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NOVEL RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF PRAZOSIN AND NSAIDS IN BULK, PHARMACEUTICAL FORMULATIONS AND HUMAN SERUM

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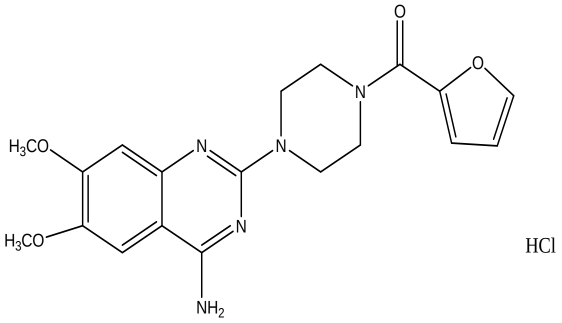
ABSTRACT

Here in this article we are describing about a simple, isocratic reverse phase high-performance liquid chromatographic (RP-HPLC) method developed and validated for the simultaneous determination of Prazosin (PRZ) and non-steroidal anti-inflammatory drugs (NSAIDs) (diclofenac sodium, flurbiprofen, mefenamic acid, meloxicam and naproxen). This method was successfully applied for quantification of respective drugs in bulk, dosage formulations and human serum. Chromatographic separation was achieved at 240nm on the Nucleosil® C_{18} (250mm x 4.6mm, 10µm), ambient temperature using mobile phase consisting of methanol: acetonitrile (85:15 v/v pH 3.0 adjusted by phosphoric acid 85 %). Flow rate was 1.0 mL min^{-1} with an average operating pressure of 140 kg/cm^2. Calibration curves were linear over range of 2.5-1000 µg mL^{-1} with a correlation coefficient 0.9997±0.0002. The limit of detection (LOD) and limit of quantification (LOQ) were in the ranges of 4.0-45.1 ng mL^{-1} and 12.1-136.6 ng mL^{-1}, respectively. Suitability of this method for the quantitative determination of the drugs was proved by validation in accordance with the requirements laid down by International Conference on Harmonization (ICH) guidelines. Method was validated in accordance with ICH and AOAC guidelines and is applicable to the routine analysis of PRZ and NSAIDs, alone or in combination.

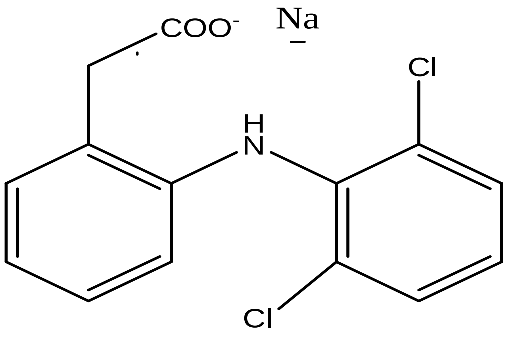
KEYWORDS: Prazosin; NSAIDs; Method Validation; Isocratic; RP-HPLC; Human Serum.

INTRODUCTION

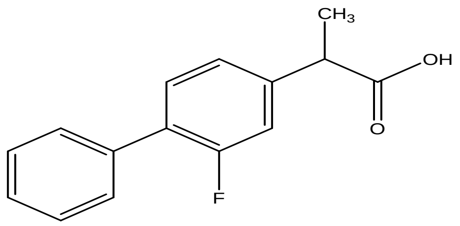
Prazosin hydrochloride (PRZ) (Figure 1) is a quinazole derivative, which reduces peripheral resistance and blood pressure (BP) by vasodilation of peripheral vessel (by blockade of α_1 - adrenergic receptors) in arterioles and veins without increasing the heart rate or significantly impairing sympathetic function.[1,2] Non-steroidal anti-inflammatory drugs (NSAIDs) are usually indicated for the treatment of acute or chronic conditions where pain and inflammation are present. At present, most commonly prescribed NSAIDs; diclofenac sodium (DIC), flurbiprofen (FLR), meloxicam (MLX), mefenamic acid (MEF) and naproxen (NAP) (Figure 1) have been used in this study. They are polyvalent drugs, able to modulate more than one molecular or cellular events thought to be concerned in inflammation.[3] NSAIDs inhibit prostaglandin mediated vasodilation and promote salt and water retention. Hypertensive patients use NSAIDs for a variety of indications. Antihypertensive and non-steroidal anti-inflammatory drugs are frequently prescribed together since hypertension and co-existing musculoskeletal problems are two of the more frequent conditions.[4,5,6] The co-administration of NSAIDs and antihypertensive agents often, but not always, results in blunting of the effect of antihypertensive therapy.[7]



