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Research Article

RP-HPLC Method for Determination of Several NSAIDs and Their Combination Drugs

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An RP-HPLC method for simultaneous determination of 9 NSAIDs (paracetamol, salicylic acid, ibuprofen, naproxen, aceclofenac, diclofenac, ketorolac, etoricoxib, and aspirin) and their commonly prescribed combination drugs (thiocolchicoside, moxifloxacin, clopidogrel, chlorpheniramine maleate, dextromethorphan, and domperidone) was established. The separation was performed on Kromasil C18 (250 × 4.6 mm, 5 μm) at 35°C using 15 mM phosphate buffer pH 3.25 and acetonitrile with gradient elution at a flow rate of 1.1 mL/min. The detection was performed by a diode array detector (DAD) at 230 nm with total run time of 30 min. Calibration curves were linear with correlation coefficients of determination (r^2) > 0.999. Limit of detection (LOD) and Limit of quantification (LOQ) ranged from 0.04 to 0.97 μg/mL and from 0.64 to 3.24 μg/mL, respectively. As an application tool of quality by design, full factorial experimental design was used for the testing of robustness of the method. The prediction profiler correlating various parameters and responses was established from the results of design of experiments (DOE).

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most frequently prescribed drugs worldwide and are used for relief of inflammatory, chronic (e.g., rheumatoid arthritis, osteoarthritis, and gout), and acute (e.g., headache, postoperative pain, and orthopedic fractures) pain conditions [1]. NSAIDs formulations are also available as over-the counter pharmaceutical preparations. The anti-inflammatory activity of NSAIDs and most of their other pharmacological effects are related to the inhibition of the conversion of arachidonic acid to prostaglandins, which are mediators of the inflammatory process. NSAIDs are potent inhibitors of cyclooxygenase *in vitro* and *in vivo*, thereby decreasing the synthesis of prostaglandins, prostacyclin, and thromboxane products [2]. Table 1 represents the classification of NSAIDs based on their chemical structure [3].

The growing demand for NSAIDs stimulates higher level of quality control of these therapeutic substances and preparations. Hence, there is need to develop new analytical methods for qualitative and quantitative analysis of NSAIDs and their combination drugs. There are a number of liquid chromatographic methods reported in the literature [4–52] for the individual assays of these drugs and also for the some of their combinations but so far no method has been reported for the simultaneous determination of cited NSAIDs in the presence of selected commonly prescribed combination drugs. In light of the increasing number of combinations, these separation procedures are extremely incompetent. Some attempts have been made to develop single method determination of several NSAIDs in biological samples like Hirai et al. who developed a HPLC method for quantification of 12 NSAIDs in urine samples [53], Lapicque et al. who reported a HPLC method for quantification of sixteen NSAIDs in plasma [54], and Kazemifard and Moore who developed a highly sensitive HPLC method with amperometric detection in plasma samples [55]. GC-MS method has also been reported for some NSAIDs in water samples [56] and plasma and urine samples [57]. It is apparent that a more convergent method is required in order to make these procedures more straightforward and efficient for the estimation of NSAIDs and their combination drugs in their formulations.

Table 1: Chemical classification of NSAIDs.

Class	Drugs
Salicylic acid derivatives	Acetylsalicylic acid (aspirin), salicylamide, sodium salicylate
Aniline and p-aminophenol derivatives aniline and p-aminophenol derivatives	Paracetamol, phenacetin
Pyrazolone derivatives	Phenylbutazone, propyphenazone
2-Arylpropionic acids derivatives (profens)	Ibuprofen, flurbiprofen, ketoprofen, naproxen
Enolic acid derivatives	Meloxicam, piroxicam, tenoxicam, droxicam, lornoxicam
Arylalkanoic acids derivatives	Indometacin, diclofenac, aceclofenac, etodolac, nabumetone, sulindac
N-Arylanthranilic acids (fenamic acids)	Mefenamic acid, tolfenamic acid, meclofenamic acid, flufenamic acid
Selective COX-2 inhibitors (Coxibs)	Celecoxib, rofecoxib, etoricoxib, valdecoxib, parecoxib
Naphthylbutanone derivatives	Nabumetone
Sulphonanilides	Nimesulide
Benzoxazocine derivatives	Nefopam

We have developed a RP-HPLC method for the quantification of fifteen drugs (NSAIDs: paracetamol (PCM), salicylic acid (SA), ibuprofen (IBF), naproxen (NPX), aceclofenac (ACF), diclofenac (DCF), ketorolac (KTL), etoricoxib (ETC), and aspirin (ASP) and commonly prescribed combination drugs: thiocolchicoside (THC), moxifloxacin (MXF), clopidogrel (CLP), chlorpheniramine maleate (CPM), dextromethorphan (DXM), and domperidone (DOM) (structures are shown in Figure 1)) using design of experiments (DOE) approach for robustness testing of the method. To assess the effect of method parameters on chromatographic separation of all the drugs, statistically designed experiments were performed by varying different method parameters such as buffer concentration, pH of mobile phase, flow rate, and column temperature. The developed method was able to determine the content of the cited drugs in different commercial dosage forms. This method would be useful for simultaneous determination of these drugs in different single or compounded formulations.

