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Spectrophotometric Quantitative Estimation and Validation of Nimesulide and Drotaverine Hydrochloride in Tablet Dosage form

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ABSTRACT
Three simple, sensitive and accurate UV spectrophotometric methods, I; first order derivative spectrophotometric, II; area under curve and III; multi-component method, has been developed for the estimation of drotaverine hydrochloride and nimesulide in tablets dosage form. Beers' law was obeyed in the concentration range 5-35 µgml^{-1} and 10-50 µgml^{-1} for drotaverine (λ_{max} = 230.5 nm) and nimesulide (λ_{max} = 331.5 nm) respectively in methanol. All the three methods allowed rapid analysis of binary pharmaceutical formulation with accuracy. Results of analysis for three methods were tested and validated for various parameters according to ICH guidelines.

Keywords: Drotaverine hydrochloride; Nimesulide; Derivative spectrophotometric method, Area under curve method, Multi-component method.

INTRODUCTION

anti -drug.

Chemically NIMS is N-(4-nitro-2-phenoxyphenyl) methane sulphonamide. It is a potent selective cyclooxygenase-2 inhibitor and is highly effective in the treatment of various forms of pain and inflammatory conditions. It is official in USP BP and IP. A survey of the literature revealed that only UV -spectrophotometric [1-4]liquid chromatographic methods [5-8], and estimation from human plasma and urine [9-10], have been reported for the estimation of nimesulide.

Drotaverine HCl (DROT) is an analogue of papaver. Chemically it is 1-[(3, 4-[diethoxy phenyl) methylene]-6, 7-diethoxy-1, 2, 3, 4-tetrahydro isoquinolene [11]. DROT generally acts as an antispasmodic agent, by inhibiting phosphodiesterase IV enzyme, specific for smooth muscles spasm and pain associated with labor. It is not official in USP, BP and IP. Literature survey revealed that chromatographic method was reported for its estimation from human plasma [12], and urine [13], and spectrophotometric methods for estimation in single [14] and combined dosage forms [15-16].

In the present work, we attempted to develop an easier, accurate, and reproducible three analytical methods with better detection range for estimation of NIMS and DROT in bulk drug and in its solid dosage forms. This paper describes UV spectrophotometric methods for the estimation of NIMS and DROT in methanol. The results of the analysis were validated by statistical methods, recovery studies and LOD, LOQ.

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MATERIALS AND METHOD

Materials

NIMS and DROT reference substance obtained from Plethico Pharmaceutical Ltd. (India). The solvent used for the experiment was methanol (AR grade). All the chemicals were used as obtained without further purification.

UV/visible double beam spectrophotometer (Shimadzu Model 1700) was employed with spectral bandwidth of 1nm and wavelength accuracy of 0.3 nm (with automatic wavelength correction with a pair of 1 cm matched quartz cells).

Preparation of Standard stock solution

The standard stock solution of NIMS and DROT (10 mg/100 ml) was prepared in methanol and diluted to get working concentrations.

Preparation of sample stock solution

Twenty tablets were taken, their average weight was determined and crushed to a fine powdered, equivalent to100mg of NIMS and 40 mg of DROT was weight and dissolved in 100 ml of methanol with vigorous shaking for 15 minute. The solution was filtered through whatman filter paper No. 41 to a 100 ml of volumetric flask and volume was made up to mark with methanol to get sample stock solution

which was further diluted with methanol to get required concentration in linearity range. Sample solutions were scanned using proposed three methods and the results were obtained and reported in Table 1.

Table 1: Results of Tablets Dosage form

Parameters	Method I		Method II		Method III	
	NIMS	DROT	NIMS	DROT	NIMS	DROT
Label claim (mg/Tab.)	100	40	100	40	100	40
Found (mg/Tab.)	99.18	39.87	99.41	39.78	98.98	39.77
%found [a]	99.18	99.82	99.41	99.45	98.98	99.43
S.D.	0.505	0.139	0.588	0.519	0.309	0.448
% RSD	0.508	0.139	0.591	0.522	0.313	0.452
S.E.	0.206	0.0005	0.240	0.212	0.126	0.183

[a] Average of six determinations, S.D.: Standard deviation, R.S.D. : Relative standard deviation, S.E.: Standard error.

Method I (Derivative Spectrophotometric Method)

In this method [17], the standard stock solution of NIMS and DROT were scanned from 200 nm to 400 nm. The spectra obtained were derivatized in first order and then overlain spectra recorded (Fig. 1). From the entire derivative spectra obtained, the wave lengths were selected in a manner such that NIMS had zero crossing point at 322 nm and DROT showed a measurable dA/dλ where as the zero crossing point of DROT at 262 nm. NIMS showed appreciable dA/dλ. Hence wavelengths 262 nm and 322 nm were selected as analytical wavelength for determination of NIMS and DROT respectively. The mixed standards were scanned in the spectrum mode, derivatized in first order with derivative interval of 6 nm and absorbances were measured at the selected wavelengths. Calibration curve for NIMS (10-50 µg/ml) and DROT (5-35µg/ml) were plotted as dA/dλ verses concentration. By extrapolating the value of absorbances, the conc. of corresponding drugs in the sample was determined.

Method II (Area calculation Method)

AUC method [17], involves the calculation of integrated value of absorbance with respect to wavelength. Area calculation processing item calculates the area of bounded by the curve and horizontal axis. Here horizontal axis represents baseline.

$$(\alpha+\beta)=\int_{\lambda_2}^{\lambda_1} A d\lambda$$

Where; α = area of portion bounded by curve data and a straight line connecting the start and end point, β = area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis, $\lambda_1$and λ_2 are wavelength representing start and end point of curve region.

This method involved calculation in regions 302 nm to 306 nm for NIMS and 244 nm to 248 nm for DROT respectively. These regions were selected on the basis of repeated observation that plot area calculation of pure single drug v/s concentration. The UV spectra of NIMS and DROT along with its AUC region are shown in (Fig. 2a) and (Fig. 2b) respectively.

$$\int_{302}^{306} A d\lambda = K_2 C_1 \quad \text{.....Eqn.1}$$

$$\int_{244}^{248} A d\lambda = K_1 C_1 \quad \text{.....Eqn.2}$$

$$\int_{302}^{306} A d\lambda = K_4 C_2 \quad \text{.....Eqn.3}$$

$$\int_{244}^{248} A d\lambda = K_3 C_2 \quad \text{.....Eqn.4}$$

Where C_1 and C_2 are concentration of NIMS and DROT respectively in µg/ml and K_1, K_2, K_3, and K_4 are constant.

Area of curve between 302 nm to 306 nm and 244 nm to 248 nm were represented by and $\int_{302}^{306} A d\lambda$ and $\int_{244}^{248} A d\lambda$ for NIMS and DROT respectively. In view of that following two final equations were developed for estimation of NIMS and DROT.

$$\int_{302}^{306} A d\lambda = 0.0.0658 C_1 + 0.1034 C_2 \quad \text{…Eqn.5}$$

$$\int_{244}^{248} A d\lambda = 0.0852 C_1 + 0.1195 C_2 \quad \text{…Eqn.6}$$

Sample solutions were scanned and area was calculated with in indicated wavelength range. Concentration of both components was calculated using above-mentioned Eqn. 5 and 6.

Method III (Multicomponent Method)

In this method [18], the six mixed standard solutions with concentration of NIMS and DROT in the ratio of 25:10, 30:12, 35:14, 40:16, 45:18, and 50:20 (µg/ml) were prepared in methanol. All the mixed standard solutions were scanned over the range of 400-210 nm. In the multi-component the wavelength selected were 230.5, 299 and 331 nm. Sampling wavelengths were selected on trial and error basis. The concentration of individual drug was feed to the multi-component mode of the instrument. The instrument collects and compiles the spectral data from mixed standards. Overlain spectra of mixed standards solution are given in (Fig. 3). Mixed standard solution of both the drug was scanned on all the selected wavelengths to study the range of Beer's Lambert' s range.

The sample solutions were scanned over the range of 400-210 nm in the multi-component mode of the instrument and concentration of each component was obtained by analysis of spectral data of sample solution with reference to that of six mixed standards, in the terms of µg/ml.

VALIDATION OF THE DEVELOPED METHODS

The developed methods for the simultaneous estimation of NIMS and DROT were validated as per ICH guidelines (ICH 1996).

Linearity

Appropriate dilutions of standard stock solutions were assayed as per the developed methods for each drug**.** To establish linearity of the all proposed three methods, six separate series of solutions of NIMS and DROT were prepared from the stock solutions and analyzed.

Accuracy

To check the accuracy of proposed method, recovery studies were carried out from the pre-analyzed sample at three different level of standard addition 80 %, 100 % and 120 % of the level claim.

Precision (Intra-day and Inter-day precision)

The Intra and Inter-day precision was determined by assay of the sample solution on the same day and different day at different time intervals respectively.

