

**DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR
SIMULTANEOUS ESTIMATION OF DROTAVERINE HCL AND NIMESULIDE
FROM TABLET DOSAGE FORM**

M. S. Charde*¹, R. A. Kundu², M. H. Ghante², R. D. Chakole³

¹Government College of Pharmacy, Amravati

²J. L. Chaturvedi College of Pharmacy, Nagpur

³Department of Pharmacy, Government Polytechnic, Amravati

Corresponding author*: manojudps@rediffmail.com

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ABSTRACT:

A new simple, specific, precise and accurate reversed-phase liquid chromatography method has been developed for simultaneous estimation of Drotaverine HCl (DRO) and Nimesulide (NIM) in tablet formulation. The separation was achieved on a 5-micron C18 column (250 X 4.6 mm) using mobile phase consisting of a mixture of water: acetonitrile: methanol 30: 35: 35 % (pH 2.5, adjusted with orthophosphoric acid). The flow rate was maintained at 1.0 ml/min, with an average operating pressure of 2630 psi. The detection of the constituents was done using UV detector at 295 nm for DRO and NIM. The retention time of DRO and NIM were approximately 5.6 and 10.6 min respectively. Recovery study values of DRO and NIM is 100.06±0.40 and 100.13±0.34 respectively, relative standard deviation of less than 2% for the assay show that the method is precise, accurate and linear in the concentration given and demonstrate the method developed is rugged and robust. Linear response obtained for DRO was in the concentration range 50-250 µg/ml and NIM in the range 125-625 µg/ml.

Keyword: Drotaverine HCl, Nimesulide, RP-HPLC, Simultaneous estimation

1. INTRODUCTION:

Drotaverine HCl chemically is (1-(3, 4-diethoxybenzylidene)-6, 7-diethoxy-1, 2, 3, 4-tetrahydroisoquinoline) hydrochloride, is an isoquinoline derivative. It is a highly potent spasmolytic agent.

Chemically, Nimesulide is 4-Nitro-2-phenoxymethanesulfonamide¹. It is a non-steroidal anti-inflammatory drug². It is used for chronic arthritis (such as rheumatoid arthritis and osteoarthritis) surgery and posttraumatic acute pain and inflammation, otorhinolaryngological inflammation resulting in pain; dysmenorrhoea, upper respiratory tract infection symptoms such as fever treatment

Literature survey revealed that few analytical methods for the determination of DRO such as spectroscopy, HPLC, HPTLC from pharmaceutical preparations. However there are number of methods for the determination of NIM such as spectrophotometry, HPLC in pharmaceutical preparations. No method is reported so far the estimation of both drugs in combined dosage form.

Thus, we proposed a very simple, fast and accurate reversed-phase HPLC method for simultaneous estimation of these drugs in

pharmaceutical preparations at single wavelength (295 nm).

The main utility of the developed method is that it can be extended successfully for in-vitro and in-vivo studies of the DRO and NIM for therapeutic drug monitoring, Pharmacokinetic and bioavailability studies.

2. EXPERIMENTAL

2.1 Instrumentation: A liquid chromatography system consisting of ALC pump with auto injector, equipped with a Diode array detector 7455 detector. Merck, KROMASIL C₁₈, 5 µ, 100A⁰, 4.6 mm × 250 mm column was used as the stationary phase.

2.2 Reagents and chemicals: Reference standard DRO and NIM were procured from Aditi Pharmaceuticals PVT. LTD., Solapur and Zim Laboratories LTD., Nagpur and tablet was procured from market. Water, methanol and acetonitrile of HPLC grade were used and the mobile phase was filter through 0.45µ membrane filter paper and degassed before used.