2. Materials and Methods

2.1. Materials and Reagents. Active pharmaceutical ingredients were kindly obtained as gift samples from MSN Laboratories Ltd., Alembic Ltd., Mayer Lab chem., Maurer Wockhardt, Sri Krishna Pharmaceuticals, and Dr. Reddy's Laboratories Ltd. HPLC grade acetonitrile (ACN) was obtained from Merck, India. High purity water was prepared using Milli-Q gradient ultrapure water system (Billerica, MA, USA).

2.2. Chromatography. The experiments were performed on waters e2695 separation module with waters 2998 photodiode array (PDA) detector. The chromatographic and the integrated data were recorded using empower 2 software. Chromatographic separations were performed on Kromasil C18 (250 × 4.6 mm, 5.0 μm) column at 35°C using 15 mM phosphate buffer pH 3.25 (A) and acetonitrile (B) as mobile phase

Table 2: Gradient programme.

	Time (min.)	Flow (mL/min.)	%A	%B
1		1.10	80.0	20.0
2	3.0	1.10	80.0	20.0
3	16.0	1.10	45.0	55.0
4	17.0	1.10	35.0	65.0
5	26.0	1.10	10.0	90.0
6	27.0	1.10	80.0	20.0
7	30.0	1.10	80.0	20.0

Table 3: Chromatographic parameters for the assayed drugs.

Drug	Rt	Rs	*N*	Tf
PCM	3.34		8439	1.13
THC	5.91	11.72	7107	1.00
SA	7.12	6.9	16342	0.92
MCX	8.77	4.16	34614	1.19
ASP	9.14	3.28	31249	1.05
DOM	10.82	6.75	64678	1.28
CPM	11.47	2.74	40661	1.50
DXM	12.34	3.66	37689	1.58
KTL	14.98	14.06	145673	1.06
ETC	15.73	1.67	144829	1.03
NPX	18.43	17.66	188879	1.22
ACF	19.57	8.69	301257	1.05
DCF	21.14	8.62	321291	1.08
IBF	21.91	4.67	311614	1.06
CLP	25.36	19.73	328259	1.07

Rt: retention time, Rs: resolution, *N*: number of theoretical plates, and Tf: tailing factor.

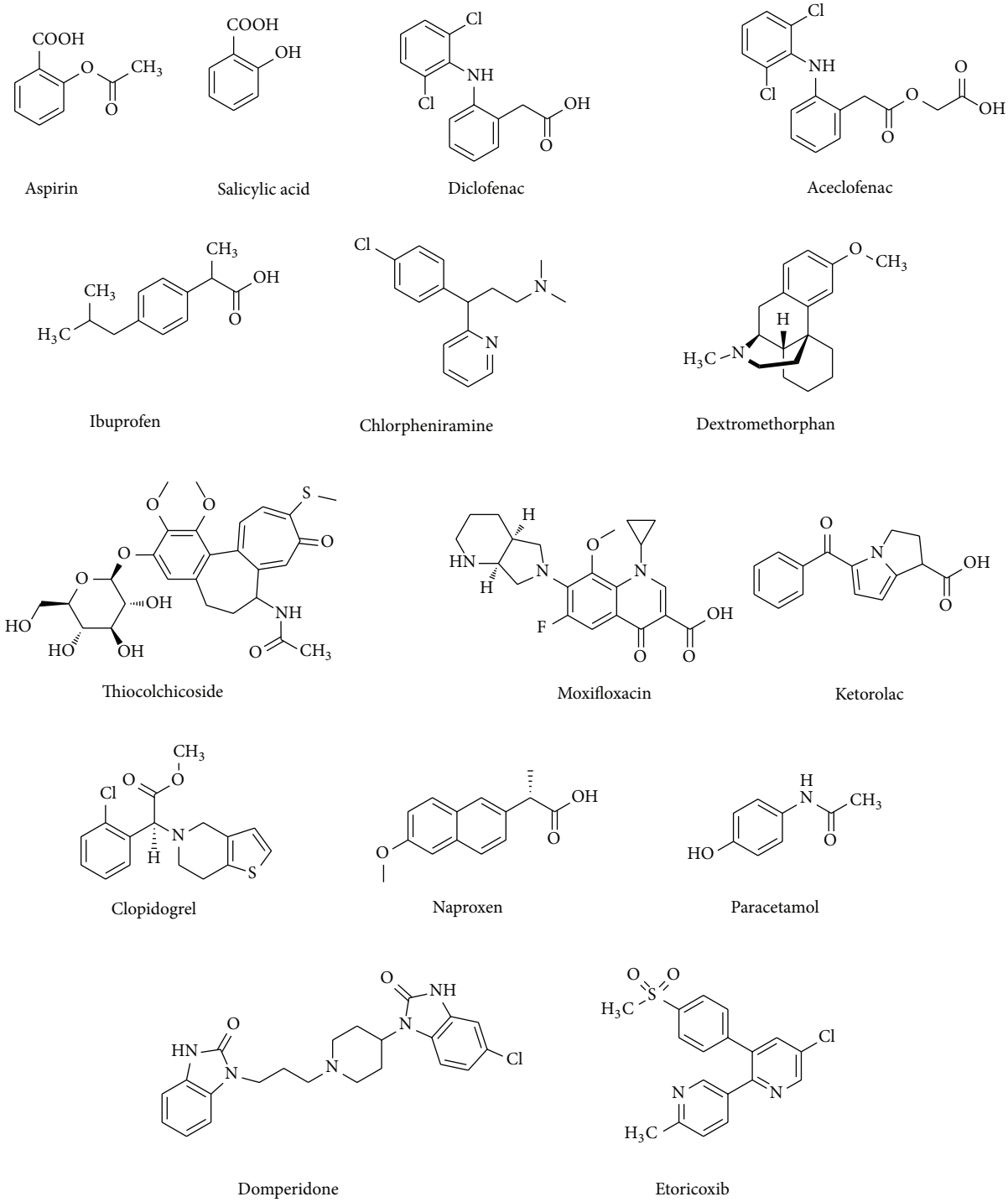

Figure 1: Chemical structure of the studied drugs.

