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Research Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF DROTAVERINE AND NIMESULIDE

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ABSTRACT

The present work describes a rapid and sensitive stability indicating high performance liquid chromatography method was developed for simultaneous estimation of drotavarine hydrochloride andnimesulide. Drugs were subjected to acidic, alkaline, neutral hydrolysis, oxidation in peroxide thermal and photolytic stress degradation studies. Separation was achieved using Methanol: Phosphate buffer pH 6.0 in ratio of 65:35 v/v as mobile phase. The method was validated as per ICH guidelines.

INTRODUCTION

Drotaverine is chemically 1-[(3,4-diethoxyphenyl) methylidine]-6,7-diethoxy-3,4-dihydro-2H-isoquinoline. It is an Anti-spasmodic and selective inhibitor of phosphodiesterase. Drotaverine is primarily indicated in conditions like Cholangitis, Cholecystitis, Cholecytolithiasis, Cystitis, Nephrolithiasis, Papilitis, Smooth muscle spasm, Stone formation, Ureterolithiasis, Urolithiasis, Vesicaltenesmus. [1] Nimesulide is chemically N-(4-Nitro-2-phenoxyphenyl) methanesulfonamide. It is a COX-2 selective, non-steroidal anti-inflammatory drug (NSAID) and is used in the treatment of acute pain, the symptomatic treatment of osteoarthrosis and primary dysmenorrhoea. [2]

Some spectrophotometric and HPLC methods have been reported for determination of droteverine alone and in combination with other drugs. [8-14] Nimesulide was determined by UV spectroscopic and HPLC methods [2-7]. A stability indicating HPTLC method and spectrophotometric methods were reported for simultaneous estimation of drotaverine and nimesulide in combination tablet [15-18].

The present study illustrates development of a stability indicating HPLC method for simultaneous estimation of drotaverine and nimesulide and related degradation products in bulk.

MATERIALS AND METHODS

HPLC System: (515 HPLC pump) WATERS® system with column oven, PDA detector and auto sampler (waters 717 plus), C18 column (250mm x 4.6 mm, i.d.,5µm) with guard column and EMPOWER 2.0 software was used. All chemical used were of HPLC grade.

Preparation of Stock Solutions

Standard stock solutions of drotavarine and nimesulide were prepared separately by transferring accurately weighed quantity of 100 mg of drotaverine and 100 mg of nimesulide to 100ml volumetric flask, powder was dissolved in sufficient quantity of mobile phase and volume was made up to the mark to get the conc. of 1000µg/ml. The solution was further diluted with mobile phase to get the suitable concentration range.

Preparationof Sample Solution

To determine the content of the drotavarine and nimesulide in pharmaceutical formulation, twenty tablets were weighed accurately, they were finely powdered and powder equivalent to 40 mg of Drotavarine and 100 mg of Nimesulide was weighed accurately and transferred to a 100 ml volumetric flask containing 25 ml of mobile phase, the solutions were sonicated for 20 min. and diluted up to the mark with mobile phase. The resulting solution was filtered through Whatmann filter paper No.41. Filtrate obtained was used as sample stock

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solution. This solution was further diluted to desired concentration range with mobile phase.

Chromatographic conditions: Different mobile phases were tested in order to find the best conditions for determination of drugs. A reverse phase C18 column equilibrated with mobile phase of Methanol-Phosphate Buffer pH 6.0 (65:35) at a flow rate of 1ml per min. was selected as optimized parameters after several experimental runs. Quantitation was achieved with UV detection at 244 nm. The sample run time was 10 min. All determinations were carried out at room temperature. Figure 1 shows chromatogram of standard drotavarine and nimesulide.

Calibration Curve

Appropriate aliquots of stock solution were taken in different 10 ml volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of 5-40mcg/ml of drotavarine and nimesulide respectively and chromatograms were recorded. Calibration curve was constructed by plotting average peak area versus concentrations.

Forced Degradation of Drotavarine and Nimesulide

In order to study the effect of stress conditions on stability of drotavarine and Nimesulidemethanolic stock solution was subjected to various stress conditions as per guidelines. The resulting solutions were injected in the system at similar experimental conditions to study the stability indicating nature of the developed method.

Methanolicdrug solutions were treated with various degradation conditions, which includes acid Hydrolysis (0.1M HCl, at room temperature for 8, hours), base hydrolysis (0.1, M NaOH, at room temperature for 8, hours), oxidation (3% H_2O_2, at room temperature for 8 hours), thermal (at 105ºC for 24 hours). In above mentioned degradation conditions, each degradation samples were neutralized then diluted with 100 ml methanol to achieve the appropriate concentration DRT and NIM respectively. Then 20 µl solution of above solutions were injected into the HPLC system and analyzed under the chromatographic condition described earlier.

