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Research Article

SIMULTANEOUS ESTIAMATION OF DROTAVERINE HCL AND NIMESULIDE IN PHARMACEUTICALS BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC METHOD

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Abstract
A simple, rapid, precise and accurate high-performance thin-layer chromatographic (HPTLC) method was developed and validated for simultaneous determination of Drotaverine hydrochloride (DRO) and Nimesulide (NIM) in pharmaceutical preparations. Separation was achieved on a Merck HPTLC plates (0.2 mm thickness) precoated with 60 F_{254} silica gel on aluminum sheet as the stationary phase using cyclohexane: methanol: ethyl acetate (5:2.:3v/v/v), as the mobile phase. Densitometric quantification was performed at λ = 295 nm by reflectance scanning. The R_F values of DRO and NIM were obtained 0.41 and 0.62 respectively. The linearity of proposed method was investigated in the range of 0.1 to 0.6 µg/spot and 0.2 to 0.7 µg/spot for DRO and NIM respectively.. The percentage recoveries for DRO and NIM were 99.91 % and 100.19 % by area and 99.63 % and 99.96 % by height, respectively. The developed method was suitably validated for precision, accuracy, specificity and ruggedness.
Keywords: HPTLC, Drotaverine HCl, Nimesulide and Validation

1. Introduction

Drotaverine HCl chemically is (1-(3, 4-diethoxybenzylidene)-6, 7-diethoxy-1,2,3,4-tetrahydroisoquinoline) HCl, is an isoquinoline derivative. It is a highly potent spasmolytic agent.
Chemically, Nimesulide is 4-Nitro-2-phenoxymethanesulfonanilide [1]. It is a non-steroidal anti-inflammatory drug [2]. It is used for chronic arthritis (such as rheumatoid arthritis and osteoarthritis) surgery and posttraumatic acute pain and inflammation; otorhinolaryngological inflammation resulting in pain; dysmenorrhoea, upper respiratory tract infection symptoms such as fever treatment.
Literature survey revealed that few analytical methods for the determination of DRO such as spectroscopy, HPLC, HPTLC from pharmaceutical preparations. However there are number of methods for the determination of NIM such as spectrophotometry, HPLC in pharmaceutical preparations. No method is reported so far the estimation of both drugs in combined dosage form.
Hence the present manuscript describes a simple, rapid, precise and accurate HPTLC method for the simultaneous determination of DRO and NIM in the same pharmaceutical preparations.

2. Experimental

2.1 Chemicals and Reagents: DRO and NIM were kindly supplied by Aditi Pharmaceuticals PVT. LTD., Solapur and Zim Laboratories LTD., Nagpur and were used without further purification. Tablet was procured from market. .All reagents used, were at least of analytical grade. Double distilled water was used throughout the system.
2.2 Chromatographic Conditions and Instrumentation: Chromatography was performed on 10 cm × 10 cm HPTLC plates coated with 0.2 mm layers of silica gel 60 F_{254} (Merck, Darmstadt, Germany). Before use the plates were washed with AR grade methanol and activated at 115 ^{0}C for 30 min. Samples were applied as bands 4 mm wide and 4 mm apart by use of a Camag Linomat V sample applicator (Muttenz, Switzerland; supplied by Anchrom Technologists, Mumbai) equipped with 100 µL syringe (Hamilton, Nevada, USA). A constant application rate of 6 sec μL^{-1} was used. Initially separate pure drug solutions were run in single solvents to ascertain the movement of each drug in the

respective solvents. Different proportions of cyclohexane: methanol: ethyl acetate were tried while mobile phase selection. Ultimately cyclohexane: methanol: ethyl acetate (5:2.:3v/v/v) was finalized as mobile phase. The spots developed were dense and compact. Linear ascending development was performed in a Camag 15 cm × 15 cm glass twin-trough chamber. Before insertion of the plate into the mobile phase, the chamber was saturated with mobile phase vapor for 20 min at room temperature (28 ± 3 ^{0}C). After saturation of the chamber, the plate was inserted into the mobile phase. The development distance was 70 mm. After development the plates were dried by hot air spray drier. Densitometric scanning was performed with a Camag TLC scanner III in reflectance-absorbance mode at λ = 210 nm controlled by CATS 4 software (Version 1.4.1; Camag) resident in the system. The slit dimensions were 3.00 × 0.45 mm and the scanning speed 20 mm s^{-1}. The radiation source was a deuterium lamp emitting continuous UV radiation between 190 and 360 nm. The amounts of the compounds chromatographed were determined from the intensity of diffusely reflected light.

