

ANALYTICAL METHOD AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF DROTAVARINE HYDROCHLORIDE AND NIMASULIDE AS API IN SYNTHETIC MIXTURE BY RP-HPLC METHOD AND UV-VISIBLE SPECTROSCOPY

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Article Received on
27 Nov. 2017,
Revised on 17 Dec. 2017,
Accepted on 07 Jan. 2018
DOI: 10.20959/wjpr20182-10705

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ABSTRACT

A novel approach was developed and validated by rapid phase high performance liquid chromatography method for the simultaneous estimation of Drotavarine Hydrochloride and Nimesulide in active pharmaceutical ingredient (API) in synthetic mixture. This study describes the simple, rapid, precise and accurate RP-HPLC method for simultaneous estimation in tablet dosage form. The separation was achieved on Nucleosil (length-250mm*4.6mm) particle size -5 μ m column with an isocratic mixture of methanol and acetonitrile 50:50, pH adjusted to and flow rate 0.6ml/min and run time 10 min and UV

detection at 298.5 nm. The retention time for Drotavarine Hydrochloride and Nimesulide was 6.28 min and 8.60 min respectively. This method was linear in the range of 2-20 μ g and 5-40 μ g/ml Drotavarine Hydrochloride and Nimesulide. The correlation coefficients were 0.9990 for Drotavarine Hydrochloride and 0.9980 for Nimesulide. This method has been validated as per ICH guideline and applied for estimation of Drotavarine Hydrochloride and Nimesulide in commercially available tablet dosage form.

KEYWORDS: Drotaverine, Nimesulide, ICH, Tablet.

INTRODUCTION

Excessive pain may produce other effects like sinking sensation, apprehension, sweating, nausea, palpitation, rise or fall in BP or tachypnea, analgesic relieve pain as a symptom

without affecting its cause.^[1,2] Drotavarine Hydrochloride 6,7-diethoxy-1-(4-ethoxyphenyl)-3,4-dihydroisoquinoline hydrochloride is a papaver analogue mainly used as an antispasmodic and smooth muscle relaxant in pain associated with gastrointestinal colic pain.^[3] Nimesulide *N*-(4-nitro-2-phenoxyphenyl)methanesulfonamide. Nimesulide is selectively inhibit Cox-2 with a potent anti-inflammatory and analgesic activity, when administered orally, rectally or topically. It is widely used for the treatment of a various inflammatory processes due to its analgesic and antipyretic properties. It is approved for use in treatment of musculoskeletal disorder, thrombophlebitis, dental pain and inflammation.^[4,7] Literature survey reveals that UV spectrophotometry HPLC method are reported for determination of Drotavarine Hydrochloride and Nimesulide.^[8,16]

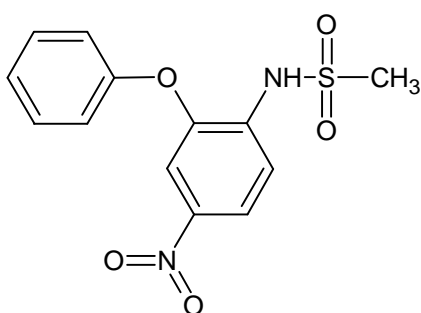


Fig-1: Structure of Nimesulide.

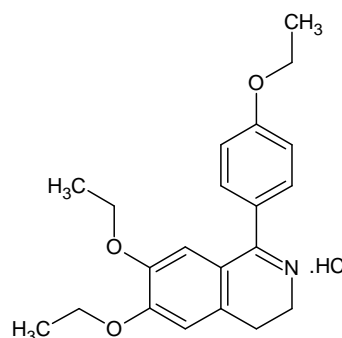


Fig-2: Structure of Drotavarine.

MATERIAL AND METHODS

CHEMICAL AND REAGENTS

Analytical pure Drotavarine HCL from alkume pharmaceutical Pvt. Ltd., Nimesulide from Dr.Reddy's Lab. Pvt. Ltd. All chemicals and reagent used were of analytical grade obtained from Merk.

INSTRUMENT

Shimadzu double beam UV-Visible recording spectrophotometer (model –UV-1601). The chromatographic separation has been achieved on Machery –Nagle, NUCLEODUR (Length - 250mm*4.6mm, 5µm). Digital pH meter SYSTRONIC(pH meter 335) has been used during analysis.

PREPARATION OF STOCK SOLUTION OF NIMESULIDE AND DROTAVARINE HCL

Accurately weighed Drotaverine HCL 50 mg and Nimesulide 50mg, were transferred in 50 ml volumetric flask, dissolved in methanol and diluted up to mark with same. The final solution contained 1000 ppm per ml of Drotaverine HCL and Nimesulide.

CONSTRUCTION OF CALIBRATION CURVE

Solutions of different concentrations of Nimesulide (5, 10, 20, 30 and 40 ppm) and of Drotaverine HCL (2, 5, 10, 15 and 20 ppm) were prepared. The volume was made up with methanol and mixed properly.

PROCEDURE FOR DETERMINATION OF WAVELENGTH OF MAXIMUM ABSORBANCE

Drotaverine HCL and Nimesulide solution of different concentrations (ppm) were taken and absorbance of the final solutions were scanned in the range 220 to 400 nm against methanol blank. Individual absorption spectra was plotted in figure-1,2,3.

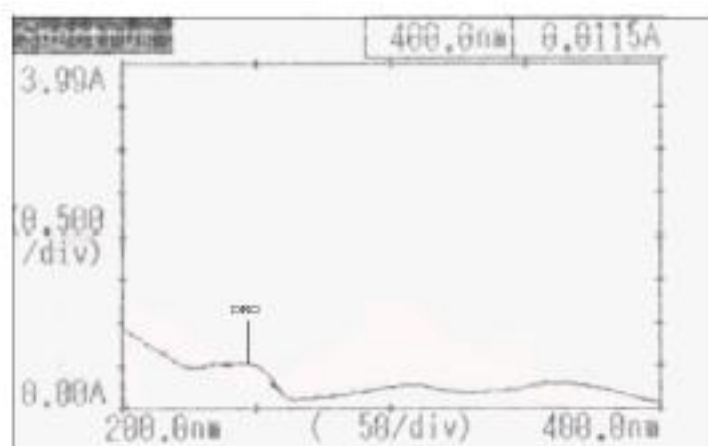


Figure -1: Spectra of absorbance of Drotaverine.

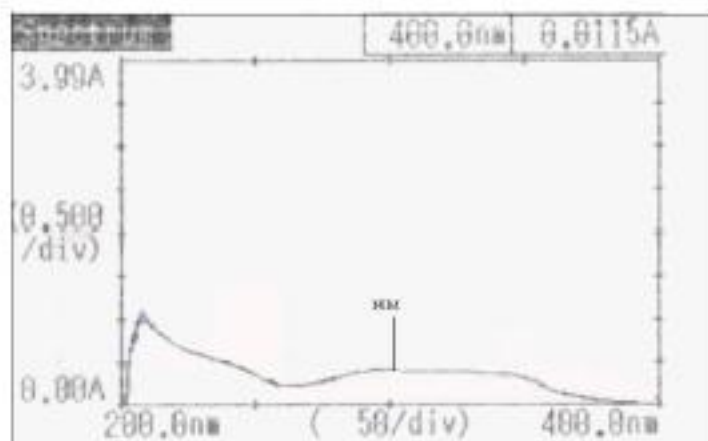


Figure 2: Spectra of absorbance of Nimesulide.

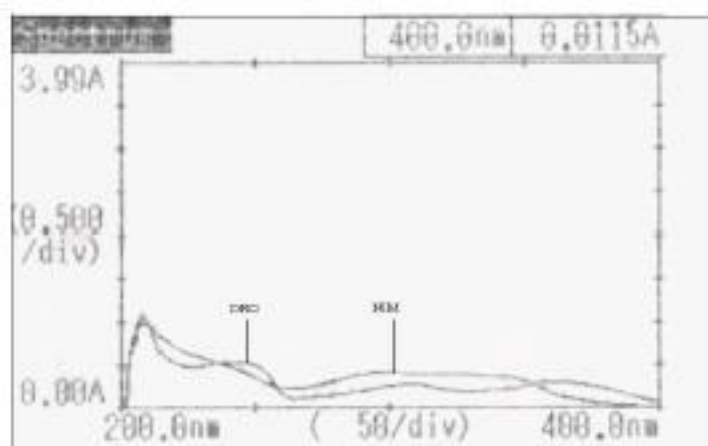


Figure 3: Overall spectra of absorbance of Nimesulide and Drotaverine HCL.

The molecular absorptivity and Sandell's sensitivity were calculated as

Molecular absorptivity (ϵ) = AM/ct , where

A = Absorbance

M = Molecular weight

C = Concentration of sample

t = path length

Sandell's Sensitivity = M/ϵ

M = molecular weight

ϵ = Molecular absorptivity

Other optical parameters i.e. Beer's limit, slope, intercept and correlation coefficient were calculated from calibration curve.

The absorbance of the solutions was measured at 245 nm and 298.5 nm against methanol as a blank in figure-4,5,6,7. The observation data of Nimesulide and Drotavarine Hcl in table -1.

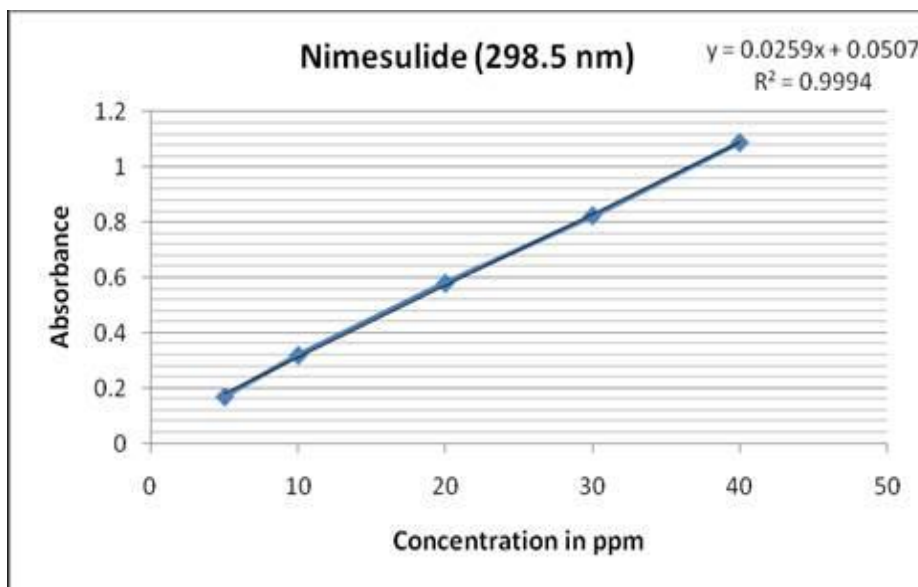


Figure 4: Calibration curve of Nimesulide at 298.5 nm.