with gradient elution at a flow rate of 1.1 mL/min. Gradient Programme is shown in Table 2. Detection of all the components was carried out at 230 nm with adequate sensitivity.

2.3. Preparation of Stock and Calibration Solutions. Composite stock solution was prepared by dissolving 25 mg of PCM and NPX, 50 mg of THC, SA, MCX, ASP, DOM, CPM, KTL, ETC, ACF, DCF, & CLP, and 100 mg of IBF & DXM in ACN : water (70 : 30 v/v). Using this stock solution, serial dilutions were made to get 8 different concentrations (1, 2.5, 5, 10, 20, 30, 40, and 50 μg/mL for PCM & NPX, 4, 10, 20, 40, 80, 120, 160, and 200 μg/mL for IBF & DXM, and 2, 5, 10, 20, 40, 60, 80, and 100 μg/mL for THC, SA, MCX, ASP, DOM, CPM, KTL, ETC, ACF, DCF, & CLP) to construct calibration curve.

Table 4: Linearity data.

Drug	Range (μg/mL)	Regression equation	Correlation coefficient (R^2)
PCM	1–50	$y = 21951x - 18472$	0.9992
THC	2–100	$y = 16869x - 14679$	0.9996
SA	2–100	$y = 23301x - 13847$	0.9995
MCX	2–100	$y = 13062x - 22221$	0.9997
ASP	2–100	$y = 17800x - 15202$	0.9992
DOM	2–100	$y = 15840x - 10582$	0.9998
CPM	2–100	$y = 11350x - 12712$	0.9994
DXM	4–200	$y = 8476x - 11714$	0.9997
KTL	2–100	$y = 7035x - 4477$	0.9994
ETC	2–100	$y = 37259x - 19456$	0.9998
NPX	1–50	$y = 17840x + 16511$	0.9996
ACF	2–100	$y = 16004x + 25084$	0.9995
DCF	2–100	$y = 18575x - 11191$	0.9995
IBF	4–200	$y = 8957x - 13038$	0.9993
CLP	2–100	$y = 11627x - 14346$	0.9992

Table 5: LOD and LOQ of the drugs studied.

Drug (μg/mL)	PCM	THC	SA	MCX	ASP	DOM	CPM	DXM	KTL	ETC	NPX	ACF	DCF	IBF	CLP
LOD	0.19	0.37	0.39	0.48	0.41	0.37	0.52	0.97	0.57	0.19	0.04	0.36	0.32	0.69	0.53
LOQ	0.64	1.22	1.3	1.61	1.36	1.24	1.75	3.24	1.9	0.65	0.14	1.18	1.05	2.3	1.77

Final dilutions of all drugs were made in phosphate buffer pH 3.25 : ACN (80 : 20 v/v). Responses were measured as peak areas and plotted against concentration.

3. Results and Discussion

3.1. Method Development and Optimization. Experiments were carried out to optimize the experimental parameters affecting the chromatographic separation of all the drugs. Initial experiments showed better resolution and peak shape with acetonitrile compared with the methanol. Therefore, acetonitrile was used as an organic modifier for method development.

The effects of different pH and mobile phase composition were tried to improve the resolution and peak symmetry, such as ammonium acetate, ammonium formate, phosphate, and trifluoroacetic acid with variable pH along with altered composition for % organic, that were tested for complete chromatographic resolution of all 15 drugs.

With ammonium acetate buffer pH 4, there was not an adequate separation between CPM, KTL, and DXM whereas with phosphate buffer pH 2.8 there was no separation at all between SA and ASP. As there were fifteen analytes with diverse physicochemical properties, trials were done to optimize the gradient to have the maximum resolution in the shortest possible run time. From all the trials, pH 3.25 with proposed gradient programme gave the best resolution for all the analytes in run time of 30 min.

The suitability of the proposed method was checked on Phenomenex C8, Grace Genesis Phenyl, Grace C18, Hiber C18, and Kromacil C18 columns with the same dimensions. C8 column led to a very poor resolution of almost all drugs and with phenyl column, there was no separation at all between CPM-DOM and ETC-KTL. Use of Hiber C18 column with proposed gradient resulted in poor separation of ASP-MCX.

Finally, the use of phosphate buffer pH 3.25 with proposed gradient on Kromacil C18 column provided an adequate peak separation, with less tailing, and resulted in the best resolution amongst the buffers tested. A typical chromatogram showing the separation of peaks of all 15 drugs is depicted in Figure 2.