2.3 Preparation of Stock and Standard Solutions: Stock solution of DRO (equivalent to 400 µg mL^{-1}) and NIM (equivalent to 1000 µg mL^{-1}) was prepared in AR grade methanol. A 2.5 mL standard stock solution of DRO and NIM was transferred into the 10.0 mL volumetric flask and diluted to volume with AR grade methanol, to yield final concentration of 100 µg mL^{-1} and 250 µg mL^{-1} for DRO and NIM respectively.

2.4 Preparation of Sample Solutions for Assay: Twenty tablets were weighed and the average weight was calculated. The tablets were then powdered and an amount equivalent to 20 mg of DRO was dissolved in a 50.0 mL volumetric flask with minimum volume of AR grade methanol. The drugs were extracted from the powder with AR grade methanol. To ensure complete extraction of the drugs the flask was sonicated for 15 min and then shaken for 30 min. The solution was then made up to volume with AR grade methanol. Aliquots of the solution was filtered through a whatman filter paper and 2.5 mL of filtered solution was transferred to a 10.0 mL volumetric flask and diluted to volume with AR grade methanol, to yield final concentration of 100 µg mL^{-1} and 250 µg mL^{-1} for DRO and NIM respectively. This sample solution (6 µL) was applied to a plate in triplicate, with standard solution, and the plate was developed and scanned under the optimized as described above.

3. Results and Discussion

3.1 HPTLC Method Development and Optimization: Normal phase HPTLC on silica gel 60 F_{254} with cyclohexane: methanol: ethyl acetate (5:2.:3v/v/v), as mobile phase enabled good separation of DRO (R_F 0.41) and NIM (R_F 0. 62). The typical HPTLC densitogram is shown in **Figure 1**. The well defined peaks were obtained only when the chamber was saturated with the mobile phase for 10 min at controlled temperature before plate development.

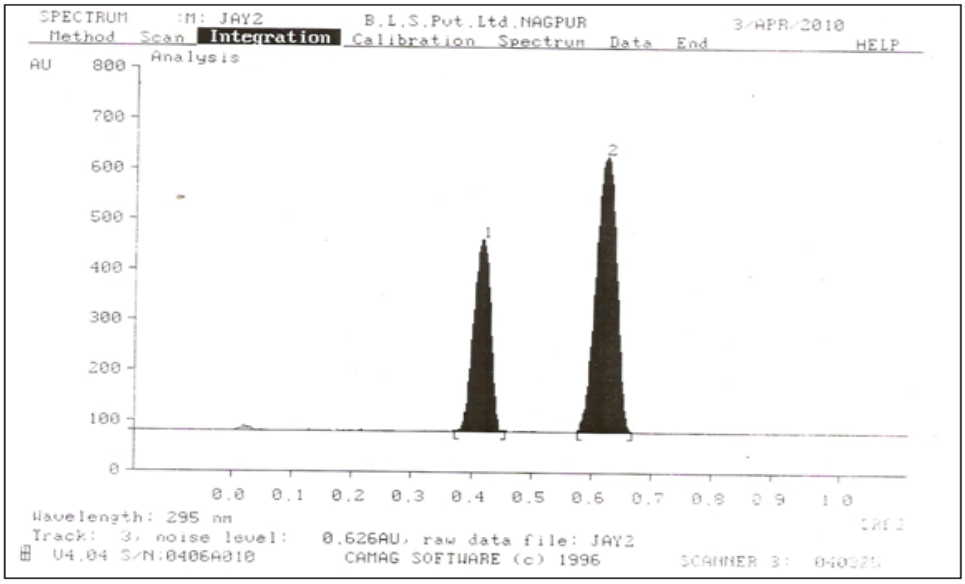

Figure 1: Typical HPTLC Densitogram of Standard Solution

3.2 Validation of the method: As recommended in the ICH guidelines [15] the all validation parameter were performed during the development of the analytical procedure. The proposed method was validated with respect to parameter such as linearity, precision, accuracy, specificity and ruggedness.

