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Research Article

Development and Validation of Analytical Methods for Drotaverine and Nimesulide Combination

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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 Acetonitrile: water: triethylamine (70:30:0.2 v/v, pH 3, 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 230 nm for DRO and NIM. The retention time of DRO and NIM were approximately 2.98 and 5.73 min respectively. Recovery study values of DRO and NIM is 99.44±0.095 and 99.62±0.085 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 10-80 µg/mL and NIM in range of 25-200 µg/ml.

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

INTRODUCTION

Drotaverine HCl chemically is (1-(3, 4-diethoxybenzylidene)-6, 7-diethoxy-1, 2, 3, 4-tetrahydroisoquin-oline) hydrochloride, is an isoquinoline derivative. It is a highly potent spasmolytic agent. Chemically, Nimesulide is 4-Nitro-2-phenoxymethanesulfonanilide [1]. It is a nonsteroidal anti-inflammatory drug [2]. 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) [3,4].

Drotaverine hydrochloride is official in Polish Pharmacopoeia. A few UV spectrophotometric [5-8] and HPLC [9-11] methods have been reported individually or in combination with other drugs for estimation of Drotaverine hydrochloride. 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.

EXPERIMENTAL METHODOLOGY

Equipment

Shimadzu isocratic liquid chromatographic condition system equipped with liquid chromatogram model LC-10 AD, Rheodyne injector model 7725i with 20µL fixed Rheodyne loop, UV-visible detector model SPD-10A, Knauer eurospher C18 column in VAMA Pharma, Nagpur.

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Chemicals and Reagents

All the chemicals used during the project work were either AR or HPLC grade. Whatman filter paper No. 41 was used throughout the project work. Membrane filter (0.45 µ) was used for HPLC. Reference standard DRO and NIM were procured from Mankind Pharma LTD. New Delhi and Aarti drugs LTD., Mumbai and tablet was procured from market (NOBEL SPAS).

Preparation of standard solutions

Solution A (DRO): Accurately weighed quantity of DRO (25 mg) was dissolved in methanol and diluted to 25 mL with methanol (conc.1000 µg/mL).

Solution B (NIM): Accurately weighed quantity, NIM (25 mg) was dissolved in methanol and diluted to 25 mL with methanol (conc.1000 µg/mL).

Solution C (Mixed DRO+NIM): Accurately weighed quantities of DRO (40 mg) and NIM (100 mg) were dissolved in methanol (30 mL) in volumetric flask (100 mL) and the volume was made up to the mark. (conc. DRO-400 µg/mL; NIM-1000 µg/mL).

Solution C1: Accurately measured 1 mL portion of standard solution C was diluted to 10 mL with mobile phase (conc. DRO-40 µg/mL; NIM-100 µg/mL).

Selection of mobile phase and optimization of chromatographic conditions

The chromatographic conditions were set as per the given parameters and mobile phase was allowed to equilibrate with stationary phase as was indicated by the steady baseline. Standard solution A, B, and C1 were injected separately (20 µL) and the chromatograms were recorded for the drugs. Various mobile phases were tried by individual solvents and combination of solvent and also by varying the flow rate. Finally optimized chromatographic conditions are as follows.

Column	: C_{18} (250.0 x 4.6 mm)
Packed particle size	: 10 µm
Detection wavelength	: 230 nm
Flow rate	: 1 mL/min
Temperature	: Ambient
Sample volume	: 20 µL
Mobile phase	: Acetonitrile: water: triethylamine (70:30:0.2 v/v, pH 3, adjusted with orthophosphoric acid)

Calibration plot for DRO and NIM

The mobile phase was allowed to equilibrate with the stationery phase until steady baseline was obtained. The various conc. of DRO (10-80 µg/mL) and NIM (25-200 µg/mL) were injected. The peak areas were recorded. The observation for standard calibration curve and the graph was plotted as the conc. of the drug Vs peak area as depicted in Fig. 1.

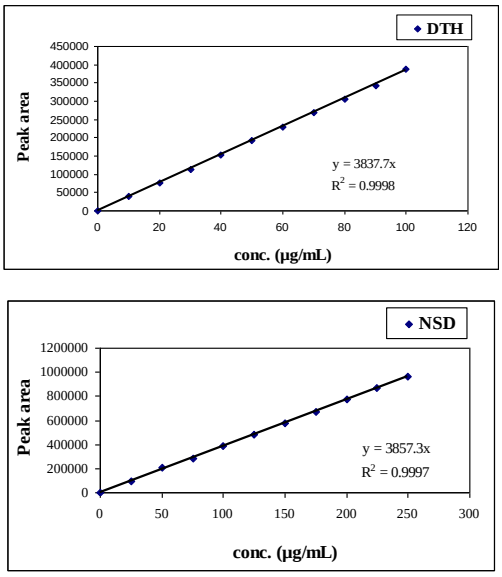

Fig. 1 Calibration Plot

Study of system suitability parameters

The system suitability parameters are used to verify whether resolution and reproducibility of the chromatographic system are adequate for analysis. The chromatographic conditions were set as per the optimized conditions and mobile phase was allowed to equilibrate with stationary phase as was indicated by the steady base line. Five replicate injections of standard solution C1 were injected and their system suitability parameters were recorded as shown in Table 1.

