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Simultaneous determination of moxifloxacin with NSAIDs in API, dosage and serum by reverse phase high-performance liquid chromatography: Application to *in vitro* drug interactions

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Abstract: Experimental design is a significant tool for optimization and validation for the development of HPLC methods to determine API in both human serum and pharmaceutical formulations. In this study, RP-HPLC method is developed and validated for the simultaneous determination of moxifloxacin and NSAIDs. In this experiment, Purospher STAR C_{18} column with optimum assay conditions (10:90, v/v, water: methanol, pH 2.75) used as mobile phase having flow rate of 1.5mL min^{-1} and screened at 240 nm. The experimental results exhibit reliability through accuracy (98-102%), precision (0.011-1.85%) and linearity (R^2>0.999) in range of 0.15-40µgmL^{-1}. The LOD and LOQ limits for moxifloxacin and NSAIDs are found to be 0.015 and 0.046 µgmL^{-1} respectively. The significant outcomes conclude that the developed method for assay is effectively suitable to human serum and pharmaceutical formulations and there is no interference from excipients of tablets and serum. The proposed method is useful for drug-interaction and investigation of moxifloxacin with NSAIDs.

Keywords: Method development, HPLC, moxifloxacin, NSAIDs, drug interaction.

INTRODUCTION

Moxifloxacin (MOX, fig. 1a) is a member of fourth-generation fluoroquinolones. It is a synthetic analog, developed by the addition of methoxy group at 8th position. It is a broad-spectrum antibacterial agent for upper respiratory tract infection (Trindade *et al.*, 2006, de Almeida *et al.*, 2006). Sultana *et al.* (2010) have developed an RP-HPLC method for MOX in API, human serum, and formulations. Literature survey showed different methods available for analysis of MOX. Khan *et al*. (2016) published an analysis of MOX with ofloxacin. Pekamwar *et al.* (2015) discovered a UV-spectrophotometric method for the analysis of moxifloxacin with cefixime in the formulation. Lee *et al*. (2016) developed the LC-MS technique for simultaneous determination of MOX and levofloxacin. A simultaneous method has been developed for the separation of MOX with ketorolac tromethamine by RP-HPLC by Patel *et al*. (2012). Syed *et al*. (2012) reported HPLC method for the investigation of prednisolone and MOX. Syed *et al*. (2017) also developed a method for the separation of MOX and dexamethasone in the dosage form. Dewani *et al*. (2011) published an analysis of MOX with its degradation product by HPLC. Shine *et al*. (2015) reported a method for isolation of MOX with bromfenac sodium by HPLC. For these determinations, they used acetonitrile as a mobile phase which can increase the cost of method. Sultana and group reported some methods for investigation of H_2 receptor antagonist and NSAIDS with fluoroquinolones (Sultana *et al.,* 2011, Gul *et al.,* 2012, Shamim *et al.,* 2012). Momin *et al*. (2018) published a method for quantitative analysis of MOX with bedaquiline (TMC207) and pyrazinamide in inhaler powder formulation powder. Simultaneous use of quinolone and nonsteroidal anti-inflammatory drugs (NSAIDs) may decrease bioavailability and also produce convulsions in patients (Nagoji *et al*., 2003). However, until now, no HPLC method has been described earlier for the simultaneous determination of MOX with NSAIDs to check the interactions and effect on bioavailability. The objective of this research work is to design an easy, economical and reproducible method for the analysis of MOX with NSAIDs simultaneously (fig. 1b-1g). Consequently, present research work is intensive on the development of a simple, quick method of HPLC for the investigation of NSAIDs with MOX in API, human serum and dosage.

MATERIALS AND METHODS

Chemicals

API of MOX was provided by Getz Pharma Pakistan (Pvt.) as gift sample. Analytical grad e methanol and ACN were purchased from Merck Germany. naproxen (Proxen 250 mg tablet), Diclofenac sodium (Fenac 50mg tablet), flurbiprofen (Vobifen 100mg tablet), mefenamic acid (Ponstan 250mg tablet), meloxicam (Xobix 7.5mg

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tablets), and ibuprofen (Brufen 200mg tablet). The expiry dates of all selected products were not prior to 2 years.

