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DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING HPTLC METHOD FOR THE ESTIMATION OF DROTAVERINE HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORM

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Keywords:

HPTLC, Drotaverine, Stability-indicating, Estimation, Tablet, Validation

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ABSTRACT: Drotaverine (DRO) is an anti-spasmodic medication inhibiting phosphodiesterase-4, an enzyme that breaks down cyclic adenosine monophosphate (cAMP). PDE_4 inhibition causes an increase in cAMP, which causes smooth muscle relaxation. For DRO, especially the HPTLC technique and stability indicating studies through HPTLC method have not been reported properly in any database. The current research focuses only on a significantly improved HPTLC-based exact, accurate, and specific approach for DRO identification in the presence of degradation constituents employing densitometric detection.DRO was spotted in 6 mm bandwidