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Spectrophotometric simultaneous estimation and validation of drotaverine hydrochloride and nimesulide in tablet dosage form using various methods

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Abstract
Three simple, sensitive and accurate UV spectrophotometric methods, I; formation and solving of simultaneous equation method, II; absorbance ratio method, III; dual wave length method, has been developed for the determination 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, simultaneous equation, absorbance ratio, dual wavelength

1. Introduction
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 [1]. DROT generally acts as an antispasmodic agent [2] 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 [3] and urine [4] and spectrophotometric methods for estimation in single [5] and combined dosage forms [6- 8].
Nimesulide (NIMS) is an anti-inflammatory drug. Chemically, NIIMS is N-(4-nitro-2-phenoxphenyl) 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 a few UV spectrophotometric [9-12] and liquid chromatography (HPLC) methods [13-18] have been reported for the estimation of nimesulide, both individually as well as in combination with other drugs in plasma.
In the present work, we attempted to develop an easier, accurate, and reproducible three analytical methods with better detection range for estimation of DROT and NIMS in bulk drug and in its solid dosage forms. This paper describes a UV spectrophotometric method of estimation of DROT and NIMS in methanol. The results of the analysis were validated by statistical methods, recovery studies and LOD, LOQ.

2. Experimental
Materials
DROT and NIMS 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.

Instrument
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).

Standard stock solution
The standard stock solution of DROT and NIMS (10mg/100mL) 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 to 40 mg of DROT and 100mg of NIMS 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.

Method I: Simultaneous Equation Method
Simultaneous equation method [19] of analysis was based on the absorption of drugs (DROT and NIMS) at the wavelength maximum of the each other. Two wavelengths were selected for the development of the simultaneous equations, 230.5 nm and 331.5 nm, λ_{max} of DROT and NIMS respectively (Fig.1). The absorptivity values E (1%, 1cm) were determined of both the drug.
The concentration of two drugs in mixture was calculated by, using following equations.

$$A_1 = 0.0552C_1 + 0.0429C_2 \quad \text{.....Eqn.1}$$

$$A_2 = 0.0233C_1 + 0.0068C_2 \quad \text{.....Eqn.2}$$

Whereas A_1 and A_2 were the absorbance of sample at 230.5 nm and 331.5 nm respectively.

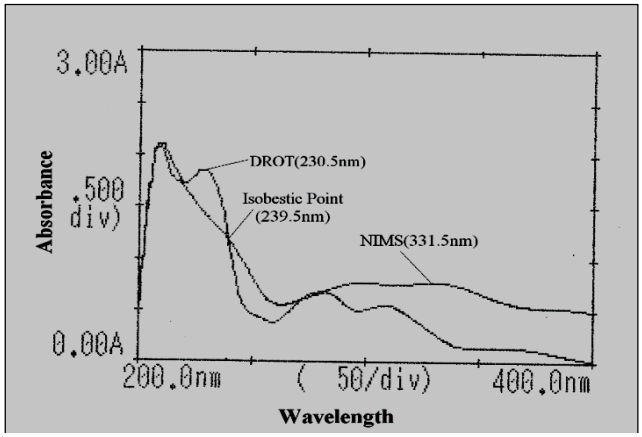

Fig 1: Overlain spectrum of DROT and NIMS

Method II: Absorbance ratio method

Absorbance ratio method [20] of analysis was based on the absorbance's at two selected wavelengths, one of which is an Isosbestic point and the other being the wavelength of maximum absorption of one of the two components. From overlain spectra (Fig.1) 239.5 nm (Isosbestic point) and 331.5 nm (λ_{max} of NIMS) were selected for the formation of Q absorbance equation (Eqn. 3 and 4). The absorbances at 239.5 nm and 331.5 nm for DROT and NIMS were measured. The absorptivity values of each drug at both wavelengths were determined which was the mean of six independent values.

The absorbances and absorptivity at this wavelength were substituted in following equations to obtain the concentration of both drugs.

$$C_{DROT} = \frac{(Q_M - Q_Y)}{(Q_X - Q_Y)} \cdot \frac{A_1}{ax_1} \quad \text{.....Eqn.3}$$

$$C_{NIMS} = \frac{(Q_M - Q_X)}{(Q_Y - Q_X)} \cdot \frac{A_1}{ax_1} \quad \text{.....Eqn.4}$$

Q_M, Q_X, and Q_Y were obtained as bellow:

$$Q_M = \frac{A_2}{A_1}, \quad Q_X = \frac{ax_2}{ax_1}, \quad Q_Y = \frac{ay_2}{ay_1}$$

Where A_1 and A_2 were the absorbance of the sample at 239.5 nm and 331.5 nm respectively, a_{X1} and a_{X2} were the absorptivity of DROT at 239.5 nm and 331.5 nm respectively and a_{y1} and a_{y2} were the absorptivity of NIMS at 239.5 nm and 331.5 nm respectively.