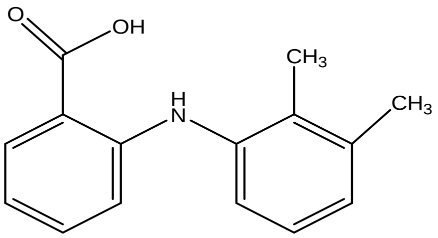
Prazosin HCl



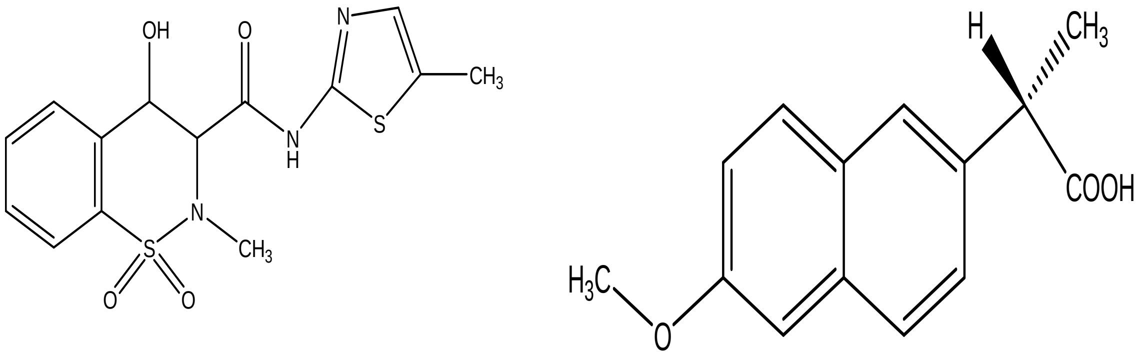
Diclofenac sodium



Flurbiprofen



Mefenamic acid



Meloxicam Naproxen

Figure1. Chemical structures of Prazosin HCl, Diclofenac sodium, Flurbiprofen, Mefenamic Acid,Meloxicam and Naproxen.

Interactions between antihypertensive agents and NSAIDs are often seen in clinical practice.[8,9] In a study of more than 2,000 referred patients receiving NSAIDs and antihypertensive therapy, the frequency of drug interaction causing resistant hypertension was found to be about 1% annually.[10] Several mechanisms have been reported to be probably involved in the elevation of BP by NSAIDs.[11] which can induce an increase in BP and may potentially reduce the efficacy of several antihypertensive drugs,[12,13] and can partially or completely antagonize the effects of many antihypertensive drugs .[7] Morgan et al.[14] described a study, which indicates that indomethacin elevates BP in elderly people treated with enalapril, but not in people whose BP is controlled with amlodipine or felodipine.

Various methods have been reported for the determination of PRZ and NSAIDs.[15,16,17,18,19,20,21,22] Farnoush *et al*.,.[23] determined PRZ in pharmaceutical preparation by potentiometric sensor. K. Sreedhar *et al*.,.[24] reported a spectrophotometric method for determination of PRZ in tablets. Bakshi *et al*., .[25] determined three alpha-adrenergic-blocker (PRZ, terazosin and doxazosin) in the presence of degradation products using a reversed-phase C_{18} column using water/acetonitrile/methanol/glacial acetic acid/diethylamine (25:35:40:1:0.017) as mobile phase for PRZ and terazosin and acetonitrile/water/glacial acetic acid/diethylamine (65:35:1:0.02) for doxazosin. Other coworker[26,27] determined PRZ in plasma by HPLC. A number of workers determined metabolism of PRZ in human liver.[28] In continuation of our previous works on simultaneous determination of NSAIDs with other co-administered drugs as paracetamol and orphenadrine[29] pyrimethamine, sulfadoxime, mefloquine and ibuprofen,[30] diltaizem and NSAIDs,[31] and NSAIDs with preservatives .[32] Now we have attempted to quantitate prazosin along with a number of NSAIDs as diclofenac sodium, flurbiprofen, meloxicam, mefenamic acid and naproxen in active pharmaceutical ingredients, dosage formulations and in human serum by a simple RP-HPLC method.

The present method was developed with an aim to ensure efficient extraction of the drugs from human serum, proper chromatographic conditions to obtain separation of all components, method to be sufficiently sensitive to measure concentrations of the investigated drugs in serum within their therapeutic range. This method has also been applied for drug-drug interaction studies between PRZ and NSAIDs in order to study the effect of these on the availability of PRZ. Such a method was needed to study co-administration of both drugs in multiple drug therapy and there is no such method reported in the literature for the simultaneous determination of PRZ and NSAIDs in active and in dosage formulations.

Aim of the study

Nowadays, a combination therapy is needed for different therapeutic purposes. Due to the prevailing drug interactions between PRZ and NSAID's, there was a need to develop and validate simple and easy method for the simultaneous estimation of both of these types of drugs in bulk materials, dosage formulations and human serum using RP-HPLC. The low LOD and LOQ values merit the method for the determination of these drugs in clinical samples. The aim of the present study was to establish an accurate, efficient, sensitive and reliable method for the separation and quantitative determination of drugs both classes concurrently. Simultaneous determination of both drugs is desirable as this would allow more efficient generation of clinical data and could be performed at more modest cost than separate assays. The applicability of the proposed method was demonstrated later for *in-vitro* interaction studies of PRZ with NSAID's.

