

VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF DROTAVERINE AND NIMESULIDE AND ITS APPLICATION IN DRUG FORMULATION

Kuldeep Chouhan, Aparna Venugopal, Lucky Soni, Chanchal Raj, Sayyed Nadeem and Navin Raj*

Technocrats Institute of Technology-Pharmacy, Bhopal, Madhya Pradesh, India

*Corresponding author

Navin Raj, Technocrats Institute of Technology Pharmacy,

Bhopal- 462 001 (M.P.), India, Tel: +91-755-2684535; Fax: +91-0755-2751679

e-mail:- pharmanavin@rediffmail.com (N.Raj)

Summary

A rapid, sensitive and specific RP-HPLC method involving UV detection was developed and validated for determination and quantification of Drotaverine and Nimesulide . Chromatography was carried out on a Phenomenex – Luna, C8 (250 x 4.6 mm i.d.,5 μ) column. using filtered and degassed mixture of methanol:water(70:30)as mobile phase at a flow rate of 1.0 ml/min and effluent was monitored at 229.5nm. The method was validated in terms of linearity, precision, accuracy and specificity. The assay was linear over the concentration range of 10.0-30.0 mcg/ml and 12.5-75.0 mcg/ml for Drotaverine and Nimesulide respectively. Accuracy of the method was determined through recovery studies by adding known quantities of standard drug to the pre analyzed test solution and was found to be 98.54-99.87% and 98.22%-98.29% for Drotaverine and Nimesulide respectively. The method requires less than 10 minutes as run time for analysis which prove the adoptability of the method for the routine quality control of the drug.

Keywords: Drotaverine, Nimesulide, Method development, Validation.

Introduction

Tablets containing the pharmaceutical association between Drotaverine and Nimesulide (40 and 100 mg, respectively) are employed as antispasmodic agent. In this combination, Drotaverine is (1Z)-1-[(3,4-diethoxyphenyl)methylidene]-6,7-diethoxy-1,2,3,4-tetrahydroisoquinoline Drotaverine (INN, also known as drotaverin) is an antispasmodic drug, structurally related to papaverine [1,2].Nimesulide {N-(4-Nitro-2-phenoxyphenyl)methanesulfonamide} is non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties [3, 4].Both drugs are insoluble in water and their chemical structures are shown in Fig. 1 (a) and(b)

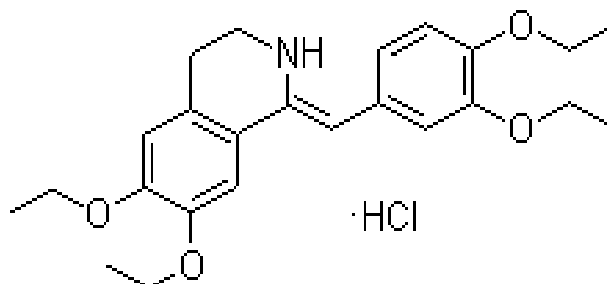


Fig 1(a) Chemical structure of Drotaverine(DRO)

(1Z)-1-[(3,4-diethoxyphenyl)methylidene]-6,7-diethoxy-1,2,3,4-tetrahydroisoquinoline

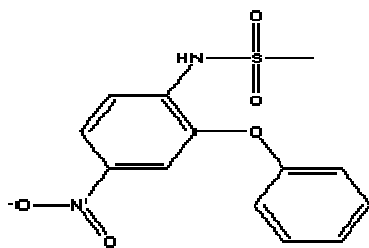


Fig.1 (b) Chemical structure of Nimesulide (NIMS)

{N-(4-Nitro-2-phenoxyphenyl)methanesulfonamide}

Many analytical methods like simultaneous estimation of Drotaverine and Nimesulide by spectrophotometric quantitative estimation [5], second order UV spectrophotometry of Nimesulide [6], Q analysis and first order derivative methods [7-8], stability indicating RP-HPLC [9] and other methods were reported for determination of DRO and NIMS alone or in combination with other analgesic and antispasmodic drugs [10-15]. Analytical method by RP-HPLC has been reported for the combination but due to the use of expensive chemicals the method was costly [16].