Instrumentation

Instrumentation used include Purospher STAR C_{18} (25cm x 4.6 mm, 5 µm) column and Shimadzu HPLC system equipped with LC-20 AT VP Pump, SPD-20AV Shimadzu UV visible detectors. The chromatographic and integrated data were recorded using a CBM-102 communication Bus Module Shimadzu to Intel Core i7, 4th generation, CPU 2.90 GHz, 4.00 GB RAM with Shimadzu CLASS-GC 10 software. Degassing of solvent system is performed on sonicator, DGU-14 AM and the solvent system was filtered by 0.45-micron membrane filter. Rheodyne manual injector fitted with a 20µL loop.

Solutions preparation

10 mg of MOX and each of NSAIDs were measured carefully and prepared the stock solution in 100 mL of solvent system. The final concentration of all solutions were 100 μgmL^{-1}. The stock solution of each drug was used to make working solutions in a concentration of 0.15-40μgmL^{-1}.

Method Development and Validation

A HPLC method has been developed for simultaneous determination of MOX and number of NSAIDs together and then optimized and validated with parameters including linearity, accuracy, precision, LOD, LOQ as per ICH guidelines.

Analysis of formulations

The 20 tablets of each drug were weighed, then converted into fine powder by crushing. The stock solution of 100 μgmL^{-1} concentration of each formulation of NSAIDs and MOX were prepared individually. These solutions were used to prepare working solution of the desired concentrations.

Method for serum

Plasma was collected from healthy volunteers and kept at a cool temperature. 10 ml of acetonitrile mix with 1 ml of plasma. After one minute of rest, it was centrifuged for ten minutes at 10,000 rpm. The supernatant solution was separate out. The prepared serum solutions were utilized for the spiking method of MOX and NSAIDs.

Interaction studies

The stock solution of MOX and selected NSAIDs were made in a concentration of 100µg mL^{-1} in a buffer of pH 4, 7.4 and 9 independently. The stock solution of each NSAIDs was mixed with MOX to become 50µg mL^{-1} concentration and heated at 37±5°C with stirring at a speed of 100 rmp. After a 30 minute interval, 4mL of solution was taken out. This procedure was continued for 3h. The solutions of analytes were diluted to make the final concentration and filter before injection. Linear equation was used to calculate the concentrations and percentage recovery of analytes in sample solution and was also evaluated. The pH effect on the recovery of MOX with NSAIDs was also observed.

STATISTICAL ANALYSIS

The intercept, slope, and standard curve were evaluated through STATICA (version 9). The regression curve plot is constructed on Microsoft Excel 2016 software. Means, SD, student's *t*-test, one-way ANOVA were calculated by SPSS version 16.

RESULTS

The results of system suitability parameters were compiled in table 1. The retention time of drugs is in the range of 2.5- 9.2 minutes and the range of capacity factor was above 2 (2.5 -8.9). The results of theoretical plates are above 2000 (3414-10096). The tailing factor of investigated analytes are less than 2 (<1.37). All peaks are resolved and the result was presented in the standard range. The separation factor of all study samples is also in the given range which was provided by the ICH guidelines. The linearity of all analytes was investigated and compiled in table 2. The concentration range of MOX, diclofenac sodium, meloxicam are 1.5 -25 μgmL^{-1}.Mefenamic acid is 0.31-5μgmL^{-1}, flurbiprofen and naproxen is 0.15-2.5 μgmL^{-1} and ibuprofen is 2.5-40 μgmL^{-1}. The regression value, standard error of estimation, standard error, LOQ and LOD values were calculated and compiled in table 2. For the investigation of accuracy of the developed method, three values of all analytes were selected. It was observed that the percent recovery of all compounds is in the range of 98.67-102.75%, which are present in the standard range (table 3). One-way ANOVA is used to check differences between API and spiking methods. Inter and intra-day precision is also investigated and provided in table 4 and the Student *t-test* was used to check differences within the day and between two days. The developed method is used for the drug interaction study. The *in-vitro* interaction of MOX was checked in the existence of NSAIDs at different physiological pH that is pH 4, 7.4 and 9. The results of the interaction are shown in table 5a-5c.

