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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION STUDIES FOR THE ESTIMATION OF NIMESULIDE AND DICYCLOMINE HYDROCHLORIDE IN FIXED DOSE COMBINATION TABLETS

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Keywords:

Nimesulide, Dicyclomine hydrochloride, RP-HPLC, Validation, fixed dose combination tablets

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ABSTRACT: A simple, accurate, and precise reversed-phase high-pressure liquid chromatography (RP-HPLC) method was developed for the simultaneous estimation of nimesulide and dicyclomine hydrochloride in fixed-dose combination tablets. The developed method was validated as per ICH guidelines Q2(R1). The chromatographic separation was achieved on Hypersil ODS C18 (200 × 4.6 mm 5 µ) column using methanol: phosphate buffer (pH 4) 40:60 (v/v) as the mobile phase at a flow rate of 1 mL/min at 25 °C and detection at 215 nm. The retention times of nimesulide and dicyclominehydrochloride were 3.32 min and 4.22 min, respectively. The linearity range of nimesulide was 2.5-80 µg/mL, and that of dicyclominehydrochloride was 150- 650 µg/mL. The percent recovery of nimesulide and dicyclomine hydrochloride was found to be in the range of 98-102%. The developed method was validated as per ICH guidelines Q2 (R1) and can be used for the estimation of nimesulide and dicyclomine hydrochloride in fixed-dose combination tablets. The developed method can be extended to other dosage forms containing nimesulide and dicyclomine hydrochloride with slight modifications in sample preparation.

INTRODUCTION: Nimesulide, N- (4- nitro-2-phenoxyphenyl) methane sulphonamideis a nonsteroidal anti-inflammatory drug (NSAID) belonging to the sulfonamide class **Fig. 1A**. It has anti-inflammatory, antipyretic, and analgesic effects. It is a preferential cyclooxygenase 2 (COX) inhibitor that inhibits the synthesis of prostaglandins.

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It is used in the treatment of primary dysmenorrhoea, musculoskeletal disorder, and inflammation. Dicyclomine hydrochloride is 2-(diethylamino) ethyl 1-cyclohexylcyclohexane-1-carboxylate hydrochloride **Fig. 1B**. It is used in the treatment of intestinal hypermotility, irritable bowel syndrome (also known as the spastic colon).

An exhaustive literature survey indicated that many methods had been reported for the estimation of nimesulide and dicyclomine hydrochloride individually and in combination with other drugs [1-8]. However, no HPLC method for the simultaneous estimation of nimesulide and dicyclomine hydrochloride has been reported so far.

Hence, there exists a need to develop a new HPLC method for the simultaneous estimation of nimesulide and dicyclomine hydrochloride in fixed-dose combination tablets and validate the developed method as per ICH guidelines Q2(R1).

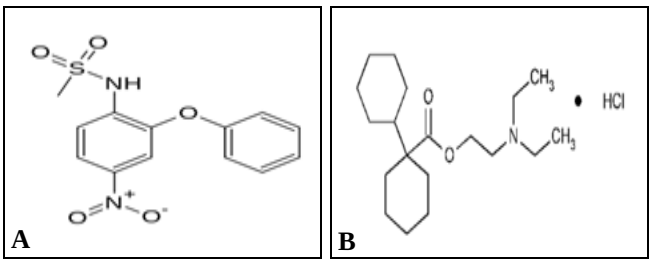

FIG. 1: STRUCTURES OF (A) NIMESULIDE (B) DICYCLOMINE HYDROCHLORIDE

MATERIALS AND METHODS:

Chemicals and Reagents: Gift samples of nimesulide and dicyclomine hydrochloride were received from Indoco Remedies, Mumbai. Methanol (HPLC grade), potassium dihydrogen orthophosphate, disodium hydrogen ortho-phosphate and orthophosphoric acid were obtained from S. D. Fine Ltd., Mumbai. NIMEK SPAS tablets containing nimesulide (100 mg) and dicyclomine hydrochloride (10 mg) were purchased from local pharmacy store in Mumbai.

Instruments: Analytical method development and validation studies were conducted on Agilent Technologies 1260 series chromatograph using a PDA detector equipped with OpenLab software.