The aim of the present work is the development and validation of a cheap, simple and reliable RP-HPLC method for the simultaneous determination of DRO and NIMS in their combined tablet formulation, and its application to the determination of both analytes in commercial brand of their combined tablet formulation. Hence, an attempt was made in this study to develop a rapid, economical, precise and accurate method for simultaneous estimation of DRO and NIMS in tablet formulation by RP-HPLC.

Materials and methods

Chemicals and reagents

All experiments were performed with pharmaceutical-grade DRO and NIMS. HPLC-grade solvents were employed for analysis. Solvents were filtered through 0.45 μm membrane filters. All dilutions were performed in standard volumetric flasks. The pharmaceutical preparations, declaring to contain 40 mg DRO, 100mg NIMS and excipients were obtained from a local drugstore.

Instrumentation and chromatographic conditions

The separations were performed with a Shimadzu R 1100 series liquid chromatograph consisting of quaternary pumps, a injector fitted with a 20 μl loop. Compounds were separated on a 250 mm \times 4.6 mm C8 column (Luna, Phenomenex, 5 μm particle size). The mobile phase was a 70:30v/v methanol: water pumped at a flow rate of 1.0 ml min⁻¹. Chromatograms were recorded employing lab solutions software.

Preparation of stock and working standard solutions

The stock solution of DRO (1.0 mg ml⁻¹) was prepared in a 50.0 ml volumetric flask by dissolving an accurately weighed amount (50.0 mg) of DRO in methanol. The stock solution of NIMS (1.0 mg ml⁻¹) was prepared in a 100 ml volumetric flask by dissolving in methanol 100.0 mg of accurately weighed NIMS. The solutions, which proved to be stable for a period of 3 months, were conserved at 4°C, in light-resistant containers and were left to attain room temperature before use. Solutions containing mixtures of DRO and NIMS were prepared by dilution of appropriate volumes of the working solutions in methanol. All the solutions were protected from light throughout the experiments.

Sample preparation

Pharmaceutical formulation of one brand was evaluated. In this, 20 tablets were accurately weighed and their average weight was calculated. The tablet powder was taken and a quantity equivalent to one tablet was weighed and transferred to a 100.0 ml volumetric flask and the volume was made up to 100.0 ml using methanol. The flask was sonicated on a water bath for 10 min at 37 °C. A 10.0 ml portion of this solution was diluted up to 100.0 ml with methanol to get concentration of 40.0 $\mu\text{g/ml}$ of DRO and 100.0 $\mu\text{g/ml}$ of NIMS. The process was repeated with five aliquots of tablet powder. The solutions were filtered through a 0.45 μm nylon membrane filter before the analysis.

Results and discussion

Selection of the mobile phase composition

After a series of screening experiments, it was observed those mixtures of met produced satisfactory separations, the addition of methanol: water (70:30) being useful for improving peak shapes. The retention times of DRO and NIMS were 7.709 and 2.269 min, respectively, as shown in the typical chromatogram of **Fig. 3**.

