6	98.5	78.6	106	47.84	87.16
60	89.8	47.32	102.7	66.6	80.9	86	59	86.4	61.7	109	46.4	105
90	96.2	46.99	97.4	71.3	75.9	75.7	66.3	88.8	53.3	85	37.38	101.8
120	90.3	56.61	101.3	65.1	73.4	74.4	105	77.6	51.5	82.6	34	80.61
150	78.8	57.2	96.53	71.5	75	73.9	78.5	68.7	65.3	62.5	56.77	95.6
180	115	59.77	77.79	62.7	80.8	88.3	77.7	66.7	52.2	82.4	38.28	88.88

Availability of naproxen decreased up to 57.72%, 15.09%, 66.22% and 59.77% in simulated gastric juice and buffers of pH 4, 7.4 and 9 respectively. Good interaction found between SPFX and flurbiprofen in simulated gastric juice (12.6%) and buffer of pH 4 (47.9%) while 64.8% and 62.7% available at pH 7.4 and 9 respectively. When ibuprofen interacted with sparfloxacin, the availability was significantly decreased up to 31.2% - 36.3% in selected simulated gastric juice and pH 7.4. While in pH 4 and 9, availability decreased to little extend. Availability of mefenamic acid significantly decreased in simulated gastric juice only. However, no or little interaction have been seen incase of pH 4, 7.4 and 9. The % availability of diclofenac Na decreased to 47.1 in simulated gastric juice, 14.5 in pH 7.4 and increased to 103% in pH 4 and 82.4 in pH 9. Meloxicam was available up to 59.31% - 104.6% at all preferred pH.

On the basis of above consequences, it can be concluded that these interactions were pH dependent and temperature also favored. Significant changes in % availability of drugs might be due to some changes in drug at its chromophoric group, resulting in the distinction of molar absorptivity value which itself is an evidence of drug interaction with NSAID's. Moreover, variation in availability had occurred due to an addition of functional group being attached to the pharmacophore of sparfloxacin and due to loss of axuochromes. Advance *in-vivo* studies are needed for further evaluation.

5. Conclusion

A new method for the simultaneous determination of SPFX and NSAIDs, in bulk materials, pharmaceutical dosage formulation and human serum has been developed for the first time. The proposed HPLC method is simple, rapid, isocratic, specific, accurate and precise. Hence, it can be recommended for the routine quality control and evaluation of clinical data as well, of these drugs. It was apparent that sparfloxacin may interact with commonly used NSAID's like ibuprofen, diclofenac sodium, flurbiprofen, Mefenamic acid and meloxicam which may result in convulsions. Therefore, above mentioned method is applicable in finding out the "*in vitro*" interactions of sparfloxacin with commonly used NSAID's.

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