Limit of detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of NIMS and DROT by the proposed

Table 2: Results of Recovery Studies

Method	Level of % recovery	% Mean Recovery[a]		S.D.[a]		% R.S.D.[a]	
		NIMS	DROT	NIMS	DROT	NIMS	DROT
I	80	100.40	100.4	0.562	0.491	0.559	0.489
	100	100.27	100.5	0.417	0.728	0.416	0.724
	120	100.04	100.41	0.286	0.441	0.286	0.439
II	80	100.07	100.44	0.121	0.584	0.122	0.581
	100	99.99	101.03	0.067	0.192	0.066	0.193
	120	100.08	100.11	0.206	0.231	0.205	0.230
III	80	100.77	100.47	0.608	0.516	0.603	0.513
	100	100.27	100.03	0.418	0.061	0.416	0.061
	120	100.57	100.03	0.417	0.061	0.415	0.061

[a] Average of three determinations, S.D.: Standard deviation, R.S.D. : Relative standard deviation.

Table 3: Intraday, Interdays, LOD and LOQ data

Method	Drug	%RSD Intraday (n=6)	%RSD Interdays (n=6)	LOD (µg/ml)	LOQ (µg/ml)
I	NIMS	0.231	0.363	0.063	0.190
	DROT	0.527	0.396	0.074	0.224
II	NIMS	0.191	0.303	0.580	1.760
	DROT	0.497	0.417	2.103	6.373
III	NIMS	0.088	0.352	0.071	0.214
	DROT	0.431	0.433	0.199	0.602

R.S.D.: Relative standard deviation, LOD: Least of detection, LOQ: Least of quantitation.

methods were determined using calibration standards. LOD and LOQ were calculated as 3.3σ/S and 10σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation.

RESULTS AND DISCUSSION

Analytical validation

Linearity

Linearity range for NIMS and DROT estimation were found to be and 10-50 µg/ml and 5-35 µg/ml respectively at their respective selected wavelengths for all proposed three methods.

Accuracy

The validity and reliability of proposed method was assessed by recovery studies by standard addition method. The means of % recovery (% RSD) were found to be low values (<2.0) for all the three proposed methods (Table 2). These results revealed that any small change in the drug concentration in the solution could be accurately determined by the proposed analytical methods.

Precision

Precision was determined by studying the intermediate precision. Intermediate precision study expresses within laboratory variation in same day and different days. In intermediate precision study, % RSD values were not more than 2.0 % in all the cases (Table 3). RSD values found for all the analytical methods for both drugs were well within the acceptable range indicating that these all methods have excellent repeatability and intermediate precision.

LOD and LOQ

From data (standard deviation of y-intercept of regression equation and slope of calibration curve), it was possible to calculate the detection and quantitation limits. For method I, the LOD, LOQ values for NIMS and DROT was found to be 0.063, 0.190 and 0.074, 0.224 (µg/ml) respectively; for method II, 0.580, 1.760 and 2.103, 6.373 (µg/ml) respectively; for method III, 0.071, 0.214 and 0.199, 0.602 (µg/ml) respectively (Table 3). These low values indicated the good sensitivity of the method proposed.

Estimation of formulation

The assay values of NIMS, DROT for method I, II and III were found to be 99.18 % , 99.82 % and 99.41 % , 99.45 % and 98.98 %, 99.43 % respectively with standard deviation<1.0 (Table 1). Assay values of formulation were same as mentioned in the label claim indicating that the inference of excipients matrix is insignificant in estimation of NIMS and DROT by all three proposed methods