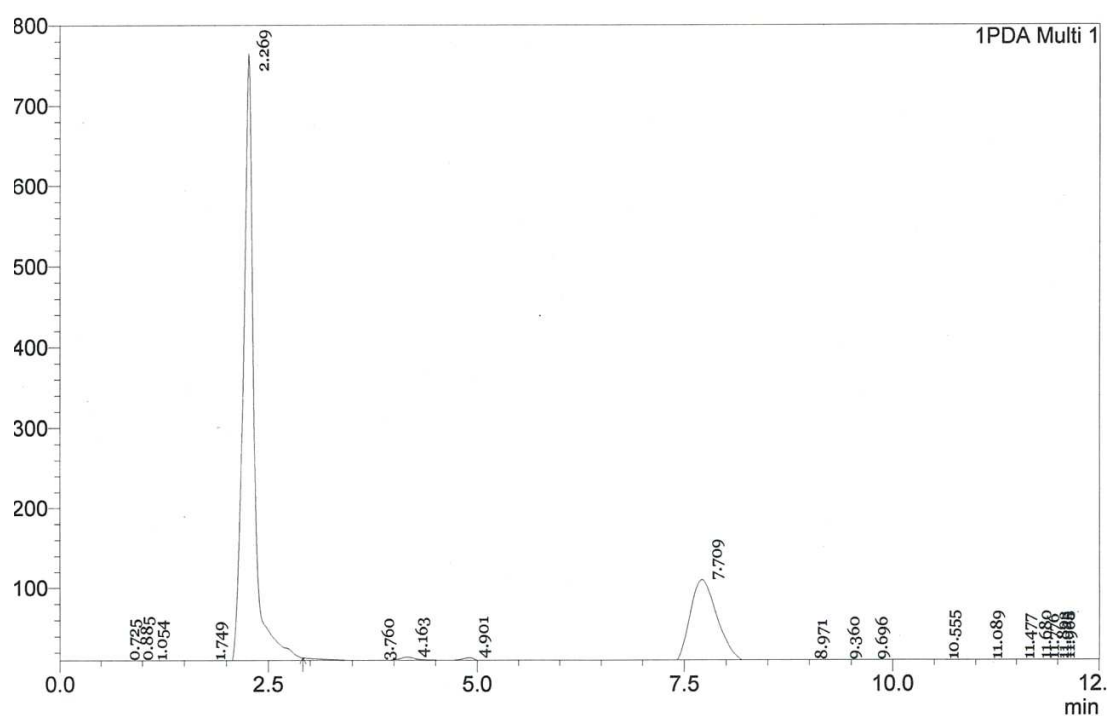


Fig no.3 Chromatogram for Drotaverine and Nimesulide

Method validation

Linearity

Linearity of the proposed method was evaluated according to the ICH guidelines, by the analysis of working solutions of DRO and NIMS at five different concentrations. Taking into account the purpose of the assay, the linear ranges were 5-30 $\mu\text{g ml}$ for DRO and 12.5-75 $\mu\text{g ml}$ for NIMS. The linearity curve for Drotaverine and Nimesulide were shown in **Fig no.4** and **5** respectively. The results show excellent correlations within the tested concentrations ranges.