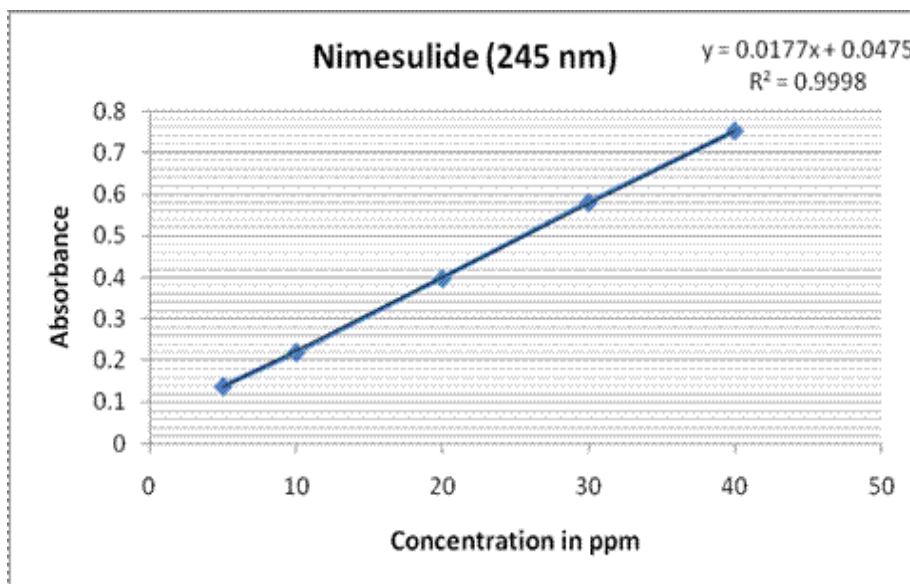


Figure 5: Calibration curve of Nimesulide at 245 nm.

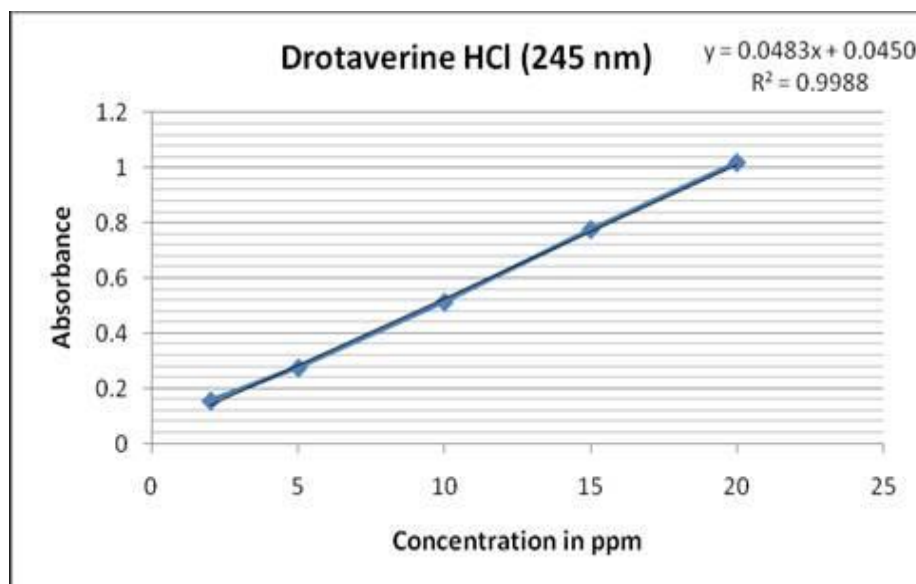


Figure 6: Calibration curve of Drotaverine HCL at 245nm.

