



## Stability-indicating HPTLC method for simultaneous estimation of Drotaverine and Nimesulide in pharmaceutical dosage form

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### Abstract

The present work describes a stability-indicating HPTLC method for simultaneous analysis of Drotaverine (DRO) and Nimesulide (NIM) in bulk and pharmaceutical dosage form. Precoated silica gel 60 F<sub>254</sub> plate was used as stationary phase. The separation was carried out using Cyclohexane: Methanol: Ethyl acetate (6: 2: 2 v/v/v) as mobile phase. The densitometric scanning was carried out at 295 nm. The R<sub>f</sub> values was found to be 0.15 for DRO and 0.53 for NIM. The linearity was obtained in the range 100-600ng/band and 200-700ng/band with correlation coefficients ( $r^2 = 0.9945$ ) and ( $r^2 = 0.9957$ ) for DRO and NIM. The method was validated as per ICH guidelines. The drug combination was subjected to forced degradation by acid, alkali, oxidation and dry heat. The degradation products were well resolved from the pure drugs with significantly different R<sub>f</sub> values.

**Key Words:** Drotaverine (DRO); Nimesulide (NIM); HPTLC; Validation; Stability Studies.

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### Introduction

Chemically, Nimesulide is 4-Nitro-2-phenoxyethanesulfonamide [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; dysmenorrhea; upper respiratory tract infection symptoms such as fever treatment [3-5]. Nimesulide alone or in combination with other drugs is reported to be estimated by spectrophotometric method [6-8], HPLC [9-11], TLC [12], HPTLC [13, 14,] GC [15] and capillary chromatographic method [16].

Chemically, Drotaverine is (1-(3,4-diethoxybenzylidene)-6,7-diethoxy-1,2,3,4-tetrahydroisoquinoline) hydrochloride, is an isoquinoline derivative. It is a highly potent spasmolytic agent [17]. Drotaverine alone or in combination with other drugs is reported to be estimated by TLC densitometry [18], HPLC [19, 20] and differential spectrophotometric method [21].

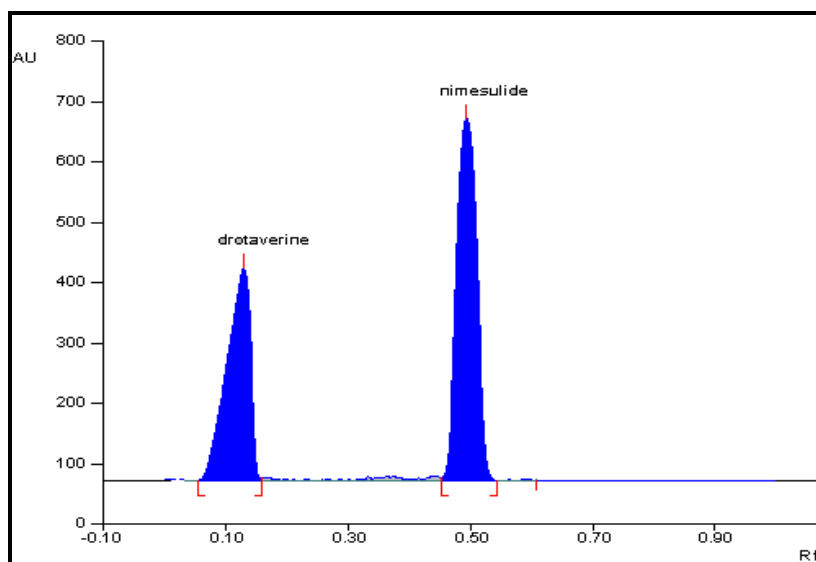
Since no HPTLC method is reported for simultaneous estimation of nimesulide and drotaverine in combination therefore, in the present work, a successful attempt has been made to estimate both these drugs simultaneously.

The present work describes a new method for simultaneous estimation of nimesulide and drotaverine in tablets using HPTLC-densitometry. The method is simple, requires less time for routine analysis of bulk and marketed formulation.