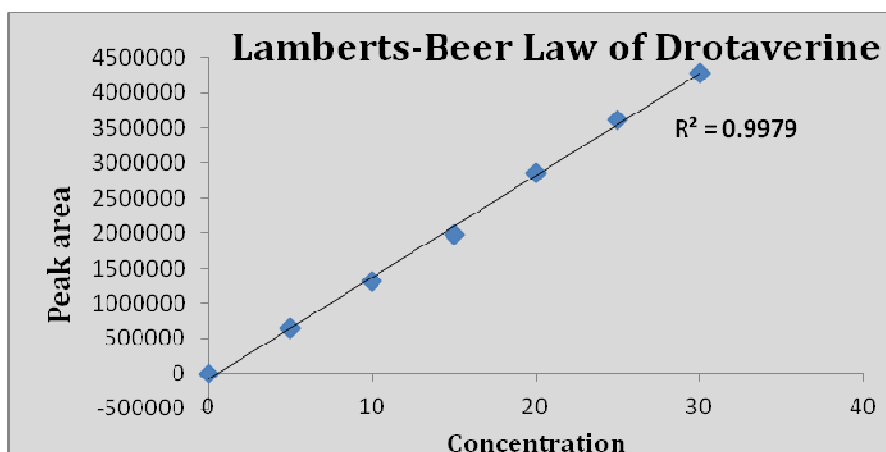


Fig-4 Linearity calibration curve of DRO

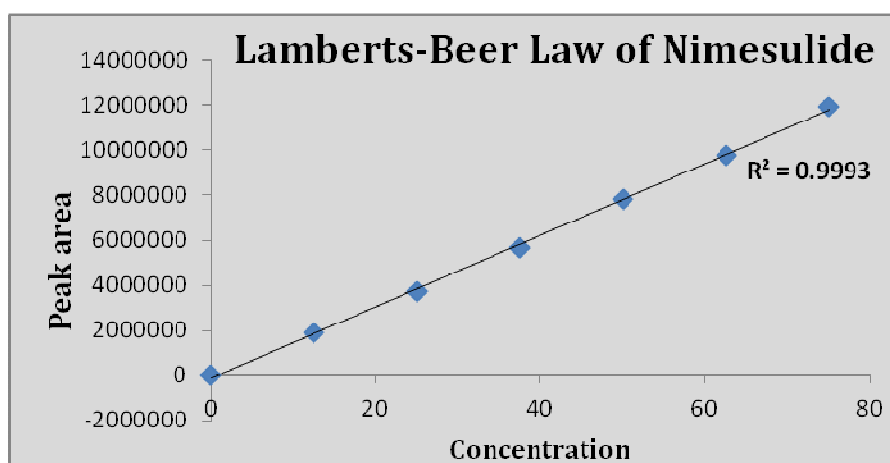


Fig-5 Linearity calibration curve of NIMS

Accuracy

The accuracy of the method was determined by measuring the drug recoveries by the standard addition method, in order to determine eventual positive or negative interferences produced by the excipients in the formulation. Known amount of standard DRO and NIMS were added in to pre-analyzed samples and subjected to proposed HPLC method. The results of recovery studies are shown in **Table-1**.

Table-1: Analysis of tablet containing Drotaverine and Nimesulide

S.No.	Wt. taken (in mg)	Amt. of pure drug added (mg)		Amt. recovered (mg)		%Recovery	
		NIMS	DRO	NIMS	DRO	NIMS	DRO
1.	120	10	3	9.9	3.0	99.0	100
2	121	20	5	19.9	5.0	99.5	100
3	165	25	12	24.9	11.9	99.6	99.1
4	165	30	18	30.1	17.9	100.3	99.44
Mean						99.64	100.13
±SD						0.535	0.442

Precision

Precision was evaluated at the repeatability and intermediate precision levels. Repeatability was studied by the determination of system precision for six replicate injections of the mixed standard solutions in groups of three, at three different levels. In inter-day precision same standard was injected on different system and the found \pm SD were 0.07 and 1.32 for Nimesulide and Drotaverine respectively. The results were depicted in **Table no.2.**

Table 2: Results for interday and intraday studies

Mean	INTERDAY		INTRADAY	
	DRO	NIMS	DRO	NIMS
\pm SD	1.32	0.07	0.09	0.79

System suitability

System suitability tests were performed in accordance with USP 30 to confirm that the equipment was adequate for the analysis to be performed. The test was carried out by injecting five replicates of a standard solution containing 100.0 μ g ml⁻¹ and 40.0 μ g ml⁻¹ of NIMS and DRO, respectively. The corresponding observed R.S.D. values were 1.48% and 1.60% which were considered satisfactory, meeting the requirements of USP 30 (R.S.D. <2%). The results were shown in **Table 3**

Table 3: Results for system suitability parameters

	DRO	NIMS
%RSD	1.60	1.48
Retention time	7.70	2.29

Assay of pharmaceutical tablet

The validated HPLC method was used for the simultaneous determination of DRO and NIMS in their combined dosage form. Five samples of each brand were weighed separately and analyzed. The results, expressed as percentage drug recovery related to label claim. These indicate that the amounts of each drug in the tablet of both brands are within the USP requirements of 90–110% of the corresponding label claims. The results were shown in **Table 4**.

Table 4: Results for Assay of pharmaceutical tablet brand

	% Label Claim	
	DRO	NIMS
Mean	99.12	98.88
±SD	0.432	0.311

Conclusion

A simple and efficient HPLC method has been developed and validated for the isocratic separation and simultaneous determination of Drotaverine and Nimesulide in their combined dosage form. The method, suitable for routine quality control, has been successfully applied to the determination of both analytes in their commercial brand of tablet containing this pharmacological association. From the results it was evident that method is more precise, accurate and inexpensive from the previously reported methods.