3.2. Validation of the Method. The method was validated by evaluating specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), robustness, and system suitability parameters in accordance with the ICH guideline Q2 (R1) [58].

3.2.1. System Suitability. The system suitability was checked by six replicate injections (standard solution of mixture of 40 μg/mL each drug). The system is deemed to be suitable for use as the tailing factor was less than 1.5 and resolution was greater than 2 for all the drugs. The chromatographic parameters for the drugs are reported in Table 3.

3.2.2. Linearity. The linearity of detector response to different concentrations of drugs was studied in the 8 different concentrations. The samples were analyzed in triplicates at all concentrations. Calibration curves were constructed and the correlation coefficient values of all the studied drugs were observed to be ≥0.999. The regression analysis data for calibration curves were calculated using the peak areas and the data are shown in Table 4.

Table 6: Intraday and interday precision of drugs.

Drug	Concentration (μg/mL)	Intraday		Interday precision (n = 9)					
				Day 0 (n = 3)		Day 1 (n = 3)		Day 2 (n = 3)	
		Mean ± SD (μg/mL)	RSD (%)	Mean ± SD (μg/mL)	RSD (%)	Mean ± SD (μg/mL)	RSD (%)	Mean ± SD (μg/mL)	RSD (%)
PCM	5	5.04 ± 0.04	0.90	5.07 ± 0.05	0.93	5.03 ± 0.04	0.85	5.12 ± 0.05	0.96
	20	20.03 ± 0.1	0.42	20.09 ± 0.1	0.72	20.12 ± 0.1	0.70	20.01 ± 0.2	0.88
	40	40.20 ± 0.3	0.79	40.12 ± 0.3	0.66	40.20 ± 0.3	0.75	40.03 ± 0.2	0.42
THC	10	10.05 ± 0.1	0.92	10.06 ± 0.1	0.59	9.97 ± 0.1	0.90	10.06 ± 0.1	0.88
	40	40.25 ± 0.2	0.44	40.18 ± 0.2	0.42	40.25 ± 0.1	0.25	40.16 ± 0.2	0.48
	80	80.22 ± 0.3	0.31	80.35 ± 0.4	0.46	80.47 ± 0.5	0.56	80.21 ± 0.2	0.24
SA	10	10.06 ± 0.1	0.56	10.13 ± 0.1	0.93	10.05 ± 0.1	0.68	10.12 ± 0.1	0.70
	40	40.18 ± 0.2	0.51	40.21 ± 0.2	0.44	40.13 ± 0.1	0.25	40.22 ± 0.2	0.38
	80	80.12 ± 0.3	0.30	80.11 ± 0.3	0.33	80.04 ± 0.2	0.21	80.17 ± 0.3	0.34
MCX	10	10.13 ± 0.1	0.81	10.12 ± 0.1	0.83	10.08 ± 0.1	0.81	10.30 ± 0.1	0.82
	40	40.28 ± 0.3	0.67	40.53 ± 0.1	0.34	40.34 ± 0.2	0.45	40.31 ± 0.2	0.50
	80	80.45 ± 0.4	0.53	80.75 ± 0.3	0.32	80.37 ± 0.4	0.47	80.51 ± 0.4	0.49
ASP	10	10.12 ± 0.1	0.67	10.07 ± 0.1	0.86	10.05 ± 0.1	0.83	10.11 ± 0.1	0.59
	40	40.20 ± 0.2	0.49	40.22 ± 0.1	0.36	40.23 ± 0.1	0.32	40.18 ± 0.2	0.42
	80	80.31 ± 0.3	0.43	80.54 ± 0.4	0.44	80.81 ± 0.2	0.19	80.42 ± 0.3	0.36
DOM	10	10.15 ± 0.1	0.55	10.20 ± 0.1	0.54	10.15 ± 0.1	0.61	10.17 ± 0.1	0.67
	40	40.19 ± 0.3	0.63	40.24 ± 0.2	0.44	40.19 ± 0.2	0.40	40.21 ± 0.2	0.38
	80	80.21 ± 0.3	0.34	80.53 ± 0.2	0.25	80.47 ± 0.1	0.13	80.34 ± 0.4	0.54
CPM	10	10.03 ± 0.1	0.73	10.06 ± 0.1	0.87	9.94 ± 0.1	0.61	10.16 ± 0.03	0.25
	40	40.12 ± 0.1	0.32	40.03 ± 0.2	0.42	40.04 ± 0.2	0.43	40.29 ± 0.2	0.51
	80	80.19 ± 0.3	0.32	80.44 ± 0.3	0.31	80.33 ± 0.2	0.30	80.21 ± 0.3	0.33
DXM	20	20.16 ± 0.1	0.67	20.17 ± 0.1	0.35	20.05 ± 0.1	0.29	20.25 ± 0.2	0.85
	80	80.11 ± 0.2	0.27	80.02 ± 0.3	0.32	80.18 ± 0.2	0.23	80.13 ± 0.1	0.17
	160	160.21 ± 0.3	0.19	160.23 ± 0.5	0.33	160.05 ± 0.3	0.17	160.51 ± 0.5	0.34
KTL	10	10.28 ± 0.04	0.34	10.24 ± 0.1	0.75	10.09 ± 0.1	0.91	10.22 ± 0.1	0.91
	40	40.27 ± 0.2	0.41	40.19 ± 0.2	0.42	40.20 ± 0.2	0.39	40.25 ± 0.2	0.39
	80	80.30 ± 0.5	0.24	80.14 ± 0.5	0.58	80.45 ± 0.1	0.11	80.14 ± 0.5	0.43
ETC	10	10.16 ± 0.1	0.92	10.22 ± 0.1	0.86	10.14 ± 0.1	0.65	10.17 ± 0.1	1.00
	40	40.01 ± 0.2	0.61	40.02 ± 0.2	0.51	40.09 ± 0.2	0.50	40.19 ± 0.3	0.62
	80	80.11 ± 0.2	0.26	79.86 ± 0.5	0.58	80.19 ± 0.2	0.23	80.15 ± 0.3	0.34
NPX	5	5.08 ± 0.05	0.90	5.06 ± 0.04	0.73	5.01 ± 0.1	0.90	5.09 ± 0.05	0.91
	20	20.01 ± 0.2	0.85	20.07 ± 0.2	0.83	20.05 ± 0.1	0.49	20.07 ± 0.2	0.89
	40	40.28 ± 0.3	0.79	40.28 ± 0.3	0.65	40.16 ± 0.1	0.30	40.28 ± 0.3	0.39
ACF	10	10.15 ± 0.04	0.37	10.14 ± 0.04	0.39	10.03 ± 0.1	0.73	10.14 ± 0.09	0.91
	40	40.27 ± 0.2	0.41	40.28 ± 0.1	0.19	40.19 ± 0.2	0.40	40.28 ± 0.1	0.44
	80	79.94 ± 0.3	0.43	80.05 ± 0.3	0.34	80.04 ± 0.3	0.33	80.22 ± 0.2	0.21