DISCUSSION

Method optimization and chromatographic conditions

The simultaneous determination of NSAIDs and MOX are performed by RP-HPLC. Initially, three different C_{18} columns were selected for this method development. NSAIDs and MOX could not be separated completely by Discovery C_{18} (25cm x 0.46cm, 5µm) (Supelco, USA) but C_{18} Hiber-RT 250-4.6 Purospher STAR-RP-18 (5µm) was effectively utilized to isolate each analyte at room temperature and this column delivered excellent results of non-polar analytes separation through the short amount of

Table 1: System suitability parameters

Analytes	Retention time (T_R)	Capacity factors (K')	Theoretical plates (N)	Tailing factor (T)	Resolution (R)	Separation factor
pH ± 0.05						
MOX	2.7±0.12	2.5±0.6	3418±71	1.11±0.05		
Diclofenac sodium	5.4±.023	5.4±1.3	7315±45	1.31±0.08	8.53±0.24	3.01±0.71
Meloxicam	4.9±0.25	4.7±1.8	5098±83	1.35±0.09	2.83±0.18	2.29±0.24
Mefenamic acid	9.5±0.17	6.5±1.0	7199±65	1.30±0.09	2.87±0.24	2.19±0.69
Flurbiprofen	6.4±0.31	6.1±1.1	8352±81	1.25±0.11	2.37±0.31	1.25±0.53
Naproxen	5.8±0.14	6.5±1.1	8989±32	1.21±0.11	2.59±0.25	1.23±0.43
Ibuprofen	7.2±0.13	8.9±1.6	10096±21	1.33±0.01	2.52±0.30	2.3±0.41
Flow rate ± 0.2 ml/min						
MOX	2.6±0.51	2.6±0.5	3414±84	1.12±0.01		
Diclofenac sodium	5.5±0.64	5.5±0.8	7293±65	1.32±0.03	8.53±0.14	3.01±0.59
Meloxicam	4.9±0.62	4.8±0.6	5046±73	1.37±0.11	2.83±0.18	2.29±0.61
Mefenamic acid	9.2±0.59	6.0±0.2	7195±81	1.31±0.05	2.87±0.14	2.19±0.58
Flurbiprofen	6.5±0.42	6.1±0.6	8253±86	1.28±0.03	2.37±0.10	1.25±0.70
Naproxen	5.8±0.57	6.9±0.4	8915±43	1.22±0.16	2.59±0.11	1.23±0.44
Ibuprofen	7.1±0.61	8.8±0.4	10092±28	1.32±0.11	2.52±0.12	2.3±0.62
Methanol percentage ± 2						
MOX	2.5±0.41	2.68±0.4	3414±58	1.12±0.11		
Diclofenac sodium	5.8±0.45	5.51±0.4	7293±59	1.32±0.015	8.53±0.15	3.01±0.56
Meloxicam	4.0±0.52	4.88±0.5	5046±66	1.37±0.14	2.83±0.29	2.29±.58
Mefenamic acid	9.3±0.39	6.01±0.4	7195±81	1.31±0.12	2.87±0.36	2.19±.81
Flurbiprofen	6.0±0.53	6.12±0.6	8253±94	1.28±0.12	2.37±0.35	1.25±0.92
Naproxen	5.2±0.48	6.95±0.3	8915±87	1.22±0.13	2.59±0.38	1.23±0.96
Ibuprofen	7.1±0.11	6.82±0.4	10092±55	1.32±0.14	2.52±0.39	2.3±0.89