Method III: Dual Wavelength Method

In this method [21], two wavelengths were selected for each drug in a way so that the difference in absorbance is zero for one drug at a time. The spectrum of DROT showed that the absorbance is identical at 271 nm (λ_1) and 288 nm (λ_2), so these two wavelengths were selected for the analysis of NIMS. All the solutions of series were scanned to ensure that absorbance difference between λ_1 and λ_2 is zero. Similarly, the NIMS solution was scanned to determined two wavelengths where absorbance was same. These two wavelengths were found to be 293.5 nm (λ_3) and 304 (λ_4) so these two wavelengths were selected for the analysis of DROT. All the solutions of this series were scanned to confirm that absorbance difference zero between λ_3 and λ_4. For DROT, the calibration curve was prepared by difference in absorbance i.e. $A_{(\lambda 3)} - A_{(\lambda 4)}$, at 293.5 nm and 304 nm (difference was zero for NIMS) plotted against the respective concentration. Similarly for NIMS, calibration curve prepared by plotting difference in absorbance i.e. $A_{(\lambda 1)} - A_{(\lambda 2)}$, at 271 nm and 288 nm (difference was zero for DROT) against the respective concentration. Sample solutions containing DROT and NIMS were scanned and concentration of was calculated from their respective calibration curve.

3. Validation of the developed methods

The developed methods for the simultaneous estimation of DROT and NIMS 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 DROT and NIMS 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 DROT and NIMS by the proposed methods were determined using standard deviation of y-intercept of regression equation and slope of calibration curve. LOD and LOQ were calculated as $3.3\sigma/S$ and $10\sigma/S$, respectively, where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation.

4. Results and Discussion

Estimation of Drug Formulation

The assay values of DROT, NIMS for method I, II and III was found to be 99.37 %, 99.28% & 99.36%, 99.04% & 99.24%, 99.29% 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 DROT and NIMS by all three proposed methods.

Table 1: Results of Tablets Dosage form

Parameter	Method-I		Method-II		Method-III	
	DROT	NIMS	DROT	NIMS	DROT	NIMS
Label claim(mg/tab)	40	100	40	100	40	100
Found (mg/Tab)	39.75	99.28	39.74	99.04	39.64	99.29
%found [a]	99.37	99.28	99.36	99.04	99.24	99.29
S.D.	0.574	0.490	0.388	0.345	0.798	0.563
%COV	0.577	0.494	0.391	0.348	0.804	0.567
SE	0.234	0.200	0.158	0.141	0.325	0.229

[a] Average of six determinations, S.D.: Standard deviation, COV: Coefficient of variation, S.E.: Standard error.

Analytical validation

Linearity

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

Accuracy

The validity and reliability of proposed method was assessed by recovery studies by standard addition method. The means of %recovery (%COV) 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, %COV 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 DROT and NIMS was found to be 0.137, 0.417& 0.133, 0.404 (µg/ml) respectively; for method II, 0.302, 0.917 & 0.133, 0.404 (µg/ml) respectively; for method III, 0.071, 0.217 & 0.055, 0.167 (µg/ml) respectively, Table3. These low values indicated the good sensitivity of the method proposed.

Table 2: Results of Recovery Studies

Method	Drug	Level of % recovery	% mean recovery ± S.D.	% COV
I	DROT	80	101.13±0.91	0.906
		100	100.35±0.57	0.576
		120	100.05±0.20	0.193
	NIMS	80	100.33±0.45	0.449
		100	100.95±1.01	1.000
		120	100.05±0.46	0.463
II	DROT	80	99.24±0.65	0.665
		100	100.08±0.85	0.851
		120	100.75±0.58	0.576
	NIMS	80	101.09±0.88	0.877
		100	99.73±0.65	0.659
		120	100.58±0.49	0.489
III	DROT	80	101.02±1.03	1.030
		100	101.02±0.539	0.534
		120	100.23±0.457	0.456
	NIMS	80	100.03±0.07	0.074
		100	100.36±1.07	1.075
		120	100.33±1.10	1.105

S.D.: Standard deviation, COV: Coefficient of variation

Table 3: Intraday, Interdays, LOD and LOQ data

Method	Drug	%COV Intraday (n=6)	%COV Interdays (n=6)	LOD (µg/ml)	LOQ (µg/ml)
I	DROT	0.166	0.537	0.137	0.417
	NIMS	0.527	0.368	0.133	0.404
II	DROT	0.769	0.467	0.302	0.917
	NIMS	0.722	0.436	0.133	0.404
III	DROT	0.477	0.109	0.071	0.217
	NIMS	0.368	0.414	0.055	0.017

COV: Coefficient of variation, LOD: Least of detection, LOQ: Least of quantitation

5. Conclusion

The proposed validated three spectrophotometric methods are simple, rapid, accurate and precise and hence can be used for the routine analysis of DROT and NIMS 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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