Table 6: Continued.

Drug	Concentration (μg/mL)	Intraday		Interday precision ($n = 9$)					
				Day 0 ($n = 3$)		Day 1 ($n = 3$)		Day 2 ($n = 3$)	
		Mean ± SD (μg/mL)	RSD (%)	Mean ± SD (μg/mL)	RSD (%)	Mean ± SD (μg/mL)	RSD (%)	Mean ± SD (μg/mL)	RSD (%)
DCF	10	10.09 ± 0.1	0.84	10.09 ± 0.1	0.77	10.03 ± 0.1	0.83	10.12 ± 0.1	0.94
	40	40.28 ± 0.1	0.16	40.28 ± 0.1	0.37	40.25 ± 0.2	0.50	40.28 ± 0.1	0.69
	80	80.28 ± 0.3	0.03	79.99 ± 0.4	0.50	80.23 ± 0.1	0.07	79.99 ± 0.4	0.19
IBF	20	20.18 ± 0.2	0.97	20.20 ± 0.1	0.33	20.11 ± 0.2	0.75	20.20 ± 0.1	0.77
	80	80.18 ± 0.3	0.40	80.25 ± 0.1	0.13	80.25 ± 0.2	0.24	80.07 ± 0.2	0.27
	160	160.21 ± 0.5	0.32	160.29 ± 0.4	0.27	160.25 ± 0.3	0.17	160.18 ± 0.5	0.29
CLP	10	10.14 ± 0.1	0.83	10.04 ± 0.2	1.69	10.23 ± 0.1	0.70	10.15 ± 0.1	0.84
	40	40.16 ± 0.2	0.49	40.23 ± 0.2	0.46	40.11 ± 0.2	0.61	40.18 ± 0.2	0.40
	80	80.14 ± 0.2	0.29	79.88 ± 0.7	0.93	80.31 ± 0.1	0.10	80.31 ± 0.4	0.37

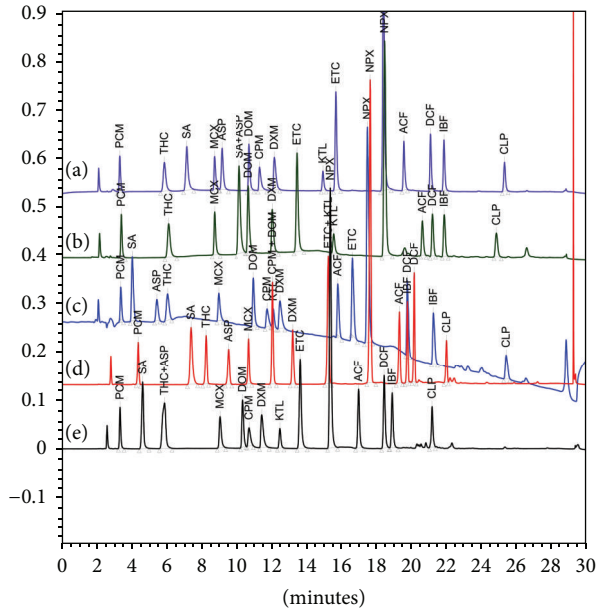

Figure 2: HPLC chromatograms showing pattern of separation in different trials (detection at 230 nm). (a) Final conditions chromatogram: pH 3.25 (phosphate buffer), Kromacil C18 column; (b) pH 2.8 (phosphate buffer), Kromacil column; (c) pH 4.0 (ammonium acetate buffer), Kromacil C18 column; (d) pH 3.25 (phosphate buffer), Grace Genesis Phenyl column; (e) pH 3.25 (phosphate buffer), Grace C18 Phenyl column.