Table 2: Regression characteristics

Analytes	Conc. range (μgmL^{-1})	$r^{2(a)}$	S. E. E.$^{(b)}$	S. E.$^{(c)}$	Intercept	Slope	LOD$^{(d)}$	LOQ$^{(e)}$
MOX	1.5-25	0.9997	0.255	0.173	2067	19975	0.202	0.611
Diclofenac sodium	1.5-25	0.9998	0.169	0.116	3309	15290	0.019	0.058
Meloxicam	1.5-25	0.9996	0.303	0.209	5959	18370	1.349	4.089
Mefenamic acid	0.31-5	0.9997	0.049	0.034	4641	76778	0.020	0.061
Flurbiprofen	0.15-2.5	0.9995	0.0319	0.024	27944	14134	0.112	0.339
Naproxen	0.15-2.5	0.9997	0.0257	0.0179	10273	22687	0.015	0.046
Ibuprofen	2.5-40	0.9995	0.569	0.487	-345.7	3473	0.172	0.521

(a) Goodness of fit, (b) Standard error of estimate, (c) Standard error, (d) Limit of detection, (e) Limit of quantification.

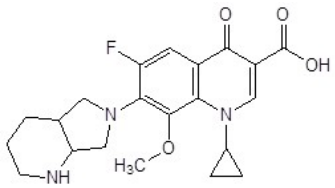

Fig. 1a: Moxifloxacin

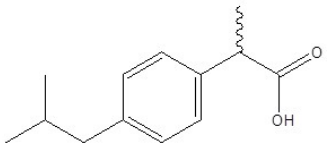

Fig. 1b: Ibuprofen

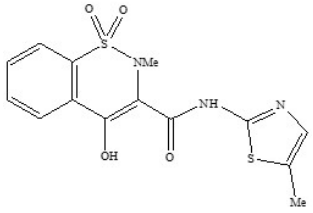

Fig. 1c: Meloxicam

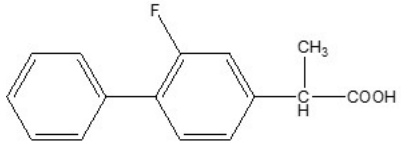

Fig. 1d: Mefenamic acid

Table 3: Accuracy of MOX and NSAIDs

Analytes	Assay (spiking method)			Assay in serum	
	Conc.(μgmL^{-1})	Conc. Found (μgmL^{-1})	Recovery (%)	Conc. Found (μgmL^{-1})	Recovery (%)
Moxifloxacin	12	12.06	100.5	12.1404	101.17
	16	16.11	100.69	16.1952	101.22
	20	20.04	100.12	20.12	100.6
Diclofenac sodium	12	12.33	102.75	12.2208	101.84
	16	16.13	100.81	16.1104	100.69
	20	19.68	98.42	19.89	99.45
Meloxicam	12	12.13	101.43	12.2064	101.72
	16	15.86	99.13	15.8208	98.88
	20	19.76	98.81	19.928	99.64
Mefenamic acid	4	4.05	101.43	4.03	100.75
	5	5.05	100.97	5.08	101.6
	6	5.9	98.95	5.9298	98.83
Flurbiprofen	2	2.04	101.91	2.0244	101.22
	2.5	2.47	98.95	2.515	100.6
	3	3.06	101.92	3.0201	100.67
Naproxen	2	2.02	100.91	2.01	100.5
	2.5	2.47	98.72	2.53	101.2
	3	3.06	102.02	2.9901	99.67
Ibuprofen	32	31.52	98.49	32.0608	100.19
	40	39.47	98.67	39.848	99.62
	48	47.48	98.92	47.9184	99.83
One-way ANOVA					
	[a] SS	[b]df	[c] MS	F	[d] Sig.
Serum					
Between groups	3.426	7	0.489	0.602	0.746
Within groups	13.014	16	0.813		
Total	16.44	23			
Tablets					
Between groups	8.495	7	1.214	0.709	0.665
Within groups	27.384	16	1.711		
Total	35.879	23			