EXPERIMENTAL

Material and reagents

All chemicals and reagents used in research work were of analytical grade. Prazosin hydrochloride (purity 99.98 %) was a kind gift from Pfizer Pakistan (Pvt) Limited. Minipress™ (Prazosin 2 mg tablets by Pfizer Pakistan Limited) while, the NSAIDs used were diclofenac sodium (99.87 %) (Voren 50 mg), flurbiprofen (99.92 %) (Ansaid 100 mg), meloxicam (99.79 %) (Melfex 7.5 mg), mefenamic acid (99.98 %) (Ponstan 250 mg) and naproxen (99.89 %) (Neoprox 250 mg) of Pfizer Pakistan (Pvt.) Ltd, Yung Shin Pharmaceuticals Ind. Co. Ltd., Parke - Davis & Co. Ltd., AGP (Pvt.) Ltd., Parke - Davis & Co. Ltd. and Merck Marker (Pvt.) Ltd. respectively. The expiry of these drugs was not less than two year at the time of study. HPLC grade acetonitrile, methanol and ortho phosphoric acid were obtained from Tedia (USA) and Merck Darmstadt, Germany.

Statistical study

Standard regression curve analysis was performed using STATISTICA version 7.0 (USA), without forcing 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.

Instrumentation

A Shimadzu HPLC system equipped with LC- 10 AT VP pump and SPD-10A VP UV–VIS detector was utilized. Chromatographic system was integrated via Shimadzu model CBM-102 to P-IV computer loaded with Shimadzu CLASS-VP software (Version 5.03) for data acquisition and mathematical calculations. Rheodyne manual injector fitted with a 20 µL loop, a Nucleosil, 100-10, C_{18} column (250- 4.6 mm, with a particle size of 10 micron) and DGU-14 AM on-line degasser. In addition, Mettler Toledo electronic balance, microliter syringe and micropore filtration assembly were used in this study. Calibrated Pyrex glassware was used for the solution and mobile phase preparation. Throughout the work only Class-A (Pyrex®) calibrated volumetric flasks and pipettes were used, as guided by Chan, Chung Chow et al. [33]

METHODS

Preparation of standard solutions

A calibrated standard solution of PRZ (100 µg mL^{-1}), DIC (600 µg mL^{-1}), NAP (100 µg mL^{-1}) and MEF (300 µg mL^{-1}) were prepared by dissolving them in methanol. These were then diluted to 2.5, 5, 8, 10, 12, 15, 20, 25, 50 and 100 µg mL^{-1} for PRZ and NAP, 7.5, 15, 24, 30, 36, 45, 60, 75, 150, 300 µg mL^{-1} for MEF and 15, 30, 48, 60, 72, 90, 120, 150, 300, 600 µg mL^{-1} for DIC. A separate stock solution of PRZ (100 µg mL^{-1}) and MLX (1000 µg mL^{-1}) were also prepared in methanol and further diluted to 2.5, 5, 8, 10, 12, 15, 20, 25 and 50 µg mL^{-1} for PRZ and 25, 50, 80, 100, 120, 150, 200, 250, 500 and 1000 µg mL^{-1} for MLX. Another stock solution of PRZ (100 µg mL^{-1}) and FLR (150 µg mL^{-1}) was also prepared in methanol. Aliquots were further diluted to the same concentration of prazosin as other NSAIDs solution and 3.75, 7.5, 12, 15, 18, 22.5, 30, 37.5, 75 and 150 µg mL^{-1} for flurbiprofen.

Fresh working solutions were prepared daily. All solutions were filtered through 0.45 µm filter and degassed using sonicator.

Preparation of sample solutions

For quality control (QC) samples, twenty tablets of each formulation were powdered finely and an amount equivalent to 10 mg of PRZ and NSAIDs each, were weighed and dissolved in methanol. Solutions with concentrations of 80, 100 and 120% were prepared, and then filtered through a 0.45 µm millipore filter, in order to separate out the insoluble excepients. Serial dilutions were prepared by the same procedure as that for calibration standards. All these solutions were stored at 20 ºC. Once prepared, analyzed daily for inter and intraday variations of the method. 20 µL of these solutions were injected into LC system and chromatographed.

Procedure for human serum

Blood 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 followed by centrifugation at 10,000 rpm for 10 minutes. It was then alienated supernatant by filtration (0.45µm pore size membrane filter). An aliquot human serum sample was fortified with PRZ and NSAIDs to get the desired concentrations.

Chromatographic conditions

Separation was carried out under isocratic elution with methanol: acetonitrile (85:15 v/v) as mobile phase. The pH of the mobile phase was adjusted to 3.0 with ortho phosphoric acid (85 %). The flow rate was 1.0 mL min^{-1}, the elution was monitored at 240 nm, and the injection volume was 20 µL, fixed via calibrated loop attached to manual injector.

RESULT AND DISCUSSION

The development of HPLC method for the determination of drugs has received considerable attention because of their importance in routine quality control analysis. HPLC methods generally require complex and expensive equipment, provision for use and disposal of solvents, labor-intensive sample preparation procedure and personal skilled in chromatographic techniques. The goal of this study was to develop a rapid, more accurate, precise reliable least time consuming HPLC method for the simultaneous determination of PRZ and NSAIDs in the form of bulk drug samples, its formulations and human serum using the most commonly employed C_{18} column with UV detector.

