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Research Article

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A new RP-HPLC method for the simultaneous determination of drotaverine hydrochloride and nimesulide in a tablet dosage form

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ABSTRACT

A simple, precise, and accurate isocratic RP-HPLC method was developed and validated for determination of Drotaverine hydrochloride (DROT) and Nimesulide (NIM) in bulk drug and tablet dosage form. Isocratic RP-HPLC separation was achieved on a Varian Microsorb mv C_{18} column (250 4.6 mm id, 5 mm particle size) using the mobile phase Water: Acetonitrile: Methanol: TEA (53:38:9:0.2) and pH adjusted to 3.0 pH with orthophosphoric acid at a flow rate of 1.5 ml/min. The retention time of drotaverine hydrochloride and nimesulide were 3.005 and 4.956 min., respectively. The detection wavelength was 306 nm and samples of 20 µl were manually injected. The method was validated for linearity, precision, accuracy, robustness, and specificity. The method was linearity in the concentration range of 4–80 µg/ml for drotaverine hydrochloride and 5-100 µg/ml for nimesulide. The limit of detection and limit of quantification for drotaverine hydrochloride were 0.1907 and 0.5781 µg/ml, respectively, and for nimesulide 0.2614 and 0.7921 µg/ml, respectively. The accuracy (recovery) was found to be in the range of 98.98–100.99 % for drotaverine hydrochloride and 98.89–101.07% for nimesulide.

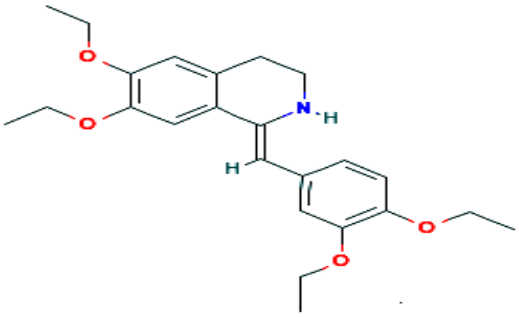
Keywords: Isocratic, Drotaverine hydrochloride, Nimesulide, Triethylamine, Orthophosphoric acid.

INTRODUCTION

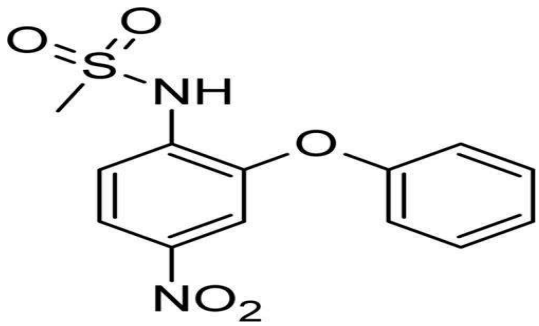
Drotaverine hydrochloride (DROT) {1-[(3, 4-[diethoxyphenyl) methylene]-6, 7 diethoxy-1, 2, 3, 4 - tetrahydroisoquinolene hydrochloride; Figure 1 (structure 1)} is an analogue of papaver, generally acts as an antispasmodic agent by inhibiting phosphodiesterase IV enzyme.[1] It is not official in USP, BP and IP. Chemically, Nimesulide (NIM) is N-(4-nitro-2-phenoxphenyl) methane sulphonamide; Figure 1 (structure 2)}.[2-4] It is a potent selective cyclo-oxygenase-2 (COX-2) inhibitor. It is official in EP.[4] It is used for chronic rheumatoid arthritis, osteoarthritis surgery, posttraumatic acute pain and inflammation, dysmenorrhoea, upper respiratory tract infection symptoms such as fever treatment.[2] Literature survey revealed that several spectrophotometric[5-11] and HPLC methods in urine and human plasma,[12-14] and voltametry [15] had been reported for the determination of DROT. While nimesulide alone or in combination with other drugs is reported to be estimated by titrimetry[16] spectrophotometric method[17-24], liquid chromatography methods[25-30] and high performance thin liquid chromatography method[31, 32] and LC-MS[33, 34] have been reported for the estimation of nimesulide.

Tablet formulations containing analgesic and antipyretic NIM along with antispasmodic DROT are used in the therapy to treat spasm. To date, there is no RP-HPLC reported method for the determination of drotaverine hydrochloride and nimesulide in combination in a tablet dosage form. Therefore, it was the purpose of this research to develop a rapid, simple, sensitive, reliable, and validated analytical method for the measurement of both drugs, which will be the first for their simultaneous analysis in API and tablet dosage form. The present RP-HPLC method

was validated following the ICH guidelines [35].



Structure 1: Drotaverine hydrochloride



structure 2: Nimesulide

Figure 1: Structure of Drotaverine hydrochloride and Nimesulide [1, 2]

EXPERIMENTAL SECTION

Chemicals and reagents used:

The reference standard of drotaverine hydrochloride and nimesulide were obtained as gift samples from Shree Pramukh laboratory. All chemicals used were of HPLC grade of Merck. triethylamine and ortho-phosphoric acid, as having HPLC grade of Merck Limited were used for chromatographic procedure. Water for HPLC was used to prepare mobile phase. Tablet dosage form manufactured by Phytochem, (brand name-ABDOMAX) was used. Each tablet containing 80 mg of drotaverine hydrochloride and 100 mg of nimesulide were used for study.

Instrumentation:

Youngling's HPLC with UV-760 detector and Manual injector of 20 µl loop. The peaks were quantified by means of PC based Autochrome 3000 software.

Chromatographic conditions:

The column used for chromatographic separations was Varian microsorb mv C 18 (4.6 mm i.d., 250 mm length, 5 µm particle size). The analytical wavelength was set at 306 nm and samples 20 µl were manually injected. The chromatographic separations were accomplished using mobile phase, consisting of Acetonitrile: Water: TEA (53:38:9:0.2) pH adjusted to 3.0 with orthophosphoric acid which is filtered through 0.45µm filter (Millipore) and degassed in ultrasonic bath. Mobile phase was pumped in isocratic-mode at a flow rate of 1.5 ml/min at ambient temperature.

Preparation of standard and sample solution:

The standard stock solutions of drotaverine hydrochloride (80 µg/ml) and nimesulide (100 µg/ml) were prepared by dissolving appropriate amounts of respective compounds in acetonitrile. Whereas in preparation of sample solution, quantity of powdered tablet equivalent to 8 mg of DROT or 10 mg of NIM was weighed and dissolved in acetonitrile. It was further diluted in order to get solution having concentration 16 µg/ml of DROT and 20 µg/ml of NIM.

RESULTS AND DISCUSSION

Optimization of analytical conditions:

The working standard stock solutions of (drotaverine hydrochloride) DROT and nimesulide (NIM) were scanned in the range of 200 to 400 nm against acetonitrile as a blank solution. The wavelength of 306 nm was found to show appreciable absorbance for drotaverine hydrochloride and nimesulide. Overlain spectrum for DROT and NIM is shown in Figure 2.

Different columns containing octyl, octadecyl, phenyl and base deactivated silane stationary phase were tried for separation and resolution. The inertsil base deactivated silane column became more advantageous over other columns. To develop a suitable LC method for estimation of drotaverine hydrochloride and nimesulide in formulations, different mobile phases were employed to achieve best separation. The selected and optimized mobile phase was Acetonitrile: Water: TEA (53:38:9:0.2) pH adjusted to 3.0 with orthophosphoric acid and conditions optimized were: flow rate (1.5 ml/min) at detector wavelength (306 nm). Run time was 7 min. Here peaks were separated and showed better resolution, theoretical plate count and asymmetry was found as 1.21 & 1.15 respectively for drotaverine hydrochloride and nimesulide. The proposed chromatographic conditions were found

appropriate for the quantitative determination of the drugs. The typical chromatogram of two drugs assayed is shown in Figure 3.

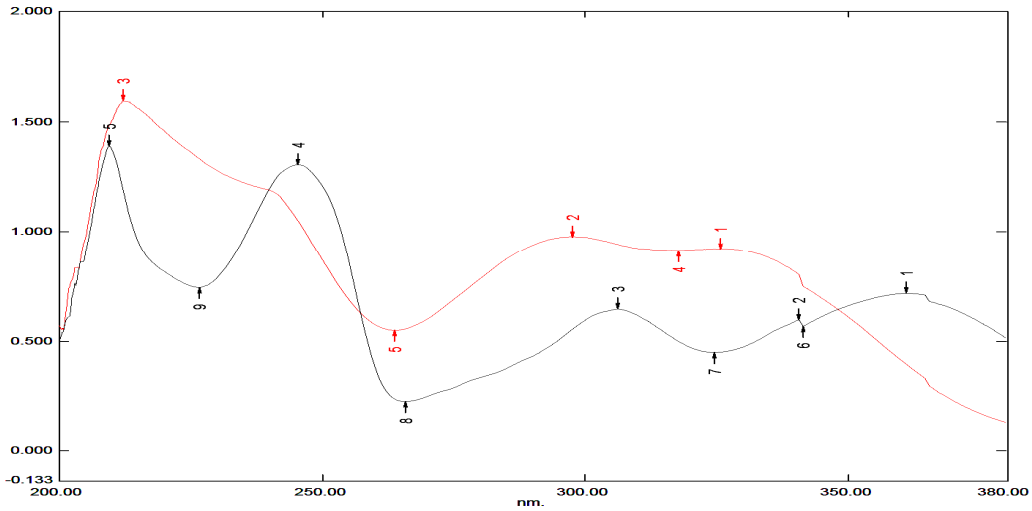


Figure 2: Overlain spectrum showing appreciable absorbance for drotaverine hydrochloride and nimesulide at 306 nm