3.2.3. Limit of Detection (LOD) and Limit of Quantification (LOQ). The LOD and LOQ were determined based on signal-to-noise ratio using analytical response of 3 and 10 times of the background noise, respectively. The data are shown in Table 5.

3.2.4. Precision. The intra-day precision was determined by injecting five replicates of three standard solutions covering low, medium, and high concentration levels on a single day. The interday precision of the proposed method was performed by chromatographing standard solutions of the same concentration levels analyzed in triplicate on each of the three consecutive days. The mean value of the concentration and % relative standard deviation (% RSD) are summarized in Table 6.

3.2.5. Accuracy. Accuracy was determined by applying the described method to synthetic mixtures of excipients (lactose, mannitol, maize starch, microcrystalline cellulose, magnesium carbonate, magnesium stearate, silicon dioxide, and titanium dioxide) to which known amounts of each drug at level described in the table were added and analyzed by the proposed method. The accuracy was then calculated as the percentage of each drug recovered by the assay (Table 7).

3.2.6. Robustness. Statistically designed experiments were performed to screen robustness of an analytical method. A full factorial design of experiments containing all possible combinations between the 4 factors and their 2 levels, leading to $n = 2^4 = 16$ experiments, was used to evaluate the robustness of the method. The variables evaluated in the study include pH, flow rate, column temperature, and buffer

Table 7: Accuracy data expressed as the percentage recovery of the amount added.

Drug	Amount added (μg/mL)	Recovery (%)	RSD (%)
PCM	10	99.97	0.76
	25	99.93	0.21
	50	100.15	0.41
THC	25	100.21	0.27
	50	100.38	0.38
	100	100.59	0.41
SA	25	101.27	1.21
	50	100.63	0.26
	100	99.90	0.24
MCX	25	99.69	0.54
	50	100.41	0.32
	100	100.45	0.55
ASP	25	101.13	0.70
	50	99.70	0.34
	100	100.02	0.21
DOM	25	99.66	0.72
	50	99.85	0.45
	100	100.40	0.77
CPM	25	101.51	0.81
	50	99.76	0.36
	100	99.85	0.42
DXM	50	101.35	1.10
	100	100.55	0.14
	150	99.88	0.28
KTL	25	101.09	1.43
	50	99.53	0.26
	100	99.85	0.21
ETC	25	98.65	0.75
	50	100.51	0.22
	100	100.49	0.31
NPX	10	98.80	1.29
	25	100.88	0.61
	50	100.51	0.20
ACF	25	100.04	0.67
	50	100.75	0.21
	100	100.29	0.05
DCF	25	101.91	0.79
	50	100.02	0.64
	100	99.95	0.35
IBF	50	100.71	0.33
	100	99.76	0.11
	150	99.67	0.14
CLP	25	99.28	0.96
	50	99.81	0.75
	100	99.84	0.18

Table 8: Full factorial design of experiments for robustness.

Exp.	Pattern	Flow rate (mL/min.)	pH	Buffer strength (mM)	Temperature (°C)
1	+ − −−	1.2	3.05	10	30
2	− − −+	1	3.05	10	40
3	+ − −+	1.2	3.05	10	40
4	− − +−	1	3.05	20	30
5	− + +−	1	3.45	20	30
6	− + ++	1	3.45	20	40
7	+ − +−	1.2	3.05	20	30
8	− − ++	1	3.05	20	40
9	+ − ++	1.2	3.05	20	40
10	+ + −−	1.2	3.45	10	30
11	− + −−	1	3.45	10	30
12	− − −−	1	3.05	10	30
13	+ + −+	1.2	3.45	10	40
14	+ + ++	1.2	3.45	20	40
15	− + −+	1	3.45	10	40
16	+ + +−	1.2	3.45	20	30

Table 9: Quantitative determination in pharmaceutical formulations.

Formulation examined	API	Label claim (mg)	Amount found (Mean ± SD) (mg)	Recovery (%)	RSD (%) ($n = 6$)
CLAVIX-AS tab	CLP	75	75.15 ± 0.1	100.17	0.14
	ASP	150	150.04 ± 0.5	100.03	0.32
DEXA-P tab	PCM	125	124.90 ± 0.3	99.92	0.21
	CPM	1	0.99 ± 0.02	98.97	1.7
	DXM	5	5.07 ± 0.1	101.48	1.81
THIOCECLO tab	ACF	100	99.92 ± 0.3	99.92	0.32
	THC	4	4.01 ± 0.1	100.33	1.37
CETADOM tab	PCM	500	499.61 ± 0.8	99.92	0.16
	DOM	10	10.03 ± 0.1	100.33	0.9
MILFLOX PLUS eye drops	MCX	5	4.92 ± 0.1	98.4	1.74
	KTL	5	5.05 ± 0.1	101	1.62
SUPAMOVE-4 cap	THC	4	4.02 ± 0.1	100.4	1.76
	DCF	50	49.70 ± 0.3	99.39	0.58
ARTIGESIC tab	IBU	400	399.35 ± 1.2	99.84	0.31
	PCM	325	325.30 ± 0.9	100.06	0.27
NUCOXIA-P tab	ETC	60	59.90 ± 0.3	99.84	0.53
	PCM	500	500.21 ± 0.6	100.04	0.12
NAPRA-D tab	NPX	250	249.72 ± 0.4	99.89	0.16
	DOM	10	9.98 ± 0.2	99.76	1.73