[a]sum of squares, [b]degrees of freedom, [c] mean square, [d] significance

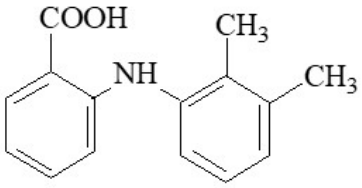

Fig. 1e: Flurbiprofen

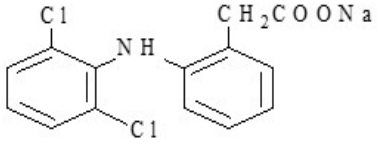

Fig. 1f: Diclofenac sodium

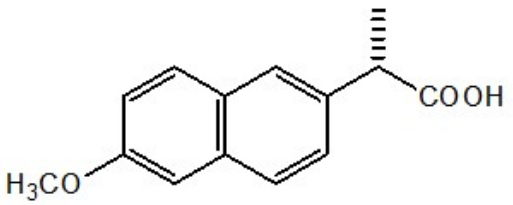

Fig. 1g: Naproxen

Table 4: Precision of MOX and NSAIDs

Analytes	Conc. (μgmL^{-1})	(%RSD)		t- stat	P (T<t) two-tail	(%RSD) Serum
		Day 1	Day 2			Day 1
MOX	1.5	0.23	1.42	-0.241	0.821	0.36
	3.1	1.68	1.89			0.32
	6.2	1.32	0.34			1.32
	12.5	0.39	0.32			1.59
	25	0.37	0.45			0.24
Diclofenac sodium	1.5	1.85	1.92	-0.752	0.494	0.012
	3.1	1.10	1.94			0.011
	6.2	1.92	1.07			0.014
	12.5	1.03	1.86			0.011
	25.0	1.06	1.79			0.019
Meloxicam	1.5	0.11	0.14	-3.066	0.037	0.015
	3.1	0.13	0.15			0.014
	6.2	0.17	0.18			0.016
	12.5	0.13	0.13			0.019
	25.0	0.16	0.19			0.012
Mefenamic acid	0.31	0.54	0.44	-0.412	0.702	1.112
	0.62	0.63	0.07			0.124
	1.25	0.15	0.67			0.122
	2.50	0.09	0.34			0.611
	5.00	1.05	1.34			0.499
Flurbiprofen	0.15	0.15	0.70	-0.149	0.889	0.982
	0.31	0.54	0.54			0.321
	0.62	1.27	0.92			1.179
	1.25	0.29	0.41			0.562
	2.50	1.00	0.80			0.182
Naproxen	0.15	1.41	1.17	-0.449	0.677	0.096
	0.31	0.94	1.12			0.326
	0.62	0.97	0.63			0.981
	1.25	0.45	1.13			0.859
	2.50	1.18	1.30			0.398
Ibuprofen	5.00	1.02	2.17	-1.367	0.244	0.452
	2.50	0.72	0.13			0.599
	10.0	0.92	1.54			0.963
	20.0	0.39	0.52			0.625
	40.0	0.65	1.40			0.924

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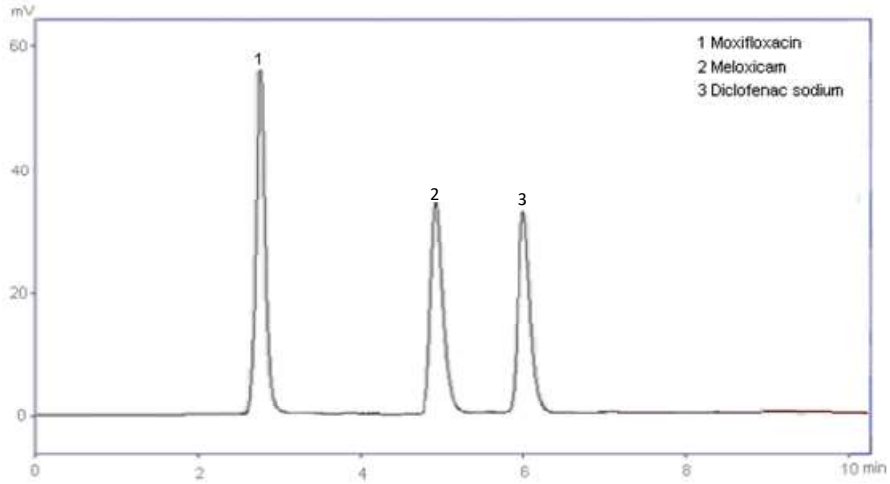