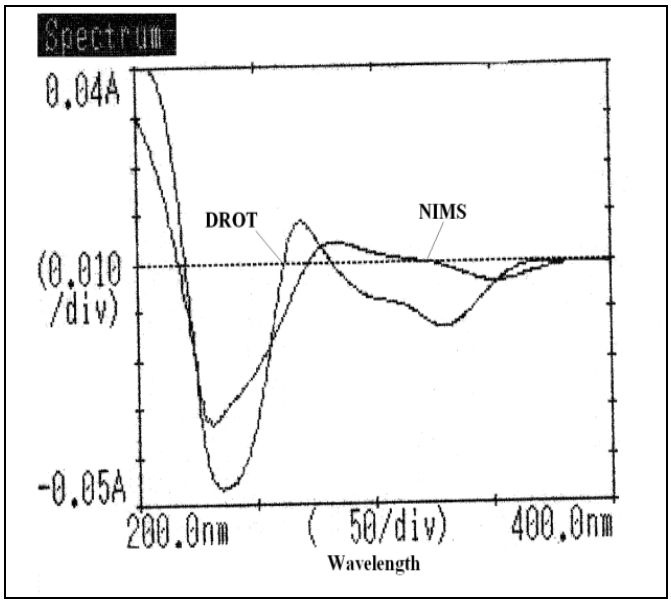
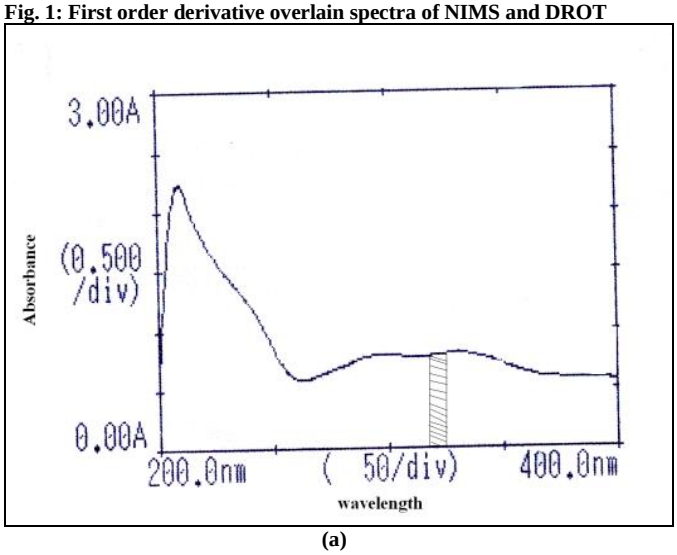
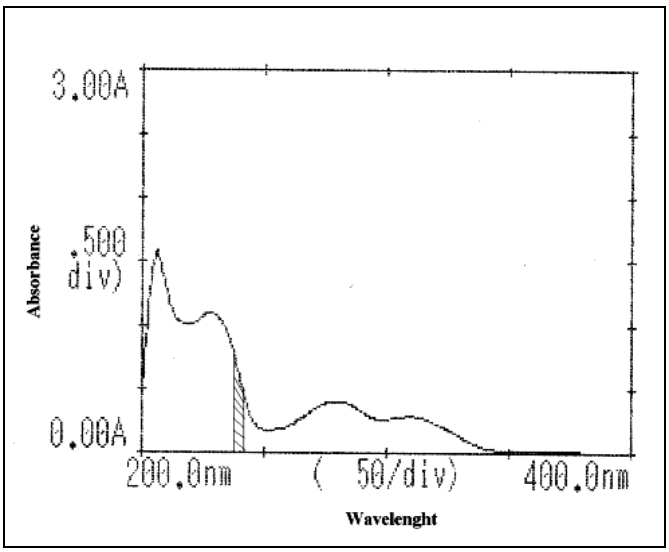


Fig. 1: First order derivative overlain spectra of NIMS and DROT



(a)



(b)

Fig. 2: UV spectra of (a) NIMS and (b) DROT along with AUC range

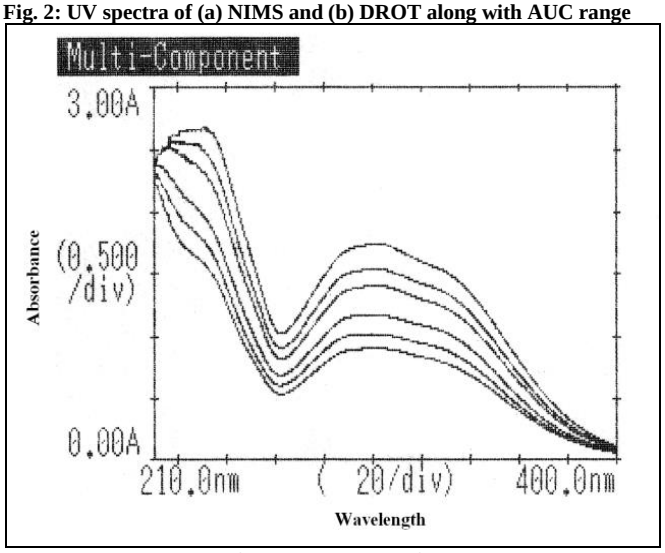


Fig. 3: Overlain spectra of mixed standards of NIMS and DROT

The proposed validated three spectrophotometric methods are simple, rapid, accurate and precise and hence can be used for the routine analysis of NIMS and DROT in tablets dosage forms. The sample recovery for all three methods was in good agreement with their respective label claims, which suggested non interference of formulation additives in estimation.

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