Method Development

Experimental design and optimization of isocratic HPLC conditions

In the present investigation the best separation of PRZ and NSAIDs was achieved using a Nucleosil®, 250-4.6, C_{18} column which provides efficient and reproducible separation of the components, it support the aim to reduce the retention time (R_t), the retention factor (K') and to improve the resolution (R) of analytes while minimizing solvent usage. A mobile phase of methanol:acetonitrile (85:15 v/v) having pH adjusted with ortho phosphoric acid to 3.0 provided a reproducible, baseline resolved peak. The lower percentage of acetonitrile in mobile phase results in peak tailing of both components and long analysis duration while higher percentage of acetonitrile in mobile phase results in very poor shape of NSAIDs peak. The criteria to select a given combination of solvents were specificity, peak resolution and omega peak (analysis time). It is obvious from the chromatograms (Fig. 2-4) that PRZ and NSAIDs eluted out forming symmetrical peaks and were well separated. pH effect was also investigating from 2.5 to 4.0, the peaks were sharp and well resolved when the pH was adjusted to 3.0. The peak area of each compound remarkably decreased when the pH value of the mobile phase was above 3.3, whereas there were little changes up to pH 3.2. Thus, pH 3.0 was chosen as an optimal. *O*-phosphoric acid was used for pH adjustment due to its inertness towards the column packing. The method was found to be rapid as PRZ and all NSAIDs eluted out within 10 minutes, which is important for routine analysis. In comparison with other published methods for determination of PRZ and NSAIDs the advantages of this method are ease of operation, short analysis time (total run time < 10 minutes, which is important for routine analysis), utilization of readily available cost-effective solvents, no matrix interferences, and satisfactory limit of quantification to enable pharmacokinetic studies of the drugs. Reliability, rapidness, simplicity, sensitivity, economical nature, acceptable resolution, good recovery and precision of this method give it an advantage over the other reported HPLC methods for simultaneous determination of PRZ and NSAIDs.

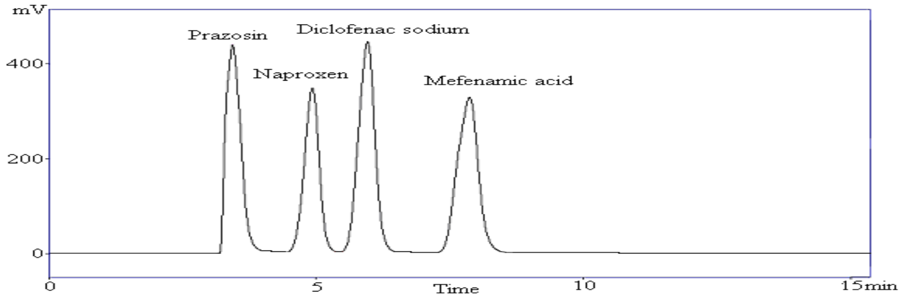


Figure 2. Representative chromatogram showing resolution between prazosin, naproxen, diclofenac sodium and mefenamic acid.
Chromatographic condition: Methanol:acetonitrile (85:15 v/v, pH 3.0)

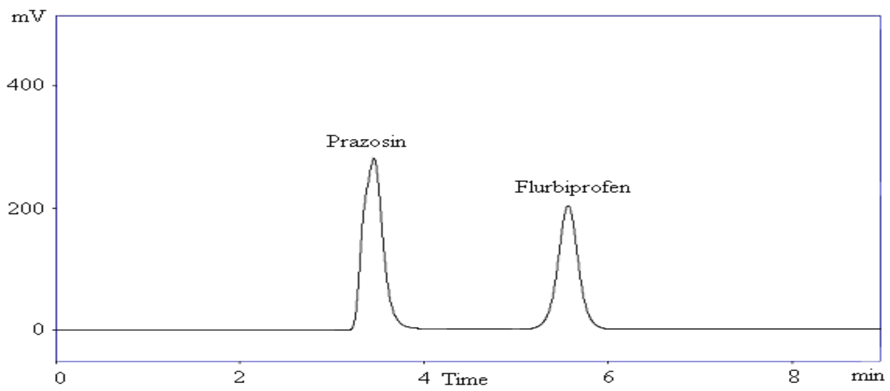


Figure 3. Representative chromatogram showing resolution between prazosin, and flurbiprofen.

Chromatographic condition: Methanol:acetonitrile (85:15 v/v, pH 3.0)

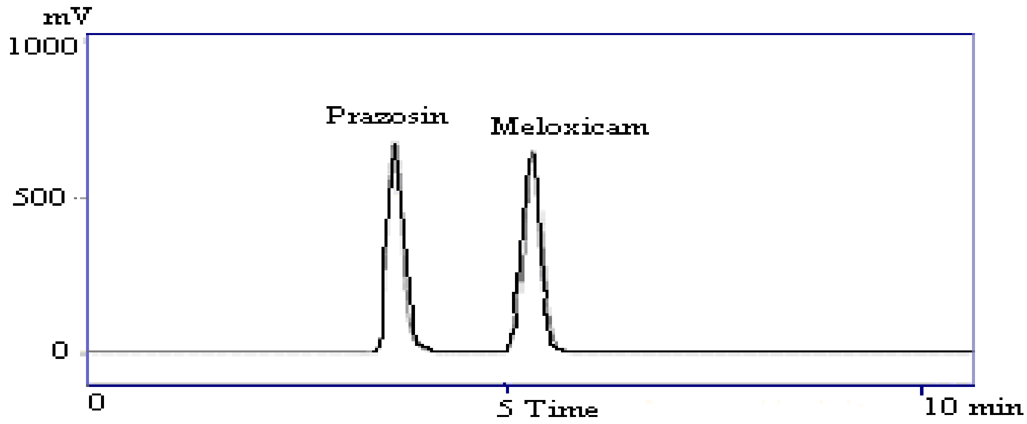


Figure 4. Representative chromatogram showing resolution between prazosin and meloxicam.

