Simultaneous Reverse Phase HPLC Estimation of Nimesulide and Diclofenac Sodium

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A simple, selective, rapid, precise and economical reverse phase HPLC method has been developed for the simultaneous estimation of nimesulide and diclofenac sodium from capsules. The analyte was resolved by using a mobile phase (methanol and water in the ratio 50:50) at a flow rate of 1 ml/min. on an isocratic HPLC system consisting of LC-10 AT liquid pump, SPD-10A UV/ Visible detector, a ODS C-18 RP column (4.6 mm I.D. x 25 cm) at a wavelength of 230 nm. The linear dynamic range for both nimesulide and diclofenac sodium was 0.5-30 μg/ml and 2.0-30 μg/ ml respectively by this method. Paracetamol was used as an internal standard.

Nimesulide[1] is a non-steroidal antiinflammatory, analgesic and antipyretic agent with minimal side effects. Chemically it is N-(4-Nitro-2-phenoxyphenyl) methane sulfonamide. It is commonly prescribed for the treatment of inflammatory conditions associated with rheumatoid arthritis, respiratory tract infections, soft tissue and oral cavity inflammations, urogenital diseases and post operative pain[2]. A few HPLC methods[3-6], a very few HPTLC methods[7-8] and a gas chromatographic method[9] has been reported for the estimation of nimesulide alone.

Diclofenac sodium[10] is a non-steroidal antiinflammatory, analgesic and antipyretic agent with minimal side effects. Chemically it is sodium [2-(2,6-dichloroanilino) phenyl]-acetate. It is commonly prescribed for the treatment of inflammatory conditions associated with rheumatoid arthritis. One HPLC method[11] and one GLC method[12] have been reported for the estimation of diclofenac sodium alone.

Fixed dose combination containing nimesulide and diclofenac sodium is available in the capsule form in the market. So far no RP-HPLC method for the simultaneous determination of these drugs from the capsules has been reported. In the present investigation, we are reporting a RP-HPLC method for simultaneous determination of nimesulide and diclofenac sodium from capsules, which is simple, rapid, selective and precise.

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Pure samples of nimesulide, diclofenac sodium and paracetamol were obtained from Dr. Reddy's Laboratories, Hyderabad. Methanol used was of HPLC grade and obtained from E. Merck Ltd., Mumbai. Water used was of triple distilled prepared by all glass distillation apparatus.

An isocratic HPLC system (Shimadzu) consisting of LC-10 AT liquid pump, SPD-10A UV/Vis detector, a ODS C-18 RP column (4.6 mm I.D. x25 cm), 25 μl Hamilton injecting syringe and window based single channel software was used. Afcoset electronic balance was used for weighing the materials.

Standard stock solutions of nimesulide and diclofenac sodium were prepared by dissolving 25 mg of the drug in 25 ml of the methanol to get 1 mg/ml solution and these solutions were suitably diluted with mobile phase to prepare stock solutions of concentration 10 μg/ml and 100 μg/ml. Stock solution of paracetamol was prepared by dissolving 25 mg of the drug in 25 ml of methanol to get 1 mg/ml solution and suitably diluted with methanol to get a solution of concentration 100 μg/ml.

Working standard solutions containing nimesulide and diclofenac sodium in various concentrations and paracetamol (internal standard) in the concentration of 5 μg/ml were prepared in the mobile phase as shown in Table 1. The chromatogram was obtained by injecting 20 μl of each solution in to the HPLC system. The ratios of AUC of nimesulide to

TABLE 1: STANDARD GRAPH FOR THE ESTIMATION OF NIMESULIDE AND DICLOFENAC SODIUM.

Concentration (μg/ml) of			Ratio of AUC of	
Nimesulide	Diclofenac Sodium	Paracetamol	Nimesulide to I.S.	Diclofenac Sodium to I.S.
0.5		5	0.85129	
1.0		5	0.17077	
2.0	2.0	5	0.32977	0.3560
5.0	5.0	5	0.854121	0.9203
10.0	10.0	5	1.855653	1.8325
20.0	20.0	5	3.24350	3.2495
30.0	30.0	5	4.96408	5.0071
Slope (m) :			0.144223	0.1748
Intercept :			0.241971	0.0287
Correlation coefficient (r) :			0.999704	0.9983

All the values are the averages of three determinations.

that of internal standard and the ratios of AUC of diclofenac sodium to that of internal standard were calculated. The results are shown in Table 2.

Contents of twenty capsules (Nicip-D, Cipla Laboratories Ltd., Mumbai) containing nimesulide and diclofenac sodium were pooled, powder equivalent to 25 mg of nimesulide was weighed accurately and was placed in a 25 ml volumetric flask. Nimesulide and diclofenac sodium contents were extracted in to methanol and the volume was adjusted to 25 ml, mixed and filtered. From the filtrate, 0.1 ml was pipetted in to a 10 ml graduated test tube and spiked with the required aliquot of internal standard solution and then the volume was adjusted to 10 ml with the mobile phase such that the concentration of internal standard in each sample was 5.0 μg/ml and 20 μl of this solution was injected into HPLC system to obtain the chromatogram. The concentration of nimesulide and diclofenac sodium corresponding to the ratio of AUC of nimesulide to AUC of internal standard and AUC of diclofenac sodium to the AUC of internal standard, respectively in the formulations were calculated from the standard graph. The results are presented in Table 1. The optimized chromatographic conditions are shown in Table 2. Recovery experiments were conducted by adding known amounts of nimesulide and diclofenac sodium to the previously analyzed pharmaceutical preparations and the results are given in Table 3.

The extracts of the formulations containing nimesulide and diclofenac sodium showed no significant peaks at the retention times other than the retention times of nimesulide

TABLE 2: OPTIMIZED CHROMATOGRAPHIC CONDITIONS.

Chromatograph	Shimadzu HPLC model LC-10AT
	Shimadzu SPD-10A UV-VIS detector
Mobile Phase	Methanol : Water (50:50)
Column	ODS C-18 (4.6 mm I.D. x25 cm)
Flow rate	1 ml/min.
Detection	U.V. set at 230 nm
Injection Volume	20 μl
Temperature	Ambient
Retention time:	
of Nimesulide	15.5-16.0 min.
of Declofenac Sodium	6.85-7.02 min.
of Paracetamol	3.26-3.40 min.
Run Time	18 min.

TABLE 3: RESULTS OF ANALYSIS OF CAPSULES CONTAINING NIMESULIDE AND DICLOFENAC SODIUM AND RECOVERY STUDIES.

Pharmaceutical formulation	Amount of Nimesulide (mg)		Amount of Diclofenac sodium (mg)		% Recovery	
	Labelled	Found	Labelled	Found	Nimesulide	Diclofenac sodium
Capsule 1 NICIP-D	100	99.87	50	49.97	99.75	99.23
Capsule 2 Emsulide Fen	100	100.25	50	49.78	100.43	99.57

All the values are the averages of three determinations NICIP-D, Cipla Laboratories Ltd., Emsulide Fen, Emcure Laboratories Ltd.

and diclofenac sodium which indicates that the generally used excipients like starch, lactose, talc and magnesium stearate in the capsules do not interfere in the estimation, using RP HPLC method. The results of recovery studies given in the Table 3 indicate that the method is accurate. As the mobile phase is only a mixture of methanol and water and the flow rate of the mobile phase is 1 ml/min the method is economical. Linearity and System suitability tests were carried out on freshly prepared standard stock solutions of nimesulide and diclofenac sodium and the results are shown in Table 4.

TABLE 4: RESULTS OF LINEARITY AND SYSTEM SUITABILITY.

Parameters	**Nimesulide**	**Diclofenac sodium**
Calibration range (μg/ml)	0.5-30.0	2.0-30.0
LOD (μg/ml)	0.17	0.09
LOQ (μg/ml)	0.56	0.2
Theoretical plates	6640	747
Tailing factor	1.222	1.2
Resolution:		
Between the peaks of I.S. and diclofenac sodium		5.281
Between the peaks of I.S. and nimesulide	9.676	

LOD: Limit of detection, LOQ: Limit of quantiation, I.S.: Internal standard.

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