Selection of Detection Wavelength: A standard solution of nimesulide (10 ppm) and a standard solution of dicyclomine hydrochloride (1000 ppm) in methanol were scanned in the UV region of 200 to 400 nm using methanol as blank. The λ_{max} of nimesulide was found to be 297 nm, and that of dicyclomine hydrochloride was found to be 215 nm **Fig. 2**.

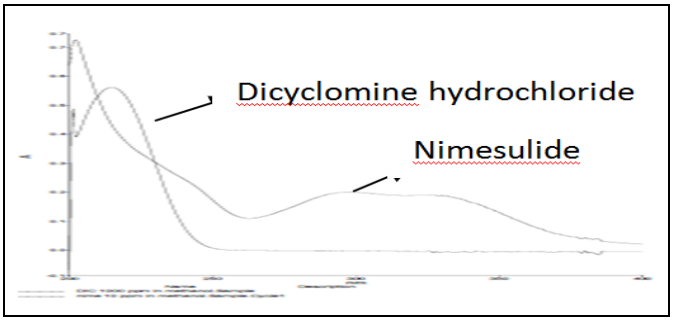

FIG. 2: UV SPECTRAL OVERLAY OF NIMESULIDE AND DICYCLOMINE HYDROCHLORIDE

Chromatographic Conditions: Chromatographic separation was achieved using Hypersil ODS C18 (200 × 4.6 mm; 5 µ particle size) column as stationary phase. Isocratic mode of elution was selected. The injection volume was 20 µL. Good chromatographic separation was achieved at mobile phase composition Methanol: Phosphate Buffer pH 4 (40:60 v/v) at 1 mL/min flow rate. The detection wavelength was selected as 215 nm.

Preparation of Standard Stock Solution of Nimesulide: A standard stock solution of nimesulide (1000 ppm) was prepared by accurately weighing 10 mg of nimesulide and transferring to 10 mL volumetric flask and making up the volume with methanol.

Preparation of Standard Stock Solution of Dicyclomine Hydrochloride: A standard stock solution of dicyclomine hydrochloride (2000 ppm) was prepared by accurately weighing 20 mg of dicyclomine hydrochloride, transferring to 10 mL volumetric flask, and making up the volume with methanol.

Preparation of Working Solution of Nimesulide: A working solution of nimesulide (100 ppm) was prepared by taking 1 mL aliquot of standard stock solution of nimesulide and transferring to 10 mL volumetric flask and making up the volume to mark with methanol.

Preparation of Working Solution of Dicyclomine Hydrochloride: A working solution of dicyclo-mine hydrochloride (1000 ppm) was prepared by taking 5 mL aliquot of standard stock solution of dicyclomine hydrochloride and transferring to 10 mL volumetric flask and making up the volume with methanol.

Preparation of Stock and Working Sample Solutions (Assay): Twenty NIMEK SPAS tablets (Label claim: nimesulide 100 mg and dicyclomine hydrochloride 10 mg) were weighed, and the average weight was determined. These twenty tablets were finely powdered, and weight equivalent to 100mg nimesulide and 10 mg dicyclomine hydrochloride was transferred to 100 mL volumetric flask. 50 mL of methanol was added to the flask and sonicated for 15 min, and volume was made up to the mark with methanol to give a concentration of 1000 ppm nimesulide and

100 ppm dicyclominehydro-chloride. The sample solution was filtered through Whatman filter paper. 0.1 mL of filtrate was transferred to 10 ml volumetric flask. 2.49 mL of working solution of dicyclomine hydrochloride was added to the flask, and volume was made with the mobile phase to concentrate 10 ppm nimesulide and 250 ppm dicyclomine hydrochloride, respectively.

RESULTS AND DISCUSSION:

Method Development and Optimization: A simple, precise RP-HPLC method was developed, which satisfies the acceptance criteria for resolution (R), tailing factor (T), and number of theoretical plates (N). Various combinations of mobile phase were tried with varying concentration and flow rate9-10. Good chromatographic separation was achieved with mobile phase composition Methanol: Phosphate Buffer pH 4 (40:60 v/v) at 1 mL/min flow rate. The detection wavelength was selected as 215 nm since both the drugs showed maximum absorbance at this wavelength. The column oven temperature was maintained at 25°C. The retention time of nimesulide and dicyclomine hydrochloride was found to be 3.32 min and 4.22 min, respectively **Fig. 2**. The chromatogram of the standard mixture solution is illustrated in **Fig. 3**.