Chromatographic condition: Methanol:acetonitrile (85:15 v/v, pH 3.0)

Method Validation

This newly developed method has been validated for determination of drug in raw materials, dosage formulations and serum. For validation of analytical methods, the guidelines of the ICH of Technical Requirements for the Registration of Pharmaceuticals for Human Use.[34] have recommended the accomplishment of system suitability, specificity, selectivity, range and linearity, precision, accuracy test, sensitivity, limit of detection and quantification of the method. These criteria were extensively studied and proved to be satisfactory.

System suitability testing

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 6 injections of the same standard. These 6 consecutive injections were used to evaluate the system suitability on each day of method validation (Table 1). The % RSD obtained by this method for PRZ, and NSAIDs were ≤ 0.744 for all peaks and tailing was ≤ 0.547.

Specificity and selectivity

The specificity and selectivity of the method was established through the study of resolution factor of the peak of PRZ from that of NSAIDs (Table 1). Method demonstrated good resolutions and was found to be free of interference from the excepients used in formulation products and thus, the method is specific for PRZ and NSAIDs.

Table 1: System suitability parameters of the proposed method

Drugs	**Retention time (Rt)**	**Capacity factor (K')**	**Tailing factor (T)**	**Resolution (R)**	**Theoretical plates (N)**	**Separation factor(α)**
PRZ	3.344	0.00	1.43	-	2010	-
DIC	5.805	0.74	0.96	1.90	2715	1.68
FLR	5.552	0.61	0.96	3.85	2500	0.00
MEF	7.672	1.29	0.96	2.68	2421	1.74
MLX	5.059	0.45	1.04	4.43	2879	0.00
NAP	4.814	0.44	0.93	3.08	2681	0.00

PRZ: Prazosin; DIC: Diclofenac sodium; FLR: Flurbiprofen;
MEF: Mefenamic acid; MLX: Meloxicam; NAP: Naproxen

Range and linearity

Linearity is generally reported as the variance of the slope of the regression line. Linearity was tested with known concentrations of PRZ, DIC, FLR, MEF, MLX and NAP i.e. 2.5, 5, 10, 15, 20, 25, 50 and 100, 15, 30, 60, 90, 120, 150, 300 and 600, 3.75, 7.5, 15, 22.5, 30, 37.5, 75 and 150, 7.5, 15, 30, 45, 60, 75, 150 and 300, 25, 50, 100, 150, 200, 250, 500 and 1000 and 2.5, 5, 10, 15, 20, 25, 50 and 100 µg mL^{-1}, respectively. Five runs were performed for every concentration. Injected concentrations versus area were plotted and the correlation coefficients were calculated which are shown in Table **2**. These analysis results reveals good linear correlations for all the drugs having correlation coefficient (r^2) value >0.9995.

Limit of detection and quantification

LOD and LOQ were evaluated as three and ten times, respectively, the ratios between the standard deviation of regression and the slope of the calibration line as depicted in Table 2. LOQ generally falls outside the optimum linear ranges for every analyte. However, as mentioned above these ranges should be regarded as limiting optimum values instead of absolute ones. Thus, analyte at concentrations higher than the LOQ can be determined without risks from the calibration graph.

Table 2: ***Regression statistics and sensitivity of the proposed method***

Drugs	**Conc. (µg mL^{-1})**	**Regression equation**	**r^2**	**LOD (ng mL^{-1})**	**LOQ (ng mL^{-1})**
In bulk					
DIC	15-600	$y = 96392x + 14045$	0.9997	7.3	22.0
FLR	3.75-150	$y = 75971x + 48522$	0.9998	30.1	91.1
MEF	7.5-300	$y = 89996x + 10337$	0.9998	4.6	14.1
MLX	25-1000	$y = 95211x - 74842$	0.9999	32.6	98.8
NAP	2.5-100	$y = 74389x + 14961$	0.9995	45.1	136.6
PRZ	2.5-100	$y = 83192x + 72797$	0.9997	4.0	12.1
In serum					
DIC	15-600	$y = 97001x + 12954$	0.9997	41.3	125.2
FLR	3.75-150	$y = 75926x + 51266$	0.9998	30.9	93.5
MEF	7.5-300	$y = 90218x + 98347$	0.9998	26.2	79.4
MLX	25-1000	$y = 95212x - 74831$	0.9999	20.5	62.1
NAP	2.5-100	$y = 74452x + 15202$	0.9995	35.3	107.0
PRZ	2.5-100	$y = 83247x + 74140$	0.9997	31.3	94.8

PRZ: Prazosin; DIC: Diclofenac sodium; FLR: Flurbiprofen; MEF: Mefenamic acid; MLX: Meloxicam; NAP: Naproxen