## Results and Discussion

### *Optimization of procedures*

Different proportions of Cyclohexane: Methanol: Ethyl acetate were tried while mobile phase selection. Ultimately Cyclohexane: Methanol: Ethyl acetate (6:2.:2v/v/v) was finalized as mobile phase. The spots developed were dense, compact and typical peak of DRO and NIM were obtained as shown in Fig No.1. Peaks were symmetrical in nature and no tailing was observed when plates were scanned at 295 nm.



**Figure 1: Chromatogram of Drotaverine ( $R_f$  0.15) and Nimesulide ( $R_f$  0.49)**

### *Linearity*

The analytical concentration ranges over which the drugs obeyed Beer Lambert's law was found to be 100-600 ng /band ( $r^2 = 0.9945$ ) and 200-700 ng /band ( $r^2 = 0.9957$ ) for DRO and NIM. The standard calibration data for both the drugs are given in Table No. 1.

**Table 1: Linear regression data for calibration curves**

Parameters	DRO	NIM
Detection Wavelength (nm)	295	295
Beer's Law Limit (ng/band)	100-600	200-700
Regression equation	$465.2x + 4100.3$	$2034.5x + 17356.6$
Correlation Coefficient ( $r^2$ )	0.9945	0.9957
Intercept (c) $\pm$ SD	$4100.3 \pm 17.47$	$16040 \pm 15.27$
Slope (m) $\pm$ SD	$465.2 \pm 1.905$	$2034.53 \pm 56.6$

**Analysis of the marketed formulation**

The spots at  $R_f$  0.15 and 0.53 were observed in the densitogram of the drug samples extracted from tablets. There was no interference from the excipients commonly present in the tablets. The content for both the drugs was found to be close to 100% and the results are summarized in Table No 2. The low %RSD value indicated the suitability of this method for routine analysis.

**Table 2: Results of marketed formulation analysis**

Marketed formulation	Label claim (mg)		Area* of densitogram		Amt. of drug estimated (mg) $\pm$ S.D*		% Mean amount estimated* $\pm$ S.D*	
	DRO	NIM	DRO	NIM	DRO	NIM	DRO	NIM
NOBELSPAS (Mankind Lab Ltd)	40	100	7630.80	17931.57	99.98 $\pm$ 0.41	99.93 $\pm$ 0.42	39.87 $\pm$ 0.22	99.65 $\pm$ 0.48

\*Average of six determination

**Precision**

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise. Results are shown in Table No 3.

**Table 3: Statistical evaluation of precision of developed method (n = 3)**

Parameters	Repeatability*		Precision			
			Intraday*		Interday*	
	DRO	NIM	DRO	NIM	DRO	NIM
<b>Conc.(ng/band)</b>	<b>100</b>	<b>250</b>	<b>100</b>	<b>250</b>	<b>100</b>	<b>250</b>
<b>Mean area <math>\pm</math> SD</b>	7642.69 $\pm$ 27.55	17879.45 $\pm$ 118.58	7658.24 $\pm$ 18.02	17766.62 $\pm$ 14.97	7653.56 $\pm$ 27.87	17736.74 $\pm$ 23.03
<b>% Content <math>\pm</math> SD</b>	100.14 $\pm$ 0.36	99.70 $\pm$ 0.66	99.98 $\pm$ 0.23	99.99 $\pm$ 0.08	99.95 $\pm$ 0.36	100 $\pm$ 0.12
<b>RSD (%)</b>	0.36	0.66	0.24	0.08	0.36	0.13
<b>S.E.</b>	15.92	68.54	10.42	8.65	16.11	13.31

\*Average of three determinations

**Recovery studies**

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 80%, 100% and 120%. Known amounts of standard DRO and NIM were added to pre-analyzed samples and were subjected to the proposed HPTLC method. Results of recovery studies are shown in Table No 4.