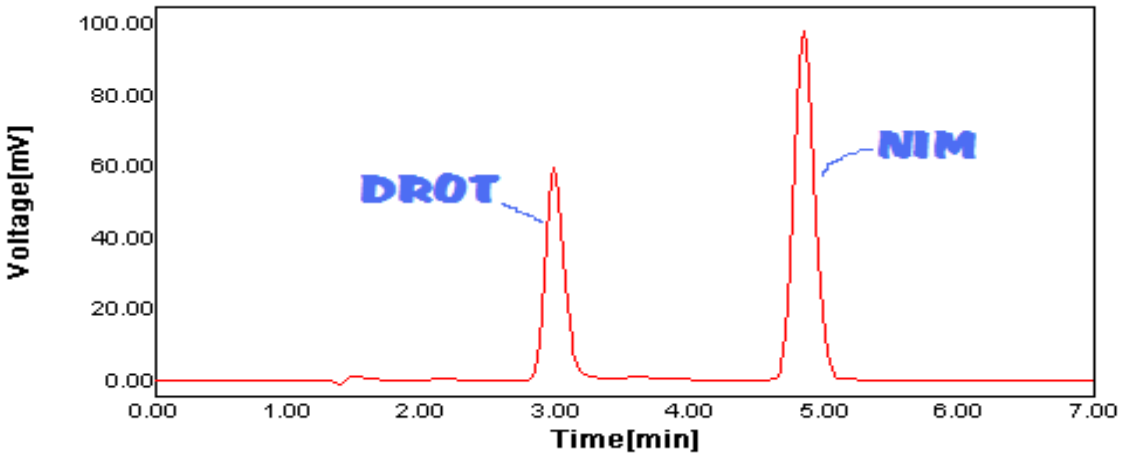


Figure 3: Typical chromatogram showing Retention time of 3.005 for Drotaverine hydrochloride and 4.956 min for Nimesulide

Method validation [33]:

System suitability:

The system suitability of the method was studied to determine the reproducibility of chromatographic system and column performance was acceptable for intended analytical application. Four parameters i.e. precision of peak area of five replicate injections, retention time of eluted drugs, number of theoretical plates, asymmetry factor and resolution between two peak of analytes were evaluated. The results are shown in Table 1.

Table 1: RESULTS OF SYSTEM SUITABILITY STUDY

Parameters	Data obtained	
	DROT	NIM
Retention Time	3.005	4.956
Theoretical plates per column	4658	6739
Symmetry factor/Tailing factor	1.21	1.15
Resolution	13.28	

Linearity:

The Linearity of analytical method is its ability to obtain test results, which are directly proportional to the concentration of analyte in the test sample .The linearity of the assay method, was established by injecting test samples in the range of 4-80 µg/ml for drotaverine hydrochloride and for nimesulide 5-100 µg/ml. Each solution was injected twice into HPLC and the average area at each concentration was calculated. The regression analysis

was carried out from graph of peak area Vs concentration; correlation co-efficient and Y- Intercept of plot was also evaluated. Linear regression equation and correlation coefficient was found to be y = 14.5310 X + 164.8519 and r = 0.9995 for drotaverine hydrochloride and for nimesulide, it was found to be y = 24.3764 X + 45.9198 and r = 0.9997; where 'y' is area of peak and 'X' is the concentration of drug solution, respectively. Calibration curves of DROT and NIM are shown in Figure 4 and Figure 5. Calibration curve data for DROT and NIM are shown in Table 2.