3.2.1 Linearity: Linearity was established by least squares linear regression analysis of the calibration curve. The constructed calibration curves were linear over the concentration range of 0.1 to 0.6 µg $spot^{-1}$ and 0.2 to 0.7 µg $spot^{-1}$ for DRO and NIM respectively. Peak height and areas of DRO and NIM were plotted versus their respective concentrations and linear regression analysis performed on the resultant curves.

3.2.2 Precision: The method and intermediate precision data are summarized in **Table 1**. Method precision was investigated by injecting 5 tablet samples (n=5) in triplicate order.

Table 1:- Result of marketed formulations

Sr. No	Amount Taken (g)	Amount of drug in 6µl (ng)		% labeled claim	
		By Height	By Area	By Height	By Area
DRO					
1	0.0201	602.88	598.32	100.48	99.72
2	0.0204	600.54	599.22	100.09	99.87
3	0.0202	594.42	598.20	99.07	99.70
4	0.0201	596.76	597.84	99.46	99.64
5	0.0205	598.68	597.30	99.78	99.55
			Mean	99.77	99.69
			S.D.	0.54	0.01
			% R.S.D.	0.54	0.01
NIM					
1	0.0502	1500.45	1496.1	100.03	99.74
2	0.0501	1480.65	1494	98.71	99.60
3	0.0501	1500.06	1494.75	100.04	99.65
4	0.0503	1491.3	1495.65	99.42	99.71
5	0.0502	1498.5	1493.55	99.90	99.57
			Mean	99.62	99.65
			S.D.	0.56	0.07
			% R.S.D	0.56	0.07

3.2.3 Accuracy: Accuracy data for the assay following the determination of each of the compounds of interest are summarized in **Table 2.** Accuracy of proposed method was ascertained on the basis of recovery studies performed by standard addition method at different levels of labeled claim (i.e. 80 to 120 % of labeled claim) in triplicate order. A known amount of each standard powder was added to samples of tablet powders, which was then mixed, extracted and subsequently diluted to volume with AR grade methanol, to yield the required concentration of both drugs.

Table 2:- Result of recovery study

Sr. No.		Amount of drug added (ng)	Amount of drug recovered (ng)		% drug recovered	
			Area	Height	Area	Height
DRO						
1	80	183	181.5	180	99.18	98.36
2	100	301	302.2	302.4	100.39	100.46
3	120	422	422.8	422.4	100.18	100.09
			Mean		99.91	99.63
			S.D.		0.64	1.12
			%RSD		0.64	1.12
NIM						
1	80	452.5	454.7	453.0	100.48	100.11
2	100	759.6	761.2	759.9	100.21	100.03
3	120	1054	1052.9	1051.5	99.89	99.76
			Mean		100.19	99.96
			S.D.		0.29	0.18
			%RSD		0.28	0.18

3.2.4 Specificity: The specificity studies were carried out by attempting deliberate degradation of the tablet sample with exposure to stress conditions for 24 hrs like acidic hydrolysis 1.0 mL of 0.1 N HCl, alkaline hydrolysis 1.0 mL of 0.1 N NaOH, oxidation 1.0 mL of 3% H_2O_2, heating at 60 ^{0}C and UV.

Sr. No.	Sample	% Labelled claim	
		DRO	NIM
1	Normal	100.10	100.01
2	Acid	32.40	53.93
3	Alkali	18.25	60.75
4	Oxide	58.81	58.81
5	Thermal	33.46	56.49

3.2.5 Ruggedness: The studies were carried out for different parameters i.e. different elapsed times (Intraday and Interday) and different analysts. The results are shown in Table 3.

Table 3:- Result of ruggedness study

Sr. No.	Parameters		Interday		Intraday		Different analyst	
			DRO	NIM	DRO	NIM	DRO	NIM
1	Area	Mean	100.13	99.73	99.87	99.31	99.27	100.23
		S.D.	0.15	0.12	0.09	0.11	0.07	0.13
		% R.S.D.	0.14	0.12	0.09	0.11	0.07	0.13
2	Height	Mean	99.33	99.42	100.04	99.16	98.34	99.43
		S.D.	0.03	0.11	0.08	0.06	0.12	0.07
		% R.S.D.	0.03	0.11	0.08	0.06	0.12	0.07

Conclusion

The proposed method is simple, rapid, accurate and superior to UV and HPLC in terms of time and cost. It was suitably developed and validated for precision, accuracy, specificity and ruggedness. The method can therefore be applied for routine quality control analysis of DRO and NIM in pharmaceutical preparations.

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