**Table 4: Result of recovery studies (n = 3)****A: Recovery studies for DRO**

Level of recovery (%)	Amount taken (ng/band)	Amt of std added (ng/band)	Total amt recovered (ng/band)	% Recovery*	SD	S.E.	% COV
80	100	80	179.40	99.66	0.50	0.29	0.27
100	100	100	199.10	99.55	1.88	1.08	0.94
120	100	120	219.86	99.93	1.26	0.73	0.57

\*Average of three determinations

**B: Recovery studies for NIM**

Level of recovery (%)	Amount taken (ng/band)	Amt of std added (ng/band)	Total amt recovered (ng/band)	% Recovery*	SD	S.E.	% COV
80	250	200	449.75	99.90	0.88	0.50	0.35
100	250	250	499.15	99.83	1.83	1.05	0.37
120	250	300	549.35	99.88	1.90	1.09	0.34

\*Average of three determinations

**Limit of detection and limit of quantitation**

The LOD and LOQ were separately determined based on the calibration curves. The standard deviation of the y- intercepts and slope of the regression lines were used. The system suitability parameters are listed in Table No 5.

**Table No. 5: System suitability parameter**

Parameter	DRO	NIM
Retention time(min.)	0.15	0.53
Limit of detection (ng)	40.26	5.2029
Limit of quantitation (ng)	121.99	15.766

**Robustness**

The robustness of the method was determined by variations in mobile phase composition ( $\pm 2\%$ ), chamber saturation period ( $\pm 10\%$ ), development distance ( $\pm 10\%$ ), time from application to development (0, 10, 20, 30 min), time from development to scanning (0, 10, 20, 30 min). One factor at a time was changed, to study the effect on the peak area of the drugs. The method was found to be unaffected by small changes with % RSD for all the parameters less than 2% indicating that method is robust.

**Stability-indicating property**

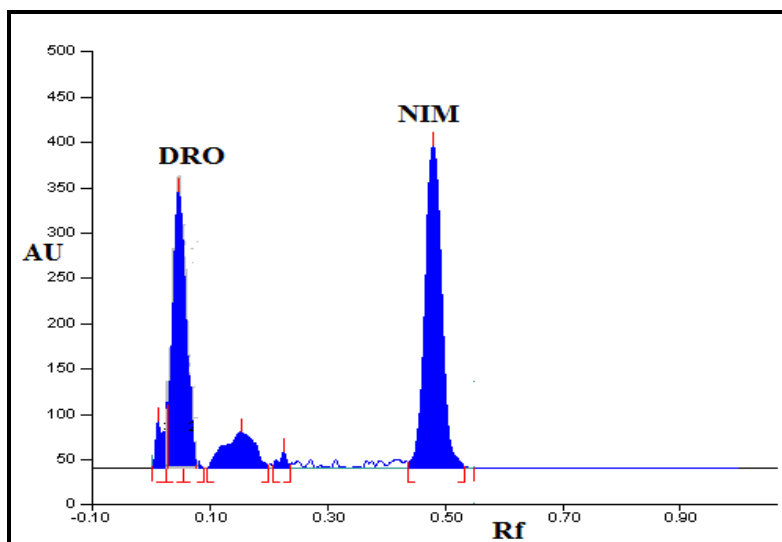
HPTLC studies of the samples obtained during the stress testing of DRO and NIM under different conditions using Cyclohexane: Methanol: Ethyl acetate (6:2:2v/v/v) as the mobile phase shows different degradation peaks as shown in figures 3-6. The amount of drug recovered after degradation studies and the  $R_f$  of degradation products are given in Table No 6.