Fig. 2a: Chromatogram of moxifloxacin with different NSAIDs

Table 5a: Interaction of moxifloxacin with NSAIDs in pH 4

Time (min)	Recovery of drugs (%)											
	[a]MOX	[b]Ibu	[a]MOX	[c]Nap	[a]MOX	[d]Mel	[a]MOX	[e]Flur	[a]MOX	[f]Dic	[a]MOX	[g]Mef
0	164.55	95.1	93.83	75.65	62.81	95.59	98.14	68.19	81.47	92.55	129.93	42.86
30	182.63	49.3	108.43	80.52	65.74	86.79	70.83	55.28	60.59	76.14	122.37	51.02
60	165.22	58.92	56.47	68.87	44.98	97.94	72.52	54.3	65.72	96.14	124.64	111.97
90	89.69	69.62	74.02	39.21	67.14	83.43	72.99	56.91	68.46	125.61	112.78	122.94
120	152.64	75.45	56.77	104.43	75.91	91.82	52.71	87.94	64.66	145.03	126.16	189.61
150	139.97	63.18	58.73	101.71	57.32	100.43	67.21	82.28	79.27	138.47	155.31	115.03
180	145.25	52.65	82.58	80.43	50.8	97.8	62.09	60.877	82.53	115.25	135.34	152.36

[a]Moxifloxacin, [b]Ibuprofen, [c]Naproxen, [d]Meloxicam, [e]Flurbiprofen, [f]Diclofenac sodium, [g]Mefenamic acid

Table 5b: Interaction of moxifloxacin with NSAIDs in pH 7.4

Time (min)	Recovery of drugs (%)											
	[a]MOX	[b]Ibu	[a]MOX	[c]Nap	[a]MOX	[d]Mel	[a]MOX	[c]Flur	[a]MOX	[f]Dic	[a]MOX	[g]Mef
0	161.33	45.48	124.05	65.07	117.47	94.83	78.65	77.21	105.53	100.66	171.95	105.79
30	177.12	99.78	155.46	71.54	112.96	86.62	64.27	90.13	106.22	89.67	272.27	106.39
60	148.03	53.39	112.72	59.64	109.76	81.23	60.19	64.98	125.55	106.42	219.45	99.52
90	159.62	74.58	109.51	49.77	108.57	185.01	57.81	70.79	93.45	83.24	327.09	112.46
120	164.88	112.78	119.4	60.56	102.89	104.71	73.51	77.77	99.07	91.16	288.44	117.06
150	162.93	78.32	131.76	56.72	100.73	106.29	73.72	85.38	126.51	85.52	249.61	115.58
180	180.79	131.46	156.93	53.32	101.86	91.32	63.87	84.91	95.43	86.64	299.57	126.07

[a]Moxifloxacin, [b]Ibuprofen, [c]Naproxen, [d]Meloxicam, [e]Flurbiprofen, [f]Diclofenac sodium, [g]Mefenamic acid

Table 5c: Interaction of moxifloxacin with NSAIDs in pH 9

Time (min)	Recovery of drugs (%)											
	[a]MOX	[b]Ibu	[a]MOX	[c]Nap	[a]MOX	[d]Mel	[a]MOX	[e]Flur	[a]MOX	[f]Dic	[a]MOX	[g]Mef
0	113.8	74.88	45.08	36.38	81.71	95.51	88.81	142.21	72.83	110.92	92.19	61.97
30	108.62	107.66	53.84	66.57	108.38	106.84	61.53	99.35	67.51	98.83	90.55	64.96
60	99.11	96.72	42.01	62.7	108.07	113.22	72.91	138.7	59.07	147.89	100.8	78.46
90	111.46	62.11	45.78	62.89	97.12	94.51	74.49	153.24	87.77	108.81	88.27	72.48
120	123.18	55.97	38.96	62.46	98.65	70.77	58.42	72.83	60.38	111.35	95.55	69.04
150	111.6	92.755	42.97	75.76	91.39	77.34	76.02	117.43	54.92	107.05	107.99	115.41
180	117.86	144.6	32.01	69.14	113.63	79.41	85.3	135.24	57.44	114.59	71.63	94.59