Table 2: CALIBRATION CURVE DATA FOR DROT AND NIM

Concentration of DROT (µg/ml)	Area of DROT (n=5)	Concentration of NIM (µg/ml)	Area of NIM (n=5)
4	232.32	5	162.71
16	399.774	20	533.68
32	618.062	40	1019.932
64	1082.616	80	2014.492
80	1330.36	100	2473.434
Mean % RSD	0.0069		0.0051

Calibration curves of DROT and NIM

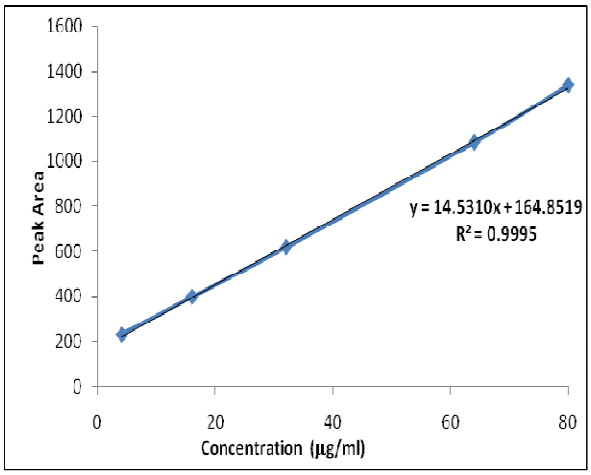


Figure 41: Calibration curve of DROT

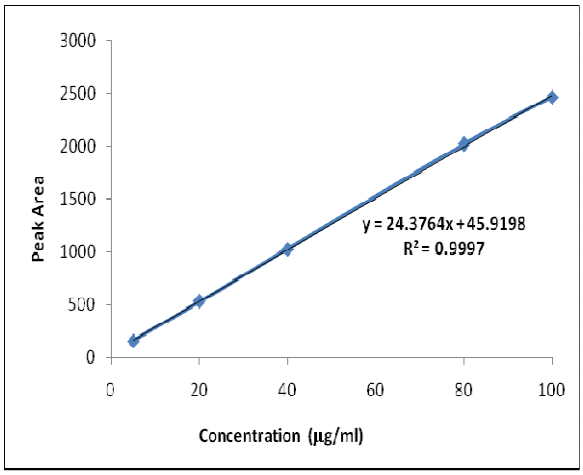


Figure 5: Calibration curve of NIM

Accuracy:

Accuracy of the methods was assured by spiking the previously analysed sample with 50%, 100%, and 150 % of target concentration. The resulting mixtures were assayed, and the results obtained for both drugs were compared to those expected. The good recoveries with the spiking method prove the good accuracy of the proposed methods. Results for recovery study are shown in Table 3.

TABLE 3: RESULTS OF ACCURACY STUDY

Drug	Amount taken conc. (µg/ml)	Amount spiked* (µg/ml)	Amount recovered* (µg/ml)	% recovery ± S.D *
Drotaverine hydrochloride	16.05	8	24.1863	100.567 ± 0.525
	16.05	16	31.9933	99.823 ± 0.644
	16.05	24	39.8819	99.580 ± 0.671
Nimesulide	19.96	10	30.1142	100.615 ±0.451
	19.96	20	39.9973	100.093 ± 1.085
	19.96	30	49.9271	99.934 ± 0.465

**Average of three experiments*

Precision:

Precision was determined by two ways; by system precision and Intermediate precision. System precision was demonstrated by making five replicate injections of standard solution. The %RSD for the analyte peak area of these replicate injections was evaluated. The results of System precision is indicating that an acceptable precision was achieved for simultaneous determination of drotaverine hydrochloride and nimesulide, as revealed by RSD < 2.0%. The intermediate precision of test method was demonstrated by carrying out precision study at three concentration level as 50 %, 100%, 150% (i.e 8, 16, 24 µg/ml and 10, 20, 30 µg/ml) for DROT and NIM respectively. Intermediate precision study includes intra-day and inter-day analysis. Results for repeatability are shown in Table

4. Results for intraday and inter day for DROT and NIM are shown in Tables 5 and 6 respectively.

TABLE 4: REPEATABILITY DATA FOR ANALYSIS OF DROT AND NIM

Sr. No	Area of DROT	% Assay	Area of NIM	% Assay
1st	395.39	99.15	529.41	99.17
2nd	396.48	99.62	532.22	99.74
3rd	396.97	99.83	538.46	101.02
4th	401.72	101.88	530.35	99.36
5th	398.18	100.35	528.16	98.91
6th	400.82	101.49	535.67	100.45
Mean	398.26	100.39	532.378	99.78
S.D.	2.5140	1.081	3.963	0.8130
% RSD	0.631	1.077	0.744	0.814

TABLE 5: RESULTS OF INTERMEDIATE PRECISION FOR DROTAVERINE

Conc. of (µg/ml)	Intraday precision		Interday precision	
	Mean ± SD	%RSD	Mean ± SD	%RSD
8	281.37 ± 0.847	0.301	281.115 ± 1.206	0.429
16	398.163 ± 1.788	0.449	398.48 ± 1.484	0.372
24	516.646 ± 2.897	0.557	512.557 ± 4.495	0.876