Accuracy and recovery

The accuracy of the proposed method was evaluated from the recovery results of spiked placebo samples. Appropriate portions of stock solution of drugs 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 99.809-100.421 %. 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 then peak area was calculated (Table 3). The average recovery for each level was calculated as indicated by Association of Official Analytical Chemists International.[35]

Table 3: Accuracy and recovery of the method

	Spiked conc.	**In bulk**		**In serum**	
Analyte		**Accuracy**	**Rec. conc.**	**Accuracy**	**Rec. conc.**
	(µg mL^{-1})	%	(µg mL^{-1})	%	(µg mL^{-1})
DIC	48	99.992	47.996	100.017	48.008
	60	100.303	60.182	99.809	59.886
	72	100.018	72.013	100.097	72.070
FLR	12	100.029	12.003	99.982	11.998
	15	99.933	14.990	100.005	15.001
	18	100.296	18.053	100.198	18.036
MEF	24	100.189	24.045	100.031	24.008
	30	100.019	30.006	100.169	30.051
	36	100.040	36.014	100.025	36.009
MLX	80	99.992	79.993	100.065	80.052
	100	99.964	99.963	99.997	99.997
	120	100.061	120.073	100.018	120.021
NAP	8	100.359	8.029	99.986	7.999
	10	99.946	9.995	99.970	9.997
	12	100.205	12.025	100.151	12.018
PRZ	8	100.000	8.000	99.980	7.998
	10	100.421	10.042	100.261	10.026
	12	99.930	11.992	99.997	11.999

DIC: Diclofenac sodium; FLR: Flurbiprofen; MEF: Mefenamic acid; MLX: Meloxicam; NAP: Naproxen; PRZ: Prazosin

Ruggedness

The ruggedness of the method was established by determining PRZ and NSAIDs, in bulk, 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. Using, two different instruments of same configuration; LC 20, on different days by different analysts (Table 4 & 5). All the results were in good limits. The assay results indicated that the method was capable with high precision did not show any notable deviations from acceptable limits.

Table4: Intra-and inter day, variation/intermediate precision of the method in pharmaceutical formulations

Drugs	Conc. (µg mL^{-1})	Day 1			Day 2			Day 3		
		Found	% RSD	% Rec.	Found	% RSD	% Rec.	Found	% RSD	% Rec.
DIC	15	15.040	0.962	100.27	14.989	0.087	99.929	14.998	0.050	99.986
	30	30.000	0.397	100.13	29.982	0.076	99.940	29.949	0.011	99.831
	60	60.206	0.941	100.34	60.059	0.343	100.098	60.079	0.388	100.013
	90	90.444	0.017	100.49	89.979	0.027	99.976	90.001	0.094	100.001
	120	120.17	0.851	100.14	119.660	0.010	99.717	120.063	0.570	100.052
	150	150.58	0.413	100.39	150.058	0.061	100.038	150.222	0.437	100.148
	300	299.67	0.088	99.89	299.988	0.081	99.996	300.205	0.331	100.068
FLR	3.75	3.757	0.431	100.18	3.756	0.515	100.153	3.759	0.165	100.247
	7.5	7.508	0.872	100.11	7.502	0.087	100.027	7.502	0.035	100.032
	15	15.011	0.171	100.08	14.997	0.066	99.977	14.995	0.150	99.969
	22.5	22.515	0.381	100.07	22.482	0.005	99.921	22.482	0.180	99.920
	30	30.131	0.001	100.44	29.991	0.071	99.969	29.987	0.153	99.955
	37.5	37.551	0.181	100.14	37.502	0.027	100.006	37.489	0.110	99.969
	75	75.249	0.398	100.33	75.009	0.014	100.013	75.011	0.074	100.015
MEF	7.5	7.497	0.083	99.96	7.498	0.012	99.974	7.499	0.045	99.990
	15	15.094	0.895	100.63	14.992	0.118	99.948	15.002	0.273	100.011
	30	30.014	0.568	100.05	29.972	0.073	99.906	30.003	0.992	100.010
	45	45.140	0.142	100.31	45.038	0.606	100.083	45.040	0.596	100.089
	60	60.012	0.126	100.02	59.971	0.102	99.951	59.975	0.142	99.958
	75	74.771	0.627	99.70	74.999	0.023	99.999	74.986	0.061	99.981
	150	150.24	0.076	100.16	149.996	0.003	99.998	149.985	0.086	99.990
MLX	25	24.972	1.001	99.889	24.996	0.027	99.985	24.952	0.780	99.807
	50	49.993	0.193	99.986	50.029	0.243	100.058	50.105	0.342	100.210
	100	100.01	0.468	100.01	100.016	0.020	100.016	100.016	0.019	100.016
	150	150.18	0.107	100.12	149.928	0.072	99.952	149.956	0.106	99.971
	200	199.68	0.012	99.84	199.999	0.008	99.999	200.038	0.079	100.019
	250	250.28	0.223	100.11	250.150	0.029	100.060	250.187	0.076	100.075
	500	499.81	0.090	99.96	499.980	0.020	99.996	499.985	0.033	99.997
NAP	2.5	2.501	0.034	100.05	2.499	0.272	99.941	2.505	0.667	100.181
	5	4.997	0.091	99.94	4.997	0.289	99.942	5.000	0.299	100.001
	10	10.006	0.992	100.06	9.999	0.075	99.987	9.991	0.225	99.912
	15	15.076	0.146	100.51	15.011	0.052	100.075	15.005	0.111	100.033
	20	20.138	0.016	100.69	20.010	0.062	100.049	20.005	0.028	100.027
	25	25.161	0.124	100.65	25.004	0.762	100.017	25.013	0.703	100.054