Method Validation

The method was validated as per the ICH [19] and USP [20] guidelines for the parameters like accuracy, linearity, precision, detection limit, quantitation limit and robustness. The accuracy of the method was determined by calculating percentage recovery of DRT and NIM. For both the drugs, recovery studies were carried out by applying the method to preanalysed drug formulation to which known amount of DRT and NIM corresponding to 80, 100 and 120% had been spiked. At each level of the amount six determinations were performed.

RESULTS AND DISCUSSION

The optimized mobile phase consisting of Methanol-Phosphate Buffer pH 5.5 (65:35) adjusted with o-phosphoric acid, at 1ml/min flow rate which gave two well-resolved peaks with minimum tailing factor for DRT and NIM (fig.1). The retention times for DRT and NIM were 3.22 min and 8.02 min, respectively.

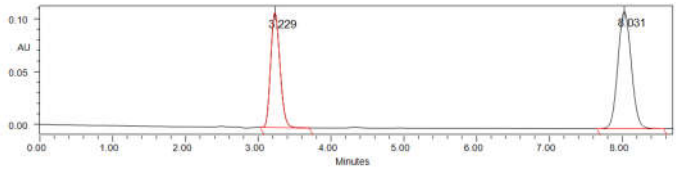

Figure 1 Chromatogram of standard Drotavarine and Nimesulide.

Linearity

The calibration curve for DRT and NIM was found to be linear over the range of 5-35 µg/ml and 5-40 µg/ ml, respectively. Calibration curve shows good linearity with the correlation coefficient of 0.9937 and 0.9979 for DrotavarineHCl and Nimesulide respectively.

The proposed method was successfully applied to the determination of DRT and NIM in their combined solid dosage form. The results for the combination were comparable with the corresponding labeled amounts.

Table 1 Results of formulation Analysis for Drotavarine and Nimesulide

Sr. No.	Amount present in (µg mL^{-1})		Total amount recovered (µg mL^{-1})		% Label claim	
	Drotaverine	Nimesulide	Drotaverine	Nimesulide	Drotaverine	Nimesulide
1	40	100	39.67	99.65	99.17	99.65
2	40	100	39.52	100.12	98.80	100.12
3	40	100	40.12	99.83	100.3	99.83
	Mean		39.77	99.866	99.4233	99.8666
	SD		0.3122	0.2371	0.7814	0.2371
	% RSD		0.7851	0.2374	0.7859	0.2374

Accuracy

Tablet sample -							
Amount of sample drug taken (µg mL^{-1})		Amount of standard drug added (µg mL^{-1})		Total amount recovered (µg mL^{-1})		% Recovery	
Drotaverine	Nimesulide	Drotaverine	Nimesulide	Drota	Nime	Drota	Nime
6	15	4.8	12	10.72	26.94	99.25	99.77
6	15	6.0	15	11.88	30.13	99.00	100.42
6	15	7.2	18	13.08	33.21	99.08	100.63
		Mean				99.11	100.27
		SD				0.1276	0.4483
		% RSD				0.1288	0.4471

Precision

Theoretical concentration (µg mL^{-1})	DrotaverineHcl				Nimesulide			
	Intra-day measured concentration		Inter -days measured concentration[a]		Intra -day measured concentration		Inter -days measured concentration[a]	
	Mean[b]	RSD %	Mean[b]	RSD %	Mean[b]	RSD %	Mean[b]	RSD %
8	7.91	1.57	7.78	0.95	21.05	0.389	20.15	0.389
32	31.94	0.39	31.79	0.05	30.98	1.12	30.75	1.57

Table 3 Intra-day and Inter-days precision of DrotaverineHcl and Nimesulide standard

[a] Inter-days reproducibility was determined from five different runs for three consecutive days

[b] Mean values represent five different DrotaverineHcl and Nimesulide standards for each concentration

Limit of Detection and Limit of Quantitation was calculated from calibration curveThe LOD for DRT and NIM were found to be 0.20 µg/ml and 0.25 µg/ml, respectively, while LOQ were 0.50 µg/ml and 0.45 µg/ml, respectively.