[a]Moxifloxacin, [b]Ibuprofen, [c]Naproxen, [d]Meloxicam, [e]Flurbiprofen, [f]Diclofenac sodium, [g]Mefenamic acid

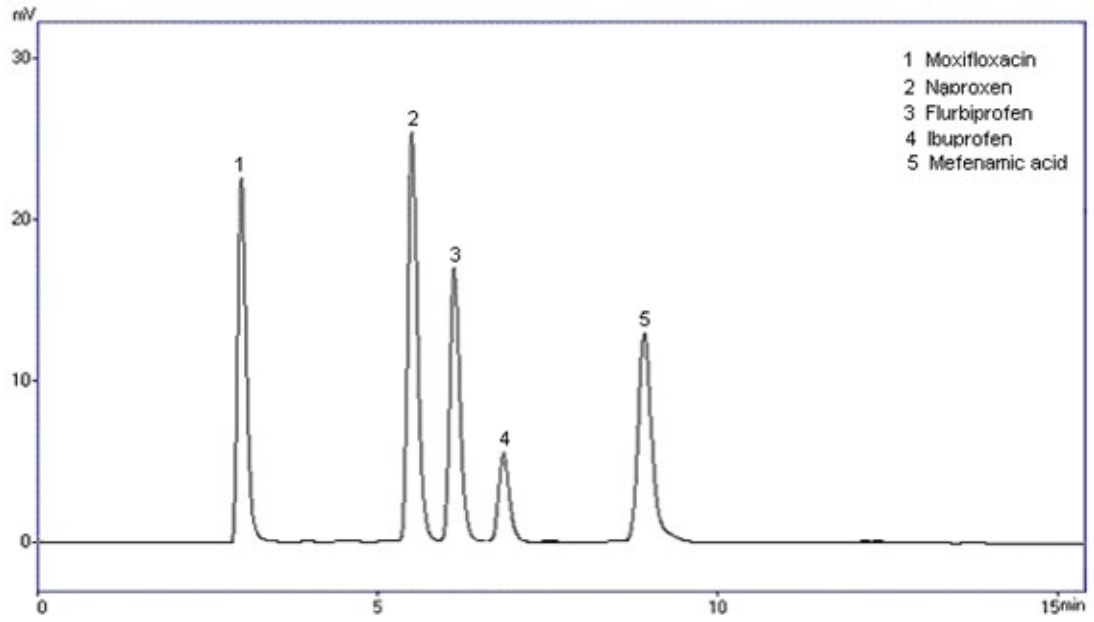

Fig. 2b: Chromatogram of moxifloxacin and different NSAIDs

mobile system usage with peak symmetry. The wave-length of individual drug is evaluated by a UV-visible spectrophotometer and compared to all spectra for calculation of the isobestic point. The 240nm is the assessed wavelength; where the absorbance of selected NSAIDs and MOX were almost equal. For the selection of the mobile phase, initially, so many compositions of water and methanol were tried. The greatest separation of analytes was evaluated by water: Methanol in the combination of 10:90, v/v. The best performance was attained at pH 2.75 having flow rate of 1.5 $mLmin^{-1}$.

Method validation

The ICH guidelines (ICH harmonized tripartite guideline 2015) are employed to validate the parameters of the developed method.

System suitability

The HPLC system was equilibrated with the initial mobile phase composition, followed by 10 injections of the same standard 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 as summarized in table 1, Fig 2a and 2b.

Linearity

Curves of calibration are built in the concentrations range 0.15-40 μgmL^{-1} for MOX and NSAIDs (table 2). The concentrations of analyst and peak-area are used to calculate the least-square linear regression study. Outstanding linearity was found in results with a correlation coefficient (r^2) > 0.999. The slope, intercept and standard curve are determined by STATICA (version 9).