strength. Table 8 represents pattern of the design of experiments. Resolution and tailing factor for all drugs in all 16 experiments were considered as responses. Data were analyzed in JMP (SAS institute) with analysis of variance (ANOVA) techniques and regression analysis combined with graphical illustrations used to determine the impact of the four variables of interest. The variables having a significant ($P < 0.05$) impact on the responses were obtained. Prediction profiler as shown in Figures 3 and 4 was one of the outcomes from the software to evaluate the impact of variables. The prediction profiler correlating various parameters and responses was obtained from the results of DOE. Based on the slope of the individual curve, impact of each variable could be easily determined. The larger the slope, the more the impact on the response.

Based on regression analysis of the data, it was concluded that pH, buffer strength, and temperature were significant parameters affecting the resolution of THC and IBF and

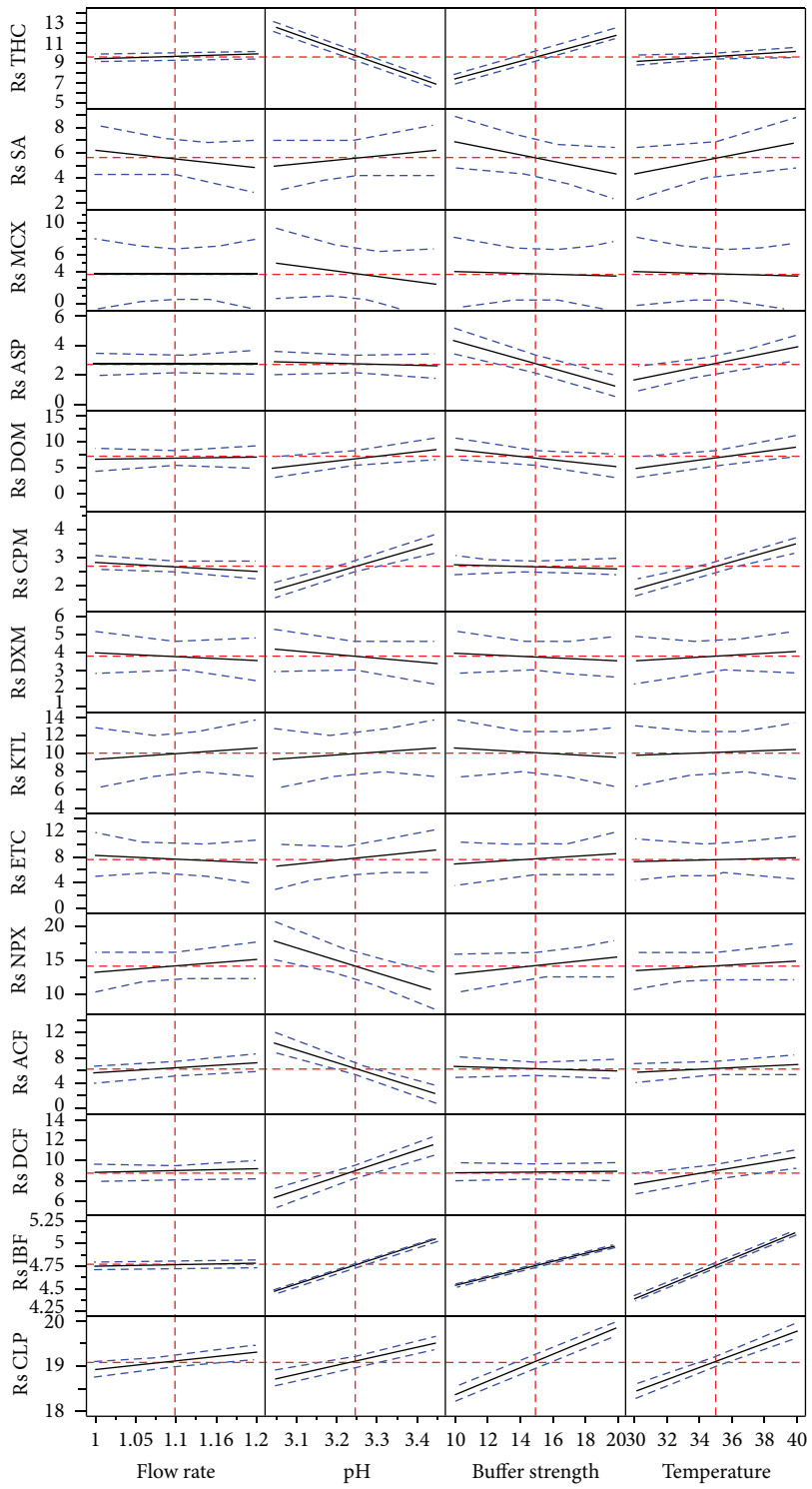

Figure 3: Prediction profiler for resolution.

tailing of NPX, IBF, & ACF whereas pH and temperature had significant effect on resolution of DCF & CPM and Tailing of DOM & CPM. pH was significant factor for the resolution of NPX & ACF and tailing of THC, SA, & ASP. However, CLP resolution was significantly affected by all parameters studied. However, none of the factors had significant effect on resolution of SA, MCX, DXM, KTL, and ETC and tailing of MCF, KTL, ETC, and CLP.