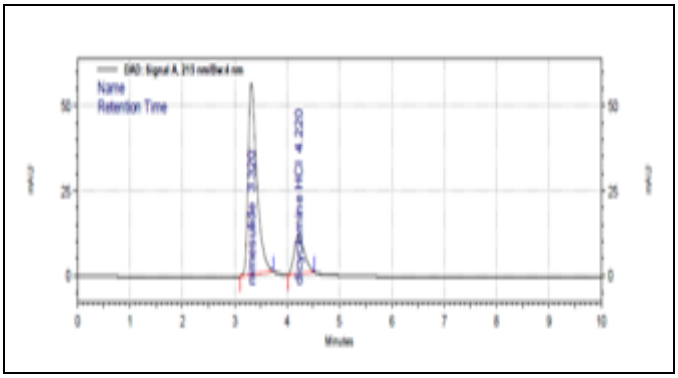

FIG. 3: CHROMATOGRAM OF STANDARD MIXTURE (NIMESULIDE 10 PPM AND DICYCLOMINE HYDRO-CHLORIDE 250 PPM)

Validation Studies: The validation of the developed analytical method was conducted as per the ICH guidelines Q2 (R10) [11].

Specificity Study: Specificity was determined by injecting a blank (mobile phase), sample solution. No peaks were observed in the retention time of nimesulide and dicyclomine hydrochloride for a blank solution. In the case of sample solution, nimesulide and dicyclomine hydrochloride were well resolved. They showed no interference with the excipients in the tablet, and hence the method was found to be specific **Fig. 4**.

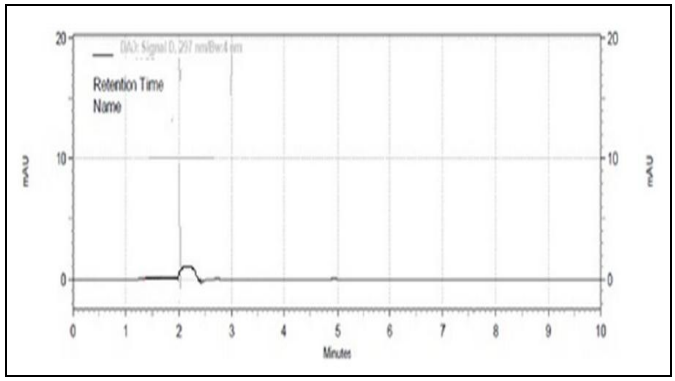

FIG. 4: CHROMATOGRAM OF BLANK (METHANOL: PHOSPHATE BUFFER pH 4)

Linearity Study: The concentration range of 2.5-80 ppm for nimesulide was prepared from a working solution of nimesulide (100 ppm), and 150-650 ppm for dicyclomine hydrochloride was prepared from working solution of dicyclomine hydrochloride (1000 ppm), which were selected for linearity studies. The sample was injected into the HPLC system, and regression was calculated from the curve plot of peak area versus concentration. A standard linear curve was obtained for the concentration range of 2.5-80 µg/mL for nimesulide and 150-650 µg/mL for dicyclomine hydrochloride, and the correlation coefficient between concentration and peak area is more than 0.99. The summary of the linearity study of the two drugs is presented in **Table 1** and **Fig. 5** and **6**.

TABLE 1: LINEARITY STUDY OF NIMESULIDE AND DICYCLOMINE HYDROCHLORIDE

Concentration (ppm)	Nimesulide	Dicyclomine hydrochloride
	Mean ± SD	Mean ± SD
150	216087± 3535.756	216087± 3535.756
250	351322 ± 1783.01	351322 ± 1783.01
350	531893 ± 5619.267	531893 ± 5619.267
450	640478 ± 9708.457	640478 ± 9708.457
550	783808 ± 2766.297	783808 ± 2766.297
650	901579 ± 12862.92	901579 ± 12862.92
Regression equation	y = 120108x +10315	y = 1381x + 18461
Regression coefficient	0.9997	0.9958

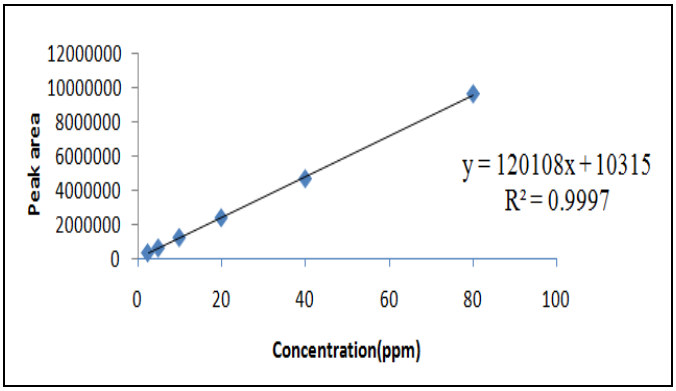

FIG. 5: LINEARITY STUDY OF NIMESULIDE

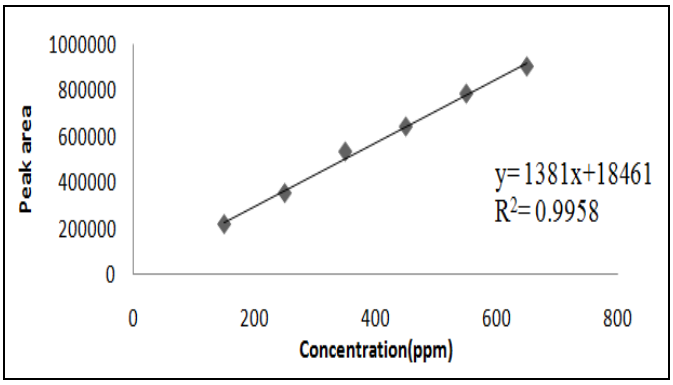

FIG. 6: LINEARITY STUDY OF DICYCLOMINE HYDROCHLORIDE

Precision Study: The precision of the developed analytical method was conducted by performing repeatability, intraday precision, and interday precision.

Repeatability (Precision): Combined standard solutions of nimesulide and dicyclomine hydrochloride 10 ppm and 250 ppm respectively were prepared and analyzed six times, and % RSD was calculated **Table 2**.

Intraday Precision (Precision): Variation of results within the same day was analyzed. Intraday precision was determined by analyzing three different concentrations (10 ppm, 20 ppm, and 40 ppm) for nimesulide and (250 ppm, 350 ppm, and 450 ppm) for dicyclomine hydrochloride at different time intervals on the same day (0 h, 4 h, 8 h).

Analysis was performed in triplicate, and % RSD was calculated. The precision of the method was verified by calculating the % RSD. The percent relative standard deviation of Nimesulidewas found to be 0.63%, and that of Dicyclomine hydrochloride was found to be 0.56% which complied with acceptance criteria. *i.e.*, %RSD should be NMT 2%, indicating that the developed method was precise **Table 2**.

Interday Precision (Precision): Variation of results on different days was analyzed. Interday precision was determined by analyzing three different concentrations (10 ppm, 20 ppm, and 40 ppm) for nimesulide and (250 ppm, 350 ppm, and 450 ppm) for dicyclomine hydrochloride for three consecutive days.

The analysis was performed in triplicate, and % RSD was calculated. Nimesulide was found to be 1.28%, and that of dicyclomine hydrochloride was found to be 0.86% which complied with acceptance criteria. *i.e.*, %RSD should be NMT 2%, indicating that the developed method was precise **Table 2**.

TABLE 2: PRECISION STUDY OF NIMESULIDE AND DICYCLOMINE HYDROCHLORIDE

Concentration (µg/ml)		Intraday Precision			Interday Precision		
		Mean ± SD	%RSD	Mean %RSD	Mean ± SD	%RSD	Mean % RSD
Nimesulide	10	1244532 ±11849.94	0.952	0.63	1259166 ±18205.59	1.44	1.28
	20	2433954 ±19532.59	0.802		2450831 ±46814.69	1.91	
	40	4675477 ±6445.995	0.137		4683119 ±23154.92	0.49	
Dicyclomine hydrochloride	250	355149 ±5136.623	1.44	0.56	354962 ±4612.839	1.29	0.86
	350	538354 ± 726.4615	0.13		539122 ±1667.05	0.30	
	450	633262 ±786.3568	0.12		636601 ±6429.356	1.00	

*The results are an average of three estimates

Limit of Detection (LOD) and Limit of Quantitation (LOQ): The detection limit and quantitation limit were determined based on the standard deviation of y-intercepts and slope of the regression line. The LOD and LOQ were estimated by using the following formulae:

$$LOD = 3.3\ \sigma / S \text{ and } LOD = 10\ \sigma / S$$

Where σ is the standard deviation of the response and S is the slope of the calibration curve **Table 3**. The limit of detection of nimesulide and dicyclomine hydrochloride was found to be 0.22 ppm and 20.96 ppm, respectively. The limit of

quantitation of nimesulide and dicyclomine hydrochloride was found to be 0.68 ppm and 63.53 ppm, respectively.

TABLE 3: LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTITATION (LOQ) OF NIMESULIDE AND DICYCLOMINE HYDROCHLORIDE

Parameters	Nimesulide	Dicyclomine hydrochloride
Mean slope	120108	1380.967
SD	8285.837	8774.234
LOD (ppm)	0.22	20.96
LOQ (ppm)	0.68	63.53

*The results are an average of three estimates

Accuracy Study: Recovery study was carried out by adding a known amount of nimesulide and dicyclomine hydrochloride (standard) to pre-analyzed sample at three different concentration levels, *i.e.,* 80%, 100%, and 120% of assay concentration and percent recoveries were.

The accuracy study was carried out in triplicate. The % recovery of nimesulide and dicyclomine hydrochloride was found to be within 98%-102% of the specified limit, indicating that the developed method was accurate **Table 4**.

TABLE 4: ACCURACY STUDY OF NIMESULIDE AND DICYCLOMINE HYDROCHLORIDE

Drugs	Level	Sample amount (ppm)	Standard amount spiked (ppm)	Total amount (ppm)	Amount recovered (ppm)	% recovery	Mean % recovery ± SD	%RSD
Nimesulide	80%	10	8	18	17.95	99.375	99.33% ±	0.31
		10	8	18	17.92	99	0.31	
		10	8	18	17.97	99.62		
	100%	10	10	20	20.14	101.4	100.96%	1.01
		10	10	20	20.17	101.7	±1.02	
		10	10	20	19.98	99.8		
	120%	10	12	22	22.2	101.66	100.71%	1.15
		10	12	22	21.93	99.41	±1.16	
		10	12	22	22.13	101.08		
Dicyclomine hydrochloride	80%	250	240	490	239.2	99.66	100.7 ±	0.89
		250	240	490	243.06	101.27	0.90	
		250	240	490	242.83	101.17		
	100%	250	250	500	249.82	99.92	100.5667	0.71
		250	250	500	253.39	101.35	±0.72	
		250	250	500	251.09	100.43		
	120%	250	260	510	259.74	99.9	100.3833 ±	0.47
		250	260	510	262.24	100.86	0.48	
		250	260	510	261.02	100.39		

*The results are an average of three estimates

Robustness Study: The robustness study was conducted by analyzing the effect of slight variation in the pH of mobile phase by ±0.1, change in flow rate ±1 ml/min, and change in mobile phase composition by ±1 on the retention time, tailing factor, theoretical plates and resolution.

The percent relative standard deviation, tailing factor was found to be NMT 2%. Hence it complied with acceptance criteria. It indicated that the method is robust in **Tables 5**, **6,** and **7**.

System Suitability Study: System suitability tests are used to ensure the reproducibility of the chromatographic system. Combined standard solutions of nimesulide and dicyclomine hydrochloride 10 ppm and 250 ppm respectively were injected six times and checked for retention time, asymmetry, resolution, and no. of theoretical plates.

The chromatogram confirmed the presence of nimesulide and dicyclomine hydrochloride at a retention time of 3.32 min and 4.22 min, respectively, without any interference. The number of theoretical plates was NMT 2000, tailing factor NMT 2, and a resolution was more than 2 **Table 8**.

Assay: The developed method was applied to simultaneously estimate nimesulide and dicyclomine hydrochloride in fixed-dose combination tablets **Fig. 7** and the results of the assay are presented in **Table 9**.