**Table 6: Results of forced degradation studies**

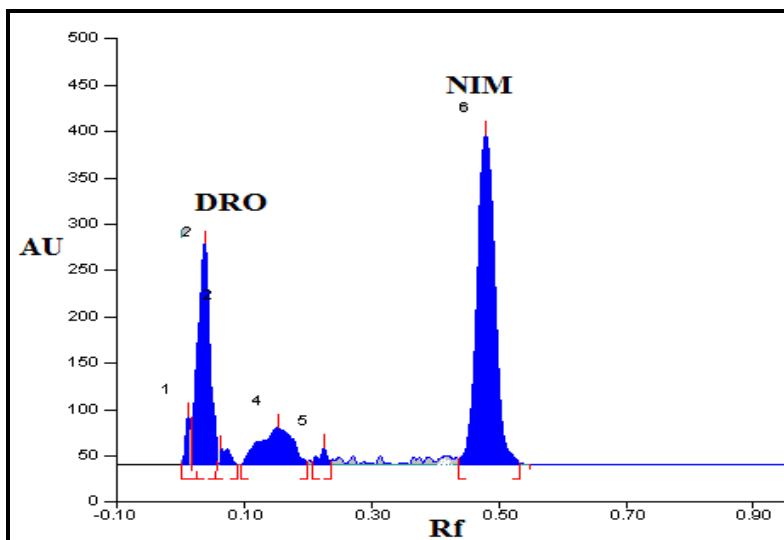
Stress condition	Time (hours)	% Assay of active substance		Mass balance (% assay + % degradation products)	<i>R<sub>f</sub></i> values of degradation products
		DRO	NIM		
Acid hydrolysis (0.1 M HCl)	24	32.40	53.93	100.00	0.02,0.18, 0.23.
Base hydrolysis (0.1 NaOH)	24	18.25	60.75	100.01	0.01, 0.04, 0.15, 0.22.
Oxidation (3% H <sub>2</sub> O <sub>2</sub> )	24	36.52	58.81	100.02	0.01,0.16.
Thermal degradation (60 <sup>0</sup> C)	24	33.46	56.49	100.01	0.01, 0.17, 0.37.

***Acid-induced degradation***

The drug combination was degraded in acidic condition and shows different degradation products at *R<sub>f</sub>* 0.02, 0.18, 0.23 as shown in Fig No.2.

**Figure 2: Chromatogram showing degradation in 0.1N HCL*****Base-induced degradation***

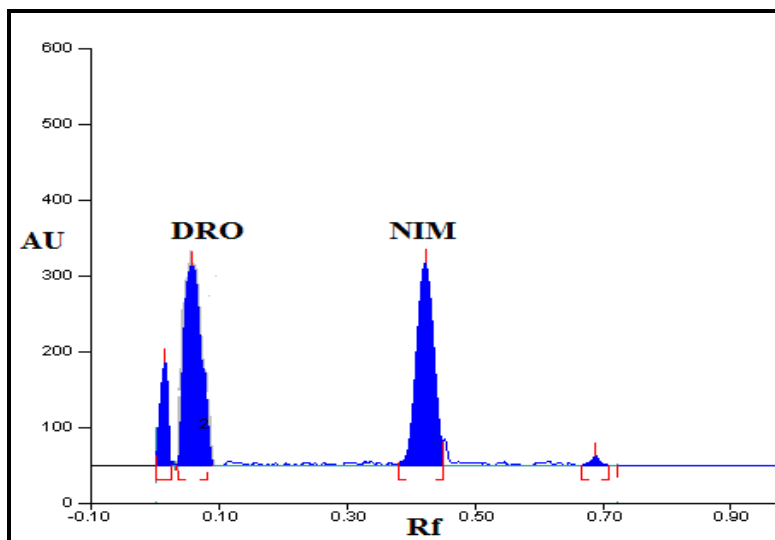
The drug combination was degraded in alkaline condition and shows different degradation products at *R<sub>f</sub>* 0.01, 0.04, 0.15, 0.22. as shown in Fig No.3.