	50	50.081	0.084	100.16	49.944	0.177	99.887	50.002	0.023	100.005
PRZ	2.5	2.507	0.019	100.30	2.500	0.075	100.016	2.499	0.011	99.971
	5	4.994	0.934	99.88	4.996	0.410	99.926	5.002	0.238	100.043
	10	10.055	0.554	100.55	9.995	0.296	99.950	10.006	0.138	100.061
	15	15.039	0.140	100.26	15.000	0.010	100.001	15.002	0.092	100.014
	20	19.953	0.077	99.77	20.001	0.186	100.007	19.996	0.043	99.980
	25	24.961	0.130	99.86	25.004	0.068	100.015	25.003	0.068	100.012
	50	49.987	0.095	99.97	49.997	0.022	99.994	50.003	0.035	100.006

DIC: Diclofenac sodium; FLR: Flurbiprofen; MEF: Mefenamic acid; MLX: Meloxicam; NAP: Naproxen; PRZ: Prazosin

Table 5: Intra-and inter day, variation/intermediate precision of the method in serum

Drugs	**Conc. (μg mL^{-1})**	**Day 1**			**Day 2**			**Day 3**		
		Found	**% RSD**	**% Rec.**	**Found**	**% RSD**	**% Rec.**	**Found**	**% RSD**	% Rec.
DIC	15	14.994	0.480	99.960	15.009	0.529	100.063	15.014	0.595	100.096
	30	30.058	0.072	100.193	30.056	0.249	100.187	30.033	0.139	100.111
	60	59.928	0.547	99.880	59.914	0.124	99.857	59.900	0.289	99.833
	90	89.997	0.455	99.997	90.018	0.089	100.020	90.015	0.171	100.017
	120	119.743	0.836	99.786	119.732	0.622	99.776	119.710	0.336	99.758
	150	149.941	0.073	99.961	149.899	0.054	99.933	149.889	0.043	99.926
	300	299.559	0.058	99.853	299.567	0.063	99.856	299.565	0.024	99.855
FLR	3.75	3.759	0.046	100.247	3.761	0.319	100.290	3.764	0.707	100.366
	7.5	7.491	0.163	99.875	7.489	0.010	99.857	7.489	0.071	99.854
	15	15.022	0.071	100.146	15.025	0.006	100.167	15.028	0.214	100.188
	22.5	22.511	0.090	100.050	22.501	0.179	100.003	22.505	0.027	100.024
	30	30.099	0.045	100.330	30.101	0.108	100.337	30.096	0.121	100.321
	37.5	37.546	0.049	100.124	37.550	0.057	100.132	37.549	0.202	100.131
	75	75.160	0.085	100.213	75.146	0.300	100.195	75.132	0.669	100.176
MEF	7.5	7.504	0.127	100.051	7.506	0.546	100.085	7.508	0.206	100.104
	15	15.031	0.029	100.207	15.030	0.318	100.198	15.034	0.034	100.229
	30	30.119	0.167	100.396	30.114	0.106	100.379	30.122	0.514	100.408
	45	44.955	0.665	99.901	44.956	0.095	99.902	44.939	0.063	99.864
	60	59.931	0.052	99.885	59.945	0.068	99.908	59.937	0.038	99.895
	75	74.665	0.341	99.553	74.662	0.051	99.550	74.664	0.133	99.552
	150	149.804	0.324	99.869	149.816	0.032	99.878	149.815	0.064	99.877
MLX	25	24.978	0.212	99.914	24.988	0.595	99.951	24.984	0.024	99.934
	50	50.012	0.010	100.025	50.016	0.058	100.032	50.018	0.029	100.036
	100	100.016	0.020	100.016	99.981	0.096	99.981	99.916	0.107	99.916
	150	150.124	0.049	100.082	150.172	0.042	100.114	150.182	0.053	100.121
	200	199.703	0.086	99.852	199.762	0.126	99.881	199.689	0.057	99.845