TABLE 5: ROBUSTNESS STUDY (VARIATION IN PH) OF NIMESULIDE AND DICYCLOMINE HYDRO-CHLORIDE

pH	Analytes	Retention time(min)	%RSD	Mean area	% RSD	Tailing factor(T)	% RSD	Theoretical Plates	Resolution
3.9	Nimesulide	3.31	0.30	1245014	0.64	1.66	0.82	3369	2.62
	Dicyclomine hydrochloride	4.16	0.24	285541	0.59	1.36	0.68	3098	
4.0	Nimesulide	3.32	0.33	1294832	0.67	1.61	0.782	3512	3.2
	Dicyclomine hydrochloride	4.20	0.25	346524	0.54	1.34	0.502	3108	
4.1	Nimesulide	3.33	0.35	1288236	0.63	1.85	0.38	3245	2.59
	Dicyclomine hydrochloride	4.41	0.27	309707	0.51	1.37	0.86	3089	

*The results are an average of three estimates

TABLE 6: ROBUSTNESS STUDY (VARIATION IN FLOW RATE - ML/MIN)

Flow rate (ml/min)	Analyte	Retention time(min)	% RSD	Mean area	% RSD	Tailing factor(T)	% RSD	Theoretical Plates	Resolution
0.9	Nimesulide	3.6	0.74	1489283	0.82	1.65	1.28	3420	2.9
	Dicyclomine hydrochloride	4.6	0.63	320010	0.58	1.40	0.86	3090	
1.0	Nimesulide	3.3	0.77	1291642	0.85	1.63	0.72	3512	3.2
	Dicyclomine hydrochloride	4.2	0.59	348672	0.52	1.38	0.52	3108	
1.1	Nimesulide	3.0	0.86	1202380	0.81	1.66	0.94	3362	2.8
	Dicyclomine hydrochloride	3.8	0.78	295353	0.51	1.39	1.32	3097	

*The results are an average of three estimates

TABLE 7: ROBUSTNESS STUDY (VARIATION IN MOBILE PHASE COMPOSITION)

Mobile phase composition	Analyte	Retention time (min)	% RSD	Mean area	%RSD	Tailing factor(T)	% RSD	Theoretical Plates	Resolution
39:61	Nimesulide	3.33	0.63	1287608	0.25	1.68	0.52	3345	2.78
	Dicyclomine hydrochloride	4.24	0.45	305252	0.18	1.34	0.69	3076	
40:60	Nimesulide	3.32	0.69	1293632	0.36	1.63	0.25	3512	3.20
	Dicyclomineh ydrochloride	4.20	0.51	347683	0.25	1.38	0.84	3108	
41:59	Nimesulide	3.31	0.71	1273078	0.42	1.72	1.32	3285	2.68
	Dicyclomine hydrochloride	4.18	0.43	293039	0.39	1.28	0.93	3025	

*The results are an average of three estimates

TABLE 8: SYSTEM SUITABILITY STUDY OF NIMESULIDE AND DICYCLOMINE HYDROCHLORIDE

Drugs	Retention time (min)	Tailing factor	Theoretical plates (N)	Mean resolution (R)
Nimesulide	3.32	1.6	3512	
Dicyclomine hydrochloride	4.22	1.4	3108	3.2

*The results are an average of six estimates

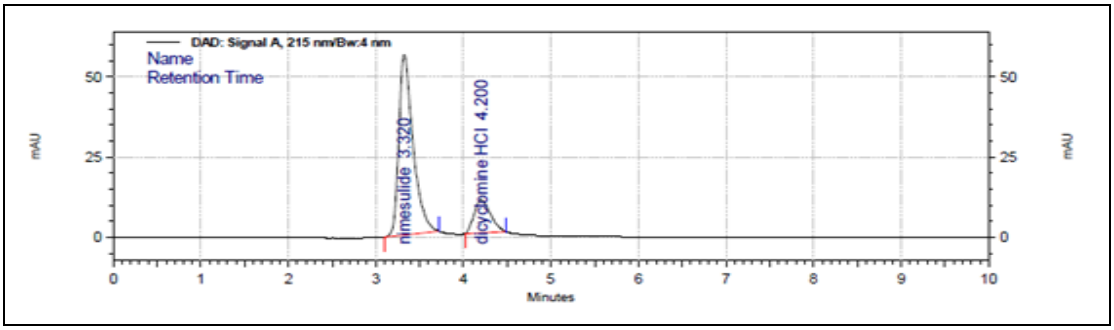

FIG. 7: CHROMATOGRAM OF SAMPLE SOLUTION (NIMESULIDE 10 PPM AND DICYCLOMINE HYDRO-CHLORIDE 250 PPM)