**Figure 3: Chromatogram showing degradation in 0.1 N NaOH**

#### *Hydrogen peroxide-induced degradation*

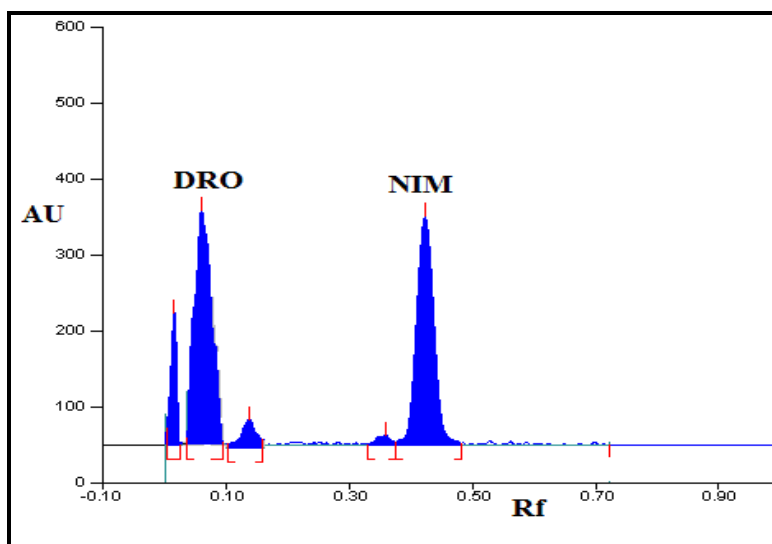
The drug combination was degraded in hydrogen peroxide (3%) at room temperature and shows different degradation products at R<sub>f</sub> 0.01, 0.16 as shown in Fig No.4.



**Figure 4: Chromatogram showing degradation in 3% H<sub>2</sub>O<sub>2</sub>**

#### *Heat degradation*

The drug combination when subjected to heat was degraded and degradation products appeared at R<sub>f</sub> 0.01, 0.17, 0.37 as shown in Fig No.5.



**Figure 5: Chromatogram showing thermal degradation**

## Materials and Methods

### *Materials*

Pure drug samples of nimesulide and drotaverine were supplied as a gift sample by Emcure Ltd. All chemicals and reagents used were of HPLC/AR grade.

### *Instrumentation and chromatographic conditions*

The standard solution ranging from 100-600ng/band and 200-700 ng/band for DRO and NIM were applied on precoated silica gel 60 F<sub>254</sub> plate in the form of bands with 100  $\mu$ l sample syringe using automatic sample applicator LINOMAT V. It was developed in a twin trough glass chamber which was already saturated for 30 min. with the mobile phase. The mobile phase consisted of Cyclohexane: Methanol: Ethyl acetate (6:2:2v/v/v). After development, plate was immediately dried with the help of dryer and was observed under UV chamber. The well resolved bands of drugs were scanned at 295 nm with Camag TLC scanner III densitometer controlled by WINCAT's software version 4.

### *Standard solutions and calibration graphs*

Stock solution was prepared by dissolving 40mg of DRO and 100 mg of NIM in 100 ml methanol, from which further dilution was done with methanol to get stock solution of 10ng/ $\mu$ l. The standard solutions were applied to reach a concentration range of 100-600ng/band and 200-700ng/band for DRO and NIM. The plate was developed on previously described mobile phase and well resolved band of drug were scanned at 295 nm with scanner. The peak areas were plotted against the corresponding concentrations to obtain the calibration curve.