3.2.7. Quantitative Determination in Pharmaceutical Formulations. The validated HPLC method was applied to the simultaneous determination of these drugs in market

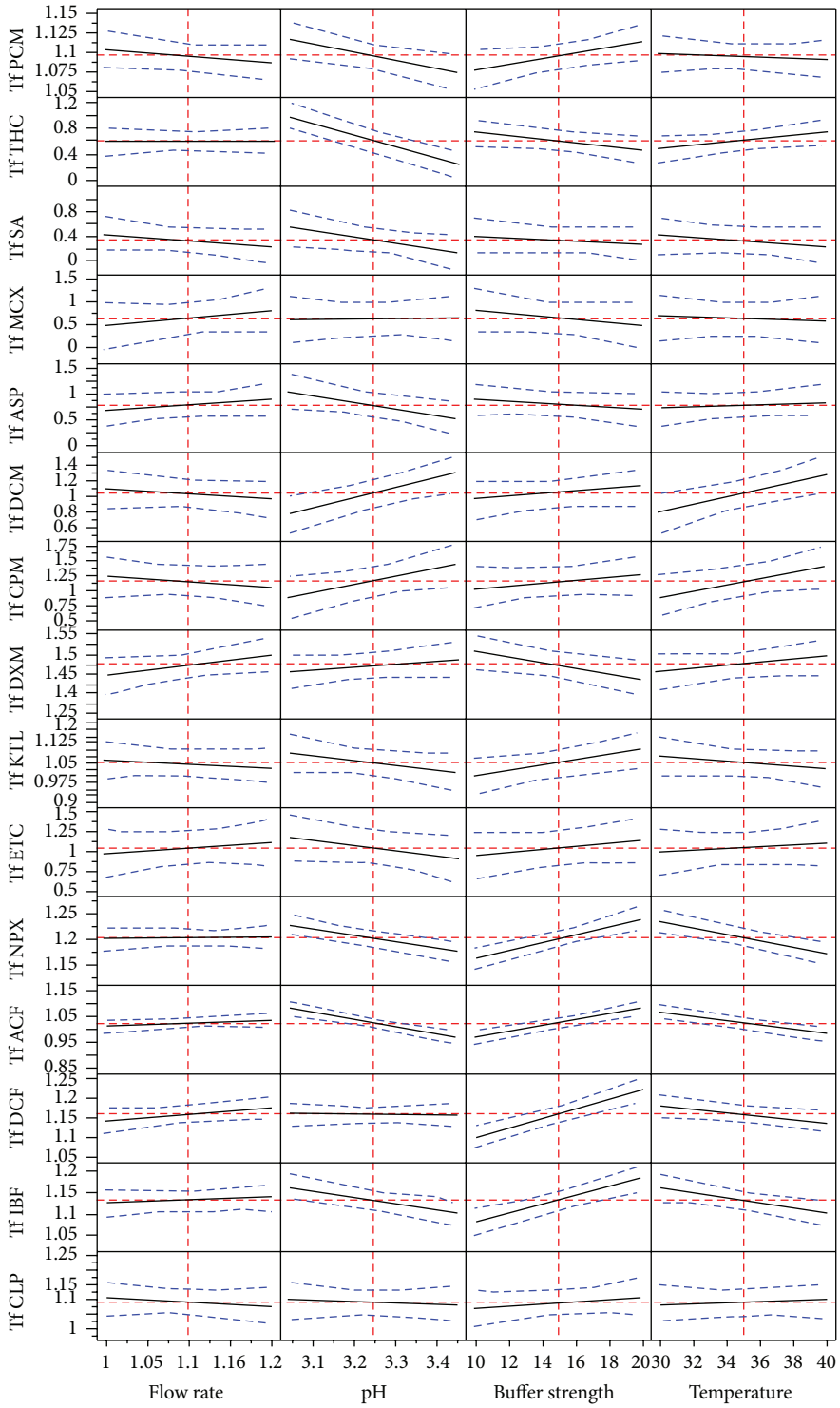

Figure 4: Prediction profiler for tailing factor.

formulations. Amount equivalent to one unit was weighed and diluted in ACN : water (70 : 30 v/v), sonicated for 15 min and further dilutions were made with phosphate buffer pH 3.25 : ACN (80 : 20 v/v) to obtain concentrations within the linearity range. All the samples were filtered through whatman (GD/X25, polypropylene, 0.45 mm) syringe filter, before injecting the samples into the HPLC instrument. The formulation assay results, expressed as a percentage of the label claim, are shown in Table 9. The results indicate that the amount of each drug in the tablets corresponds to the requirement of 90–110% of the label claim.

4. Conclusion

A simple, accurate, precise, and robust RP-HPLC method has been developed and applied for simultaneous analysis of some NSAIDs and their combinations. The methodology was evaluated for specificity, linearity, precision, accuracy, and range in order to establish the suitability of the analytical method. Robustness of the method was evaluated using statistical experimental designs to designate the factors that influence the method's robustness significantly. The developed method was employed for simultaneous determination in their combined dosage forms. This method can be applicable in routine quality control of these drugs.

Conflict of Interests

The authors have no conflict of interests in this paper.

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