The results of system suitability test parameters are summarized in Table 2

Table 2 System suitability test parameters

Parameter	drotavarine	nimesulide
Retention Time (min) ± %RSD	3.22±0.10	8.031±0.20
Tailing Factor ± %RSD	1.19±0.18	1.07±0.14
Theoretical Plates ± %RSD	35911±0.88	40240±0.95
Resolution ± %RSD	1.89522	1.65±0.18

Stress Studies

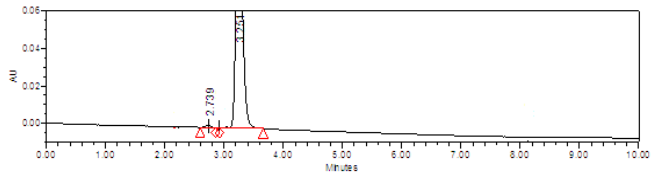

Fig 2 Chromatogram of Acid induced hydrolytic sample of drotaverine hydrochloride

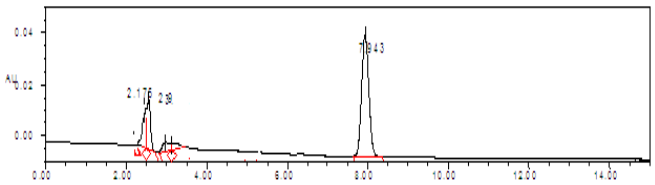

Fig 3 Acid degration of Nimsulide

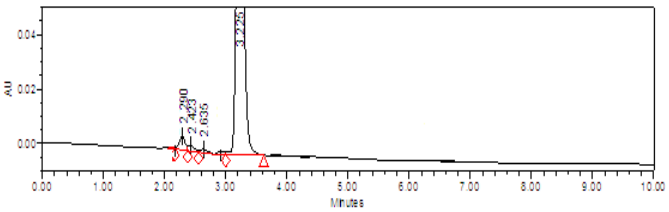

Figure 4 Chromatogram showing Alkali induced degradation of drotavarine

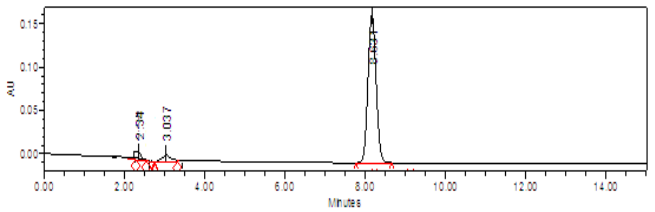

Fig 5 Chromatogram showing Alkali induced degradation of Nimesulide

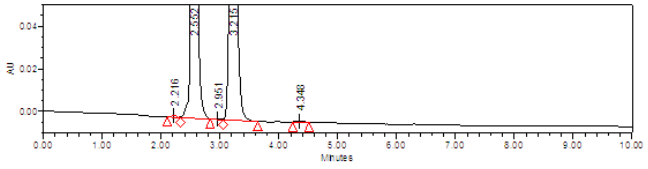

Fig 6 Chromatogram showing oxidation induced degradation of Drotavarine

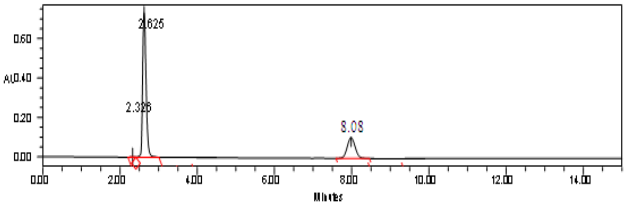

Fig 7 Chromatogram showing oxidation induced degradation of Nimesulide

The degradation study indicated that DRT was susceptible to acid and base hydrolysis and , peroxide induced oxidation while it was stable to dry heat under experimental conditions. In acid, base, hydrolysis, Oxidation the drug degrades as observed by the decreased area in the peak of the drug when compared with peak area of the same concentration of the nondegraded drug, without giving any additional degradation peaks. NIM was found to be susceptible to Acid, Base and peroxide induced degradation however it showed stability towards neutral hydrolysis as well as dry heat degradation.. Percent degradation was calculated by comparing the areas of the degraded peaks in each degradation condition with the corresponding areas of the peaks of both the drugs under non degradation condition.

CONCLUSION

In this reported study, a stability-indicating HPLC method was developed for the simultaneous determination of DRT and NIM and validated as per ICH guidelines. Statistical analysis proved that the method developed was accurate, precise, and repeatable. The developed method was found to be simple, sensitive and selective for analysis of DRT and NIM in combination without any interference from the excipients. The method was successfully used for determination of drugs in a pharmaceutical formulation. Assay results for combined dosage form using proposed method showed 99.42 of DRT and 99.86 of NIM respectively. The results indicated the suitability of the method to study stability of DRT and NIM under various forced degradation conditions viz. acid, base, dry heat, neutral, photolytic and UV degradation. It can be concluded that as the method could separate the drugs from their degradation products; it may be employed for analysis of stability samples of DRT and NIM.

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