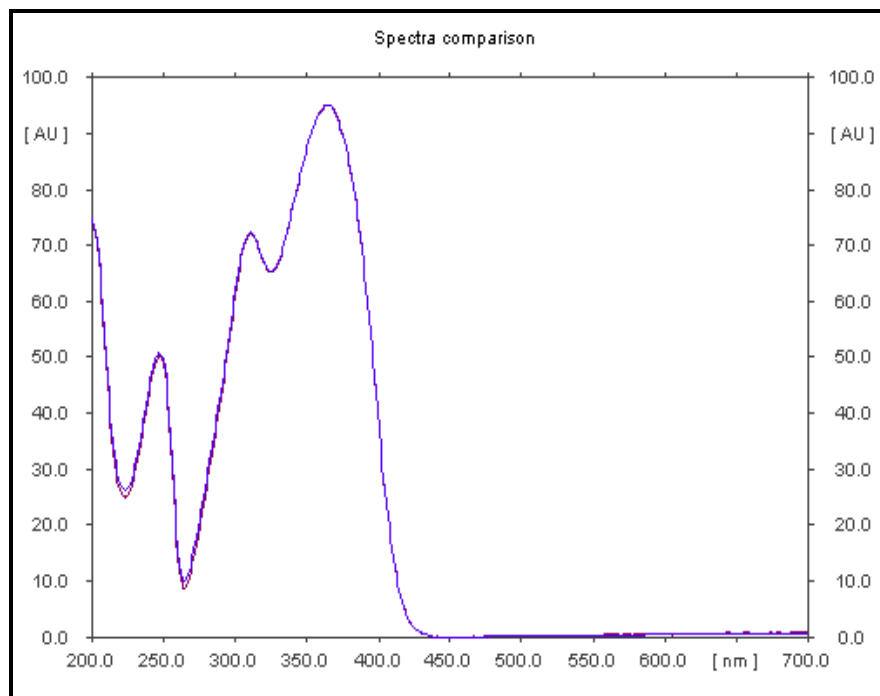
### *Method validation*

The method was validated in compliance with ICH guidelines [22].

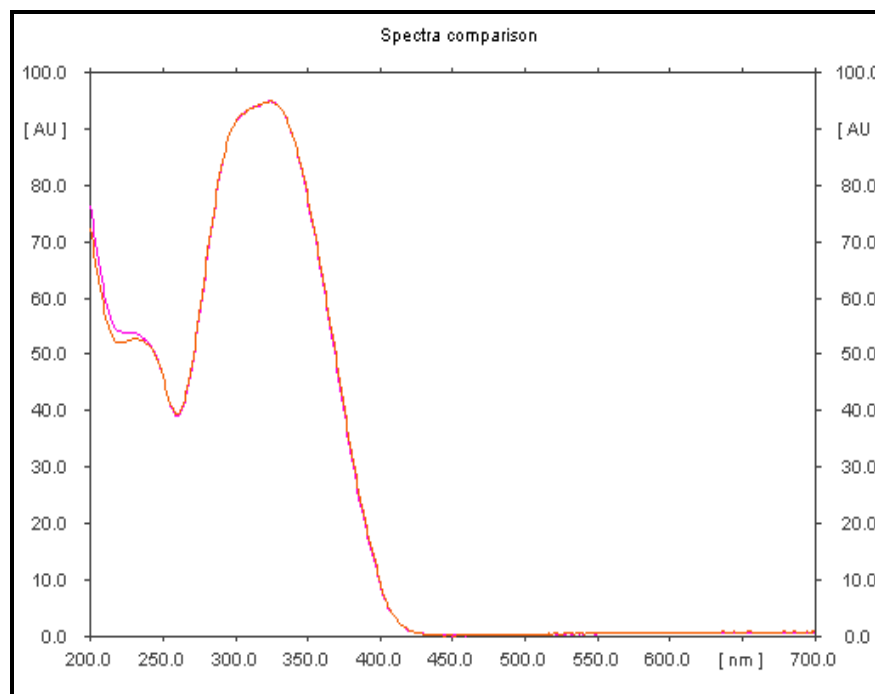
### *Specificity*

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for DRO and NIM in sample were confirmed by comparing the R<sub>f</sub> and spectra of the spots with that of standard. The peak purity for DRO and NIM were assessed by comparing the spectra at

three different levels, i.e., peak start (*S*), peak apex (*M*) and peak end (*E*) positions of the spot. The spectrum for DRO & NIM are shown in Fig No.6 & 7.



**Figure 6: Spectrum of DRO standard and sample measured from 200 to 400 nm**



**Figure 7: Spectrum of NIM standard and sample measured from 200 to 400 nm**

## Conclusion

The proposed HPTLC method was validated as per ICH guidelines. The standard deviation, %RSD and standard error calculated for the method are low, indicating high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy of the proposed methods. The results of the stress studies indicated the specificity of the method. Hence, it can be concluded that the developed HPTLC method is accurate, precise, selective and can be employed successfully for the simultaneous estimation of Drotaverine and Nimesulide in tablet formulation.

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