	250	250.364	0.012	100.146	250.368	0.094	100.147	250.301	0.039	100.120
	500	499.785	0.023	99.957	499.818	0.050	99.964	499.892	0.010	99.978
NAP	2.5	2.502	0.036	100.065	2.493	0.584	99.701	2.492	0.119	99.697
	5	4.996	0.007	99.928	4.996	0.030	99.928	4.996	0.040	99.920
	10	10.057	0.763	100.568	10.060	0.136	100.600	10.060	0.209	100.599
	15	15.058	0.074	100.390	15.058	0.108	100.384	15.060	0.225	100.401
	20	20.061	0.484	100.306	20.057	0.084	100.283	20.056	0.036	100.280
	25	25.063	0.340	100.251	25.064	0.066	100.257	25.065	0.076	100.260
	50	50.064	0.005	100.127	50.074	0.119	100.148	50.058	0.655	100.116
PRZ	2.5	2.497	0.611	99.878	2.494	0.249	99.748	2.493	0.213	99.738
	5	4.997	0.470	99.940	4.999	0.327	99.979	5.000	0.113	99.992
	10	9.997	0.436	99.971	10.001	0.074	100.013	9.994	0.309	99.941
	15	14.926	0.046	99.504	14.931	0.102	99.540	14.928	0.144	99.578
	20	20.016	0.136	100.080	20.017	0.077	100.086	20.015	0.161	100.077
	25	24.997	0.623	99.989	25.003	0.071	100.014	24.998	0.083	99.993
	50	49.932	0.022	99.865	49.929	0.032	99.858	49.931	0.060	99.861
DIC: Diclofenac sodium; FLR: Flurbiprofen; MEF: Mefenamic acid; MLX: Meloxicam; NAP: Naproxen; PRZ: Prazosin										

Robustness

Robustness of the method was accomplished by designed modifications made to the method parameters such as composition, flow rate and pH of the mobile phase (Table 6) and it was found that the % R.S.D values did not exceed more than 2.0 % .[35]

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

Drugs	Retention time (Rt)	Capacity factor (K')	Tailing factor (T)	Resolution (R)	Theoretical plates (N)	Separation factor(α)
A: pH of mobile phase 3.0 ± 0.2						
PRZ	3.344 ± 0.10	0.00	1.43 ± 0.03	-	2010 ± 40	-
DIC	5.805 ± 0.03	0.74 ± 0.01	0.96 ± 0.01	1.90 ± 0.02	2715 ± 32	1.68 ± 0.01
FLR	5.552 ± 0.04	0.61 ± 0.01	0.96 ± 0.01	3.85 ± 0.01	2500 ± 29	0.00
MEF	7.672 ± 0.03	1.29 ± 0.01	0.96 ± 0.01	2.68 ± 0.01	2421 ± 65	1.74 ± 0.02
MLX	5.059 ± 0.03	0.45 ± 0.02	1.04 ± 0.02	4.43 ± 0.02	2879 ± 38	0.00
NAP	4.814 ± 0.05	0.44 ± 0.01	0.93 ± 0.01	3.08 ± 0.02	2681 ± 45	0.00
B: Flow rate 1 ± 0.2 (mL min^{-1})						
PRZ	3.344 ± 0.15	0.00	1.43 ± 0.02	-	2010 ± 65	-
DIC	5.805 ± 0.05	0.74 ± 0.02	0.96 ± 0.01	1.90 ± 0.02	2715 ± 45	1.68 ± 0.02
FLR	5.552 ± 0.07	0.61 ± 0.01	0.96 ± 0.01	3.85 ± 0.01	2500 ± 21	0.00
MEF	7.672 ± 0.08	1.29 ± 0.02	0.96 ± 0.02	2.68 ± 0.01	2421 ± 42	1.74 ± 0.01
MLX	5.059 ± 0.05	0.45 ± 0.01	1.04 ± 0.01	4.43 ± 0.02	2879 ± 80	0.00
NAP	4.814 ± 0.10	0.44 ± 0.03	0.93 ± 0.01	3.08 ± 0.02	2681 ± 55	0.00

C: Percentage of methanol in mobile phase 85 ± 5 (V/V)						
PRZ	3.344 ± 0.08	0.00	1.43 ± 0.02	-	2010 ± 32	-
DIC	5.805 ± 0.01	0.74 ± 0.01	0.96 ± 0.01	1.90 ± 0.02	2715 ± 24	1.68 ± 0.01
FLR	5.552 ± 0.02	0.61 ± 0.01	0.96 ± 0.01	3.85 ± 0.01	2500 ± 25	0.00
MEF	7.672 ± 0.01	1.29 ± 0.02	0.96 ± 0.01	2.68 ± 0.01	2421 ± 55	1.74 ± 0.01
MLX	5.059 ± 0.02	0.45 ± 0.01	1.04 ± 0.02	4.43 ± 0.02	2879 ± 34	0.00
NAP	4.814 ± 0.01	0.44 ± 0.02	0.93 ± 0.01	3.08 ± 0.02	2681 ± 37	0.00
PRZ: Prazosin; DIC: Diclofenac sodium; FLR: Flurbiprofen; MEF: Mefenamic acid; MLX: Meloxicam; NAP: Naproxen						

CONCLUSION

A simple and reliable HPLC method for monitoring of PRZ, DIC, FLR, MEF, MLX and NAP in human serum and pharmaceutical dosage formulation has been developed. A fully validated RP-HPLC procedure for the assay of these drugs in bulk, tablets and human serum is described for the first time. Hence, it can be recommended for the routine quality control analysis of these drugs, and samples with low volume of blood or plasma. Simplicity of the separation procedure; shorter run time and the low volume of injection make this method suitable for quick and routine analysis. The intra-run and inter-run variability and accuracy results were also in acceptable limit. In addition, this method has the potential application to clinical research of drug combination, multi-drug pharmacokinetics and interactions studies.

Conflict of interest statement

It is hereby declared that there is no conflict of interest among authors.

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