2.3 Chromatographic parameter:

Column : C₁₈ (5 µ, 4.6 X 250 mm)

Detection Wavelength: 295 nm

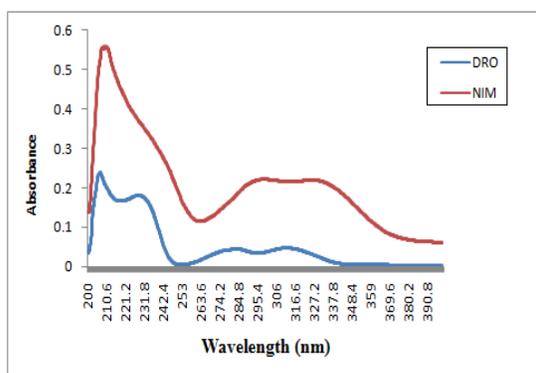
Injection volume : 20 µl

Pressure : 2680 psi
Flow rate : 1.0 mL/min
Temperature : Ambient
Mobile phase: water: acetonitrile: methanol (30:35:35) pH2.5 adjusted with orthophosphoric acid

2.4 Procedure:

Selection of Analytical wavelength: From the overlain spectra (Fig.1), the selected analytical wavelength (λ) was 295 nm.

Figure 1: Overlain spectra of DRO and NIM.



Standard stock solution: An accurately weighed 50 mg of DRO and 125 mg of NIM was transferred to 50.0 mL calibrated volumetric flask and dissolved in small volume of methanol and volume was made up to the mark with methanol.

Working standard solution: 5.0 mL of standard stock solution was further diluted to 50.0 mL with mobile phase, the chromatogram of this solution was recorded (Fig. 2)

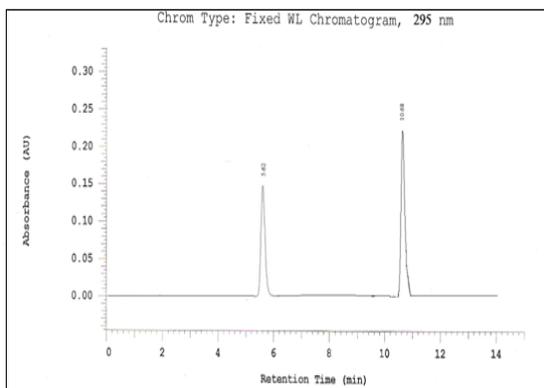
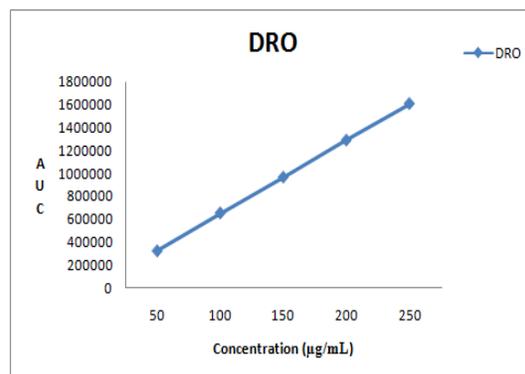


Figure 2: chromatogram of DRO and NIM.

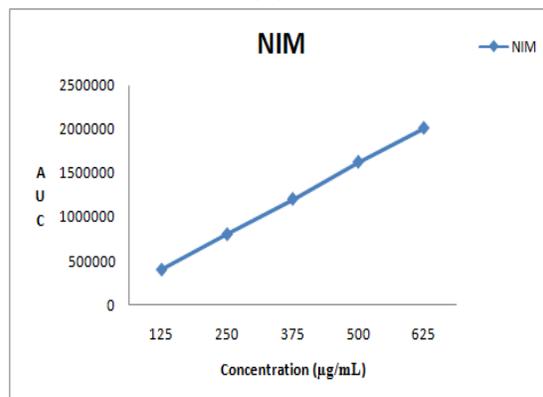
From the standard stock solution different concentrations of DRO and NIM were prepared in the ratio 1:2.5 respectively. Each

concentration was repeated five times and mean area was calculated. Peak area concentration of individual was plotted which gives linearity of the above method.