Accuracy

The accuracy of developed methods was evaluated as the percentage recovery by assessment of all investigated analytes in presence of various commonly used tablets' excipients at three levels of concentrations that are 80 %, 100% and 120%. Every analytic injected 5 times and accuracy was calculated in the range of 98-102%. As a result, no significant difference was observed between amounts added and recovered. One-way ANOVA is used to determine for the variances between recovered amount and added amount. The result of one-way ANOVA is insignificant (F-2.212, p>0.1) without serum and F-2.252, p>0.1 with serum. Dunnett's test analysis showed that percent recovery of all analytes has not any significant difference (p>0.5) between the added amount and recovered amount. Therefore, excipients of formulation do not produce any effect with active pharmaceutical ingredient present in tablets. The results are compiled in tables 3 and indicates high recovery which specified that the developed method is very accurate.

Precision

Each model is run 5 times and Intra-day and inter-day reference standard deviation values are calculated. The results were in the range of (0.005 to 1.88) % (table 4) that showed best precision. The variation in the results is investigated by using the Student *t*-test. The two-tailed value is higher than the *t*-stat value which reveals that no significant changes are observed in intra-day and inter-day precision (with df=4).

Limit of detection (LOD) and quantification (LOQ)

The impurity was determined by the LOD and LOQ (ICH, 2006 Q2 (R1), ICH, 1992, Dong 2006). LOD is expressed as a concentration that gives a signal-to-noise ratio of 3:1. LOQ is measured in terms of signal to noise ratio of 10:1

(Plackett, Burman, 1943-1946). The LOD = 3.3μ/*S* and LOQ =10 μ*/S;* (μ is SD (standard deviation) lowest concentration and *S* is the slope of the standard curve. The LOQ and LOD were calculated from the calibration curve (table 2).

Specificity and selectivity

The resolution factor of the peak of MOX with selected NSAIDs was used to identify specificity and selectivity of the developed method. The technique was confirmed excellent resolutions > 2 (table 1).

Robustness

It was achieved by creating small changes in the combination of water and methanol in the solvent system and flow rate. The results of the above investigation show that the developed method is very stable. After a parameter was deliberately altered in ±0.2 ml/min flow rate, ±0.05in pH 2.75 and ± 2ml in mobile phase from its given state, changes in retention time of ±0.1% was less. These results indicated improved robustness of the method (table 1).

Applications

Human serum

The established method is analyzed on spiking the drugs in human serum. There are no significant differences in the recovered amount of drug and amount of drug in spoke serum. It is indicating that the developed method is applicable in human serum (table 3, 4).

Interaction with Non-steroidal anti-inflammatory drugs (NSAIDs)

The developed technique was used to investigate for drug interaction study of selected NSAIDs (ibuprofen, naproxen, mefenamic acid, diclofenac sodium, meloxicam, and flurbiprofen) with moxifloxacin in buffers of pH 4, 7.4 and 9. Interactions of drugs are evaluated by focusing on the percent recovery and AUC (area under the curve). Ibuprofen is available up to 80.43, 72.48, 89.8, and 82.92% in buffers of pH 4, 7.4 and 9 respectively. The Obtainability of naproxen decreased up to 22.12 and 23.52 % in pH 4 and 7.4 respectively while it increased up to 27.8% in pH 9. When meloxicam interacts with moxifloxacin, the availability is decreased up to 12.72-27.66% in all selected pH. Availability of flurbiprofen was decreased in pH 4, 7.4 and 9 up to 15.84-24.69%. However, in simulated gastric juice, availability is increased to 24.91%. The percent availability of diclofenac sodium is decreased to 24.69 in pH 4, 15.84 in pH7.4 and increased to 114% in pH 4.percent availability of Mefenamic acid is up to 65.21- 126.61% at all preferred pH (table 5a-5c).

CONCLUSION

A simple and reliable HPLC method is developed for simultaneous determination of moxifloxacin, meloxicam, diclofenac sodium, ibuprofen, naproxen, mefenamic acid, and flurbiprofen in a very lesser amount of time with high linearity in API, formulation, and serum. The LOQ, minor analyte amount, and little chromatographic time are proved that the proposed method is the best for the routine assay. The results of intra-run and inter-run precision and accuracy are within acceptable limits. The established method is also suitable for *in-vivo* and *in-vitro* interaction, bioavailability, and stability analysis of NSAIDs and MOX.

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