TABLE 6: RESULTS OF INTERMEDIATE PRECISION FOR NIMESULIDE

Conc.(µg/ml)	Intraday precision		Interday precision	
	Mean ± SD	%RSD	Mean ± SD	%RSD
10	287.83 ± 2.255	0.783	290.61 ± 1.407	0.484
20	535.03 ± 2.385	0.445	536.67 ± 3.643	0.679
30	778.18 ± 3.755	0.482	778.64 ± 2.986	0.383

Limit of detection and limit of quantitation:

Limit of detection and limit of quantitation was established based on the residual standard deviation method. LOD and LOQ for drotaverine hydrochloride were 0.1907 and 0.5781 µg/ml, respectively, and for nimesulide 0.2614 and 0.7921 µg/ml, respectively

Specificity:

Specificity was carried as interference from placebo; first only placebo & then injecting synthetic mixture containing placebo and API's as tablet ratio and method was found to be specific.

Application of Proposed Method to Tablet Dosage Form:

Twenty tablets were weighed accurately and powdered. Powder equivalent to 8 mg of DROT and 10 mg of NIM into 100 ml volumetric flask and diluted to 100 ml with acetonitrile. This solution is sonicated for 20 mins. The solution was filtered through Whatman filter paper No. 41. Transfer 10 ml of solution into 50 ml volumetric flask and dilute to mark with mobile phase to get a final concentration 16 µg/ml of DROT and 20 µg/ml of NIM. Results are shown in Table 8.

TABLE 8: APPLICATION OF METHOD TO TABLET DOSAGE FORM

Sr. No	Drotaverine hydrochloride			Nimesulide		
	Amount labeled (mg)	Amount found (mg)	% Amount Found S.D. (n=3)	Amount labeled (mg)	Amount Found (mg)	% Amount Found S.D. (n=3)
ABDOMAX tab.	80.0	100.0	79.91	99.43	99.89 ±0.545	99.43 ± 0.536

Robustness:

Robustness of test method was demonstrated by carrying out system suitability under normal conditions and each of altered conditions as follows. Flow rate was changed by -10% and +10%; Organic phase ratio of mobile phase was changed by -5% and +5% absolute; result of robustness study are summarized in Table 4, result indicates that method is robust for simultaneous determination of DROT and NIM. The data for robustness analysis for DROT and NIM are shown in Table 7.

TABLE 7: RESULT SUMMARY OF ROBUSTNESS STUDY

Robustness of HPLC method for Drotaverine hydrochloride					
Concentration (µg /ml)					
Sample		Flow Rate		Mobile Phase Composition (Water : ACN : MeOH : TEA)	
Sr. No.	Control*	1.4 ml/min	1.6 ml/min	55: 37: 8: 0.2	51: 39:10: 0.2
1	3.007	3.125	2.907	3.091	2.955
2	3.01	3.114	2.91	3.11	2.953
3	3.003	3.109	2.912	3.097	2.958
MEAN	3.0066	3.116	2.9096	3.0993	2.9553
SD	0.0035	0.0081	0.0025	0.0097	0.0025
RSD	0.1168	0.2626	0.0864	0.3133	0.0851
Robustness of HPLC method for Nimesulide					
Concentration(µg /ml)					
Sample		Flow Rate		Mobile Phase Composition (Water : ACN : MeOH : TEA)	
Sr. No.	Control*	1.4 ml/min	1.6 ml/min	55: 37: 8: 0.2	51: 39:10: 0.2
1	4.957	5.134	4.909	4.995	4.901
2	4.959	5.127	4.892	4.991	4.911
3	4.953	5.13	4.899	4.994	4.908
MEAN	4.9563	5.1303	4.9	4.9933	4.9066
SD	0.0030	0.0035	0.0085	0.0020	0.0051
RSD	0.0616	0.0684	0.1743	0.0416	0.1045

****Control condition:** mobile phase composition (Water: ACN: MeOH: TEA) 53:38:9:0.2 and flow rate 1.5 ml/min.*

CONCLUSION

The data demonstrate that the new RP-HPLC method we have developed showed acceptable linearity, specificity, accuracy, precision and robustness in the concentration range of 4-80 µg/ml for drotaverine hydrochloride and 5-100 µg/ml for nimesulide, as per the requirement of ICH guidelines. The method described is rapid since chromatographic run time is 7 min. In conclusion, the proposed method could be routinely used for the analysis of drotaverine hydrochloride and nimesulide in tablet dosage form.

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