Figure 3: Linearity curve of DRO (A) and NIM (B) by area



(A)



(B)

2.5 Estimation of Drugs from pharmaceutical dosage form:

Twenty tablets were accurately weighed and the average weight was calculated. Tablets were ground to fine powder and weighed equivalent to 20 mg of DRO and 50 mg NIM, was transferred to 50.0 mL volumetric flask and dissolved in small volume of methanol and volume was made upto the mark with the same solvent. The solution was sonicated for 10 min and filtered through whatman filter paper. The 2.5 mL of above solution was pipetted out and diluted to 10.0 mL with mobile phase (100:250µg/ml = DRO: NIM). Sample solution of 20µl was injected and chromatograms were recorded. The summary of these results is presented in Table-1 along with statistical validation.

Table-1 Estimation of Drugs from pharmaceutical dosage form

Sr. No.	Weight of tablet powder (g)	Amount of drug estimated (mg)		% Estimation of drugs	
		DRO	NIM	DRO	NIM
1	0.0707	9.96	25.04	99.62 %	100.16 %
2	0.0705	9.87	24.97	98.70 %	99.88 %
3	0.0704	9.81	25.12	98.10 %	100.48 %
4	0.0705	9.79	25.08	97.60 %	100.32 %
5	0.0706	9.88	25.18	98.80 %	100.72 %
			Mean	98.56 %	100.31 %
			S.D.	0.07	0.03
			% R.S.D	0.07	0.02

2.6 Validation of the method: Accuracy of the method was checked by recovery studies. Precision of the method was studied by analysis of multiple samplings of homogenous sample and expressed as % R.S.D. Specificity of the method was established by various parameters like resolution, plate count and expressed as % R.S.D.

Ruggedness of the method was determined by carrying out the experiment on the different instruments by different analyst and on different days, showed that the method was rugged. Robustness of the method was determined by making slight changes in chromatographic conditions.

2.7 Recovery studies: To study the accuracy, reproducibility and precision of the above proposed method recovery studies were carried out by standard addition method at different level of labeled claim (i.e. 80 to 120% of labeled claim).

2.8 System suitability; System suitability test were carried out as per USP guidelines. The result was found in concordance with the limits specified.

Table-2 System suitability parameters

Sr. No.	Parameters	DRO	NIM
1	AUC (Area under curve)	642168	800952
		642014	801142
		642324	801782
		642876	800832
		642987	801465
2	Mean	642473.8	801234.6
	S.D.	433.73	388.34
	% R.S.D.	0.06	0.04
3	Retention time	5.62	10.68
4	Capacity factor	2.87	5.77
5	Theoretical (plate/col.)	5843.8	5844.2
6	Assymmetric factor	1.462	1.534

3. RESULTS AND DISCUSSION

The mobile phase water: acetonitrile: methanol 30: 35: 35 % (pH 2.5, adjusted with orthophosphoric acid) gave a good resolution and sensitivity of DRO and NIM. Under the conditions the analyte peaks were well defined and resolves. The elution order was DRO ($t_r = 5.62$) and NIM ($t_r = 10.68$) at a flow rate of 1.0ml/min. The optimum wavelength for detection was at 295 nm. Linearity obtained for DRO was in the range 50-250 $\mu\text{g/ml}$ and NIM in concentration range of 125-600 $\mu\text{g/ml}$. The mean recoveries obtained for DRO and NIM was 100.09 ± 0.30 and 99.91 ± 0.29 respectively. It confirmed that the method is accurate and free from any positive or negative interference of the excipients. The low value of S.D. obtained confirmed the precision of the method. Low values of S.D. and % R.S.D. less than 2% showed that there were no marked changes in the chromatographic parameters which demonstrate that the method developed is rugged and robust. Data from system suitability studies indicate conformity to compendial requirements.

CONCLUSION

The proposed method gives a good resolution between DRO and NIM within a short analysis time (<11 mins). The method is very simple and rapid and no where involves complicated sample preparation of mobile phase preparation. The linearity and reproducibility data of the drugs carried out by this method shows that no major interference is caused in the estimation of the drugs. Therefore the method can be use for routine quality control of these drugs.

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