Acknowledgement

The authors are thankful to the Management and Director Dr.A.Balasubramaniam for providing necessary facilities.

References

- [1]The Merck Index, 13th edition, Merck Research Laboratories, 609: 1174
- [2]Singh KC, Jain P, Goel N, Saxena A.Drotaverine hydrochloride for augmentation of labor. *Int J Gynecol Obstet* 2004; 84 (1): 17–22.
- [3]British Pharmacopoeia, Vol-I, 2002:1215.
- [4] Rainsford KD and Members of the Consensus Report Group on Nimesulide. Nimesulide, a multifactorial approach to inflammation and pain, scientific and clinical consensus. *Curr Med Res. Opin* 2006; 22 (6):1161-1170.
- [5] Prasad RK, Sharma R. Spectrophotometric Quantitative Estimation and Validation of Nimesulide and Drotaverine Hydrochloride in Tablet Dosage form. *Indian J Pharm Sci* 2010; 2(1):67-70.
- [6] Altinöz, Durson OO. Determination of Nimesulide in pharmaceutical dosage forms by Second order derivative UV spectrophotometry. *J Pharm Biomed Anal* 2000; 22(1):175-182.
- [7] Mahaparale S, Telekone R, Raut R, Danle S, Kasture P. Spectrophotometric determination of drotavrine hydrochloride and paracetamol in tablet dosage form. *Indian J Pharm Sci* 2010; 72(1):133-136.
- [8] Vivek SR, SantoshVG, Upasna PP, Mahima RS. Simultaneous estimation of Drotaverine hydrochloride and acefenac in tablet dosage form by spectrophoyometry. *Eurasian J Anal* 2010; 4(2):184-190.
- [9] Kabeer AS, Sachin DP.Stability-indicating RP-HPLC for simultaneous determination of Drotaverinehydrochloride, domperidone and paracetamol in pharmaceutical dosage form. *Der Pharmacia Lettre* 2010; 2(4):355-364.
- [10] Pathade A, Dakhore A, Fule S, Alaspure R.Validated RP-HPLC method for simultaneous determination of Drotaverine hydrochloride and paracetamol in tablet dosage form. *Int J Pharm Sci* 2010; 3(1):115-117
- [11] Gandhi S, Deshpande P, Ramjane V, Parab J.Simultaneous estimation and validation of Drotaverine hydrochloride and acefenac in tablet dosage form using RP-HPLC. *Int J Pharm Pharm* 2010; 4(3):49-52.
- [12] Permanand DC, Senthikumar KL, Senthikumar B, Thirumrty R.Simultaneous estimation and validation of Drotaverine hydrochloride and mefenamic acid in tablet dosage form using RP-HPLC. *Der PharmaChemica* 2010;2(5):170-177.
- [13] GhargeD, Dhabale P.Simultaneous determination of Nimesulide and paracetamol for tablet dosage form by RP-HPLC method. *Int J Pharm Tech Res* 2010; 2(2):1330-1333.
- [14] Sunil RD, Janaki VS and Vidhya KB. Simultaneous determination of Levocetirizine hydrochloride and Nimesulide in formulation by RP-HPLC method. *Pharmacieglobale International Journal of Comprehensive Pharmacy* 2011; 2(2):1-4.
- [15] Shingare MS, Naidu KR, Kale UN.RP-HPLC Method for simultaneous estimation of TizanidineHCl and Nimesulide in Tablets. *Indian J Pharm Sci* 2003; 65(3):315-318.
- [16] Prasad KR, and Sharma R. Simultaneous estimation and validation of Drotaverine hydrochloride and Nimesulide in tablet dosage form using RP-HPLC. *Indian J Pharm Sci* 2010; 2(1):59-64.