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Table 1. Results of system suitability parameters

SN	Retention time (min)		Asymmetry		Peak area		Resolution
	DRO	NIM	DRO	NIM	DRO	NIM	
1.	2.995	5.735	2.71	2.92	155681.6	393467.0	6.17
2.	2.982	5.730	2.89	2.92	153061.4	386566.3	6.20
3.	2.991	5.733	2.74	2.97	154892.6	392356.0	6.15
4.	2.982	5.730	2.89	2.92	153061.4	386566.3	6.20
5.	2.985	5.732	2.63	2.78	155573.2	393295.7	6.18
Mean	2.987	5.732	2.772	2.902	154454.0	390450.2	6.18
SD	0.0057	0.0021	-	-	1306.8	3570.7	-
RSD (%)	0.1908	0.0366	-	-	0.8460	0.9145	-
SEM	0.0025	0.0009	-	-	584.41	1596.9	-

Estimation of DRO and NIM in standard laboratory mixture

Five different laboratory mixtures of DRO and NIM were prepared by weighing the quantities of drug samples so as to get final conc. of 40µg/mL and 100µg/mL of DRO and NIM, respectively. The chromatogram, obtained by using standard laboratory mixture, is shown below (Fig. 2).

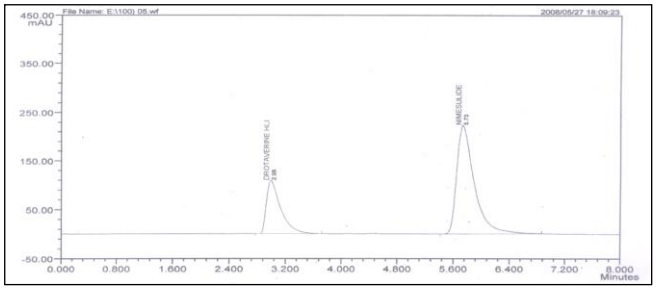

Fig. 2. Chromatogram of laboratory mixture of DRO and NIM

The peak area of standard laboratory mixture and sample laboratory mixture was compared. The amount of each drug estimated in laboratory mixture was calculated using following formula.

$$\text{Estimation (\%)} = \frac{A_t}{A_S} X \frac{d_S}{d_t} X \frac{W_S}{W_t} X\ 100$$

Where, A_t = area count for sample solution; A_s = area count for standard solution; d_t = dilution factor of standard solution; d_s = dilution factor of sample solution; W_t = weight of sample (g); W_s = weight of standard drug (g).

The results of estimation of drugs in laboratory mixture are shown in Table 2.

Table 2. Estimation of DRO and NIM in laboratory mixture

SN	Wt. of sample drug (g)		Peak area		Estimation (%)	
	DRO	NIM	DRO	NIM	DRO	NIM
1.	0.040	0.100	155792	393315	99.82	100.0
2.	0.040	0.100	155709	393576	100.2	99.62
3.	0.040	0.100	154955	393592	99.03	100.0
4.	0.040	0.100	155617	394179	100.4	99.68
5.	0.040	0.100	156170	394108	99.31	99.96
Mean					99.77	99.86
SD					0.6002	0.202
RSD(%)					0.6015	0.2031
SEM					0.268	0.0907

Simultaneous estimation of DRO and NIM in tablet by proposed RP-HPLC method

Twenty tablets were weighed. Average weight was calculated. Tablets were finely powdered and mixed thoroughly. An accurately weighed quantity of tablet powder (0.3021g) was transferred in volumetric flask (100 mL) and dissolved in the 30 mL of methanol. The volume was made up to the mark with methanol. The flask was shaken vigorously for 15 min. The solution was filtered through membrane filter and 1 mL of clear filtrate

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was diluted to 10 mL with mobile phase. Five replicate sample solutions were prepared in similar manner.

Equal volumes of standard stock and sample solutions were injected separately after the equilibrium of stationary phase. The chromatograms were recorded (Fig. 25). The response i.e., peak area of major peaks were measured. The amount of drug in the tablet formulation was calculated by using the following formula (Table 3).

$$\text{Label claim}(\%) = \frac{A_t}{A_s} X \frac{d_s}{d_t} X \frac{W_s}{W_t} X \frac{A}{LC} X100$$

Where, A_t = area count for sample solution; A_s = area count for standard solution; d_t=dilution factor for standard solution; d_s = dilution factor of sample solution; W_s = weight of standard (g); W_t= weight of tablet sample (g); LC = label claim of tablet; A = average weight of tablet

Table 3. Estimation of DRO and NIM in tablet

NOBEL SPAS				Average weight = 0.3021g	
Sr. No.	Wt. of tablet powder (g)	Peak area		Label claim (%)	
		DRO	NIM	DRO	NIM
1.	0.3030	152877.1	386528.1	100.01	99.77
2.	0.3029	152653.3	387425.3	99.95	100.03
3.	0.3034	153127.6	386487.2	100.03	99.64
4.	0.3037	153021.4	388526.4	99.88	100.07
5.	0.3022	152187.5	386541.8	99.86	100.04
Mean				99.94	99.91
SD				0.07570	0.1933
RSD(%)				0.075745	0.1934
SEM				0.03385	0.08643

Validation of proposed analytical 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.

Recovery studies

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

RESULTS AND DISCUSSION

The mobile phase Acetonitrile: water: triethylamine (70:30:0.2 v/v, pH 3, 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 (tr = 2.98) and NIM (tr = 5.73) at a flow rate of 1.0ml/min. The optimum wavelength for detection was at 230 nm. Linearity obtained for DRO was in the range 10-80 µg/mL and NIM in concentration range of 25-200 µg/ml. The mean recoveries obtained for DRO and NIM was 99.44±0.095 and 99.62±0.085 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 compendia 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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CONFLICT OF INTEREST

Declared None

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