TABLE 9: ASSAY OF MARKETED FORMULATION (TABLETS)

S. no.	Amount of drug present in marketed formulation(mg/tab)		Amount of drug found (mg/tab)		% drug content	
	NIME	DICH	NIME	DICH	NIME	DICH
1	100	10	99.00	9.91	99	99.10
2	100	10	99.48	9.94	99.48	99.40
3	100	10	99.20	9.97	99.20	99.70
	Mean ± SD	99.22± 0.24	9.94 ± 0.03	99.22 ± 0.24	99.4 ± 0.3	Mean ± SD
	%RSD	0.24	0.30	0.24	0.30	%RSD

*The results are an average of three estimates

CONCLUSION: A simple, accurate, and precise RP-HPLC isocratic method for the simultaneous estimation of nimesulide and dicyclomine hydrochloride in fixed-dose combination tablets was developed and validated as per ICH guidelines Q2 R1. The developed method had a short run time of 10 mins making it feasible for routine analysis and quality control testing of tablets containing nimesulide and dicyclomine hydrochloride in pharmaceutical industries.

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CONFLICTS OF INTEREST: The authors have no conflict of interest to declare.

REFERENCES:

1. Deshpande S, Biradar G, Patel V and Chowdari V: Development and validation of stability indicating RP-HPLC-PDA method for simultaneous determination of paracetamol and dicyclomine in combined dosage form. Int J Pharm Sci 2015; 5(1): 858-63.
2. Ragab GH, El-Henawee MM, Amin AE and Sultan AF: Stabilityindicating HPLC method for determination of nimesulide in bulk powder and in tablet dosages form. Asian J Pharm Anal Med Chem 2014; 2(4): 230-9.
3. Bhosale V, Chandewar AV, Dewani AP and Gadewar CK: Development and validation of a stability indicating assay method using HPLC for determination of a model drug in bulk and pharmaceutical dosage form. Int J Pharm Chem Anal 2015; 2(1): 34-7.
4. Prajapati D and Raj H: Simultaneous estimation of mefenamic acid and dicyclomine hydrochloride by RP-HPLC method. Int J Pharm Biol Sci 2012; 3(3): 611-25.
5. Dave CA, Tiwari SK, Brahmbhatt KD, Patel PM and Shah SB: Development and validation of RP-HPLC method for estimation of omeprazole and dicyclomine hydrochloride in pharmaceutical dosage form. IJPS 2013; 4(3): 247-56.
6. Donda ST,Baviskar VB, Deshmukh PK, Bari SB and Patil PO: Development and validation of a reversed-phase HPLC method for the simultaneous estimation of dicyclomine hydrochloride and famotidine in bulk and tablets. J Chil Chem Soc 2014; 59(4): 2662-5.
7. Vijjigiri C, Bhaskar D and Kamarapu SK: Method development and validation of RP-HPLC method for simultaneous estimation of dicyclomine hydrochloride and diclofenac potassium in tablet dosage forms. Int J Pharm Biol Sci2013; 3(4): 255-64.
8. Charan DC, Satyabrata S, Narayan UL and Garnaik B: Simple and rapid spectrophotometric estimation of nimesulide in bulk drug and liquid dosage formulations. Asian J Pharm Clin Res 2011; 4: 100-1.
9. Raghunath M, Dhamne A and Patil J: Simple UV spectrophotometric method for estimation of dicyclomine-hydrochloride in bulk and tablet formulation. Int J Pharm Res All Sci 2015; 4(3): 109-13.
10. Khan T and Rangnekar S: Analytical method development and validation studies of ticagrelor tablets by RP-HPLC. Int J Appl Pharmaceutics 2017; 9(4): 10-21.
11. ICH Harmonised Tripartite Guideline- Validation of Analytical Procedures: Text and Methodology Q2 (R1).

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