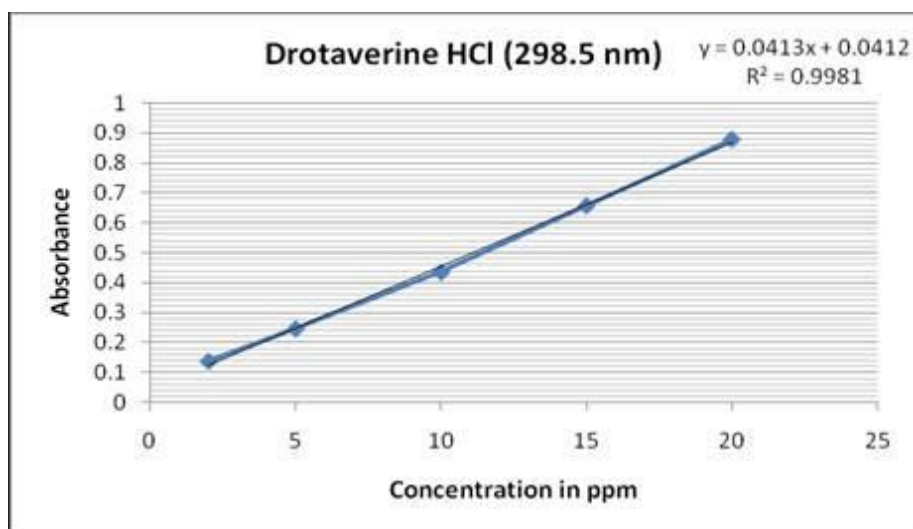


Figure 7: Calibration curve of Drotaverine HCL at 298.5nm.

Table 1: Optical and Regression Parameters.

Parameters	Drotaverine		Nimesulide	
	245nm	298.5nm	245nm	298.5nm
Beers's law limit	2-20	2-20	5-40	5-40
Molar absorptivity ($\text{mole}^{-1} \text{cm}^{-1}$)	2.494×10^4	2.160×10^4	6.659×10^3	9.203×10^3
Sandell's sensitivity ($\text{mg}/\text{cm}^2/0.001$ absorbance unit)	0.0174	0.020084	0.0463	0.033499
Regression equation ($Y=a + bc$)				
Slop (b)	0.0483	0.0413	0.0177	0.0289
Intercept (a)	0.0450	0.0434	0.0478	0.0507
Correlation coefficient (r^2)	0.9988	0.9989	0.9998	0.9994

PREPARATION OF SYNTHETIC MIXTURES OF DROTAVERINE AND NIMESULIDE

The synthetic mixture of Drotavarine HCl and Nimasulide was prepared in the ratio of 2:5 common excipients such as 8% starch 7% magnesium stearate and 15% lactose (for 1000 μ g/ml) used in tablet formulation, were added in the synthetic mixture (1,2,4,6,8 ml) was transferred to a series of the 10 ml with solvent system. The absorbance of these solutions were measured at 245 nm & 298.5 nm. At 245 nm & 298.5 nm two simultaneous equations were formed using absorptivity co-efficient values. The synthetic mixture of the combination of both the drugs were prepared in the ratio of 2:5 (drotaverine: nimesulide).

VALIDATION OF THE DEVELOPED METHOD ACCORDING TO I.C.H GUIDELINES

Following parameters were taken into consideration for validation of proposed methods:

1. Specificity:- For specificity determination, small amount of excipients such as 8% starch, 7% magnesium stearate and 15% lactose (for 1000 μ g/ml) were added, then filtration was done and any change in absorbances were observed and the data were obtained. The results obtained for the specificity study from five samples studies (n = 3) after addition of excipients had a very negligible change in concentration from the concentration before addition of excipients.

2. Precision

Repeatability was assessed using: Six time repetition of target concentration 100% that is (5 μ g/ml). Intermediate precision was assessed by intra-day and inter day analysis.

In the study of the repeatability precision which was conducted on the solution having the concentration value 100% of the target concentration (n = 6). The intra-day analysis was conducted at three different time (10 pm, 1 am, 4 am) on the solution having the concentration value 80%, 100% & 120% of the target concentration (n = 3).

TABLE

Inter-Day Precision

Method: The inter-day analysis was conducted on the solution having concentration value 80%, 100% & 120% of the target concentration (n = 3), at three different days. The data obtained are given in table 24.

3. Linearity

Linearity range was found to be 2-20 µg/ml for Drotaverine at 245 nm and 298.5 nm. The correlation coefficient was found to be 0.9988 & 0.9989 which adhere good linearity between above the range. The slope was found to be 0.0483 & 0.0413 and intercept was found to be 0.0450 & 0.0434 which was close to zero intercept. While linearity range was found to be 5-40 µg/ml for Nimesulide at 298.5 nm and 245nm. The correlation coefficient was found to be 0.9994 & 0.9998 which adhere good linearity between above the range. The slope was found to be 0.0259 & 0.0177 and intercept was found to be 0.0507 & 0.0475 which was close to zero intercept.

4. Limit of detection and limit of quantification

The detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

$$\text{L.O.D.} = 3.3(\text{SD}/S)$$

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

$$\text{L.O.Q.} = 10(\text{SD}/S)$$

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

5. Accuracy

The results of analysis, obtained in three group containing three replicate experiments with API and different tablet dosage forms, had good agreement with the labeled amount of the drug. In nine different 10 ml volumetric flasks, 0.2 ml of the pre-analyzed tablet solution (100 µg/ml) was taken and added 0.5, 1.0, 1.5 ml of standard solution of bulk (API) mixture (100µg/ml) then the volume was made up to 10 ml with methanol. The data were obtained.

RESULT AND DISCUSSION

For RP-HPLC method different phases tried and the mobile phases containing (50:50v/v) methanol and acetonitrile was found to be optimal for obtaining well defined and resolution peak with mean retention time 6.28 min and 8.6 min for Drotavarine and Nimasulide

respectively represented in chromatogram of the mixture is shown in figure. Result were found to be linear in the concentration range 5-40 μ g for Nimasulide and 2-20 μ g for Drotavarine. The summary of validation parameter of RP-HPLC method given in table-2 which is shown in figure-1.

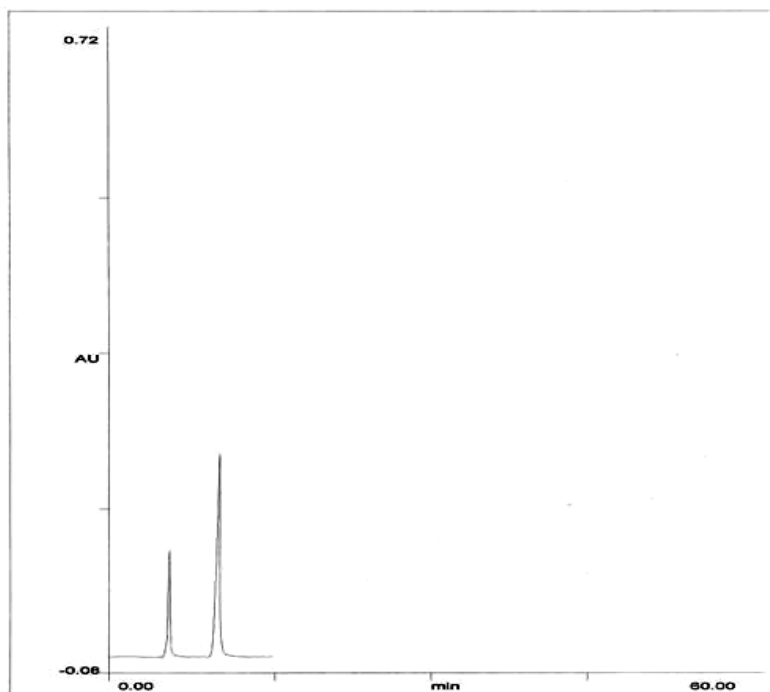


Table -2: The summary of validation parameter of proposed RP-HPLC method.

Parameter	Nimasulide	Drotavarine
Linearity	5-40 μ g/ml	2-20 μ g/ml
Correlation Co-efficient	0.9994-0.9998	0.998-0.9989
Slope	0.0450-0.0434	0.0483-0.0413
Intercept	0.0507-0.0475	0.0450-0.0434
Limit of detection	0.0294 μ g/ml	0.073 μ g/ml
Limit of quantification	0.0892 μ g/ml	0.0223 μ g/ml
%Accuracy	100.15%	99.62%
%Precession		
Intraday	1.033	0.882
Interday	0.751	0.491

CONCLUSION

The proposed method are simple, precise, accurate, economical, rapid and reproducible for determination of Drotaverine HCL and Nimasulide in the synthetic mixture showed on interference from the additive and excipient. Hence, RP-HPLC method is very convenient for the assay and evaluation of drugs in pharmaceutical preparation.

ACKNOWLEDGEMENT

The authors are thankful Department of pharmacy, Narayan Institute of Pharmacy, Jmuhar, sasaram, Bihar, India for providing research facilities and encouragement and to our friends those who helped us to complete this research.

REFERENCES

1. Singh KC, Jain P, Goel N, Saxen A. Drotaverine HCL for augmentation of labour. *Int J Gynaecobstet*, 2004; 84: 17-22.
2. Amin AS, El –Sheikh R, Zahran F, Gouda AA. Spectrophotometric determination of Piperazine HCL, dextromethorphan HBr and Drotavarine HCL in their pharmaceutical preparation *spectrochim. Acta A miolbiomol spectrosc*, 2006; 67: 1088-1093.
3. Abdellatef E, Ayad M, Soliman M, Youssef F, spectrophotometric and spectrodensitometric determination of paracetamol and drotaverine HCl in combination *spectrochimica Acta A*, 2007; 66: 1147-1151.
4. Merk index: 15th edition, 2013.
5. Martindel: The complete drug reference, The pharmaceutical Press, London, UK, 37th edition, 2013.
6. Niranjana R, Kamat PK, Nath C, Shukla R, Evaluation of gage lipid and Nimasulide on production of inflammatory mediators and GFAP expression in LPS stimulated rat astrocytoma, cell line (C6) *journal of ethnopharmacology*, 2010; 127(3): 625-630.
7. Tzanavaras PD, Nimje H, Wate SP, Dharkar DP, Determination of Nimesulide and tizanidine in Tablet, *Indian journal of pharmaceutical science*, 2007; 69(2): 281-283.
8. Daabees H.G., selective differential spectrophotometric methods for determination of Niclosamide and Drotaverine Hydrochloride, ‘*Analytical Letters*’, 2008; 33(4): 639-656.
9. Nagori B.P., Khandelwal P., Sharma S., Second derivative spectrophotometric method for the estimation of Drotaverine Hydrochloride in tablet formulations, “*Indian Drugs*”, 2008; 45(10): 798-799.
10. Dahivelkar P.P., Mahajan V.K., “Spectrophotometric method for simultaneous estimation of Drotaverine Hydrochloride and Mefenamic acid in bulk and tablet formulation”, “*Indian Drugs*”, Nov. 2006; 43: 896-899.
11. Fadia H.M., “Determination of Nifuroxazide and Drotaverine Hydrochloride in pharmaceutical preparations by three independent analytical methods”, *J. of Pharmaceutical and Biomedical Analysis*, 2006; 89: 78-81.

12. Kharitonov S.V., Transport properties and electroanalytical response characteristic of drotaverine, "J. of Analytical and Bioanalytical Chemistry", 2005, Springer Berlin Heidelberg, p-160-161.
13. Chaple D.R., Deshpande S.S, "Simultaneous spectrophotometric estimation of Nimesulide and Diclofenac Sodium in tablet dosage form", Indian Drugs, 2003; 41: 108-110.
14. Kumar Ashok, "A spectrophotometric method for simultaneous estimation of Nimesulide and Paracetamol in tablet dosage form", Indian Drugs, 2003; 40: 727-729.
15. Lallal J.K., "Modified high performance liquid chromatographic method for analysis of Drotaverine", J. of Pharmaceutical and Biomedical Analysis, 1993; 11: 385-388.
16. Mazeij, "A new Method for HPLC determination of Drotaverine", J of Pharmaceutical Sciences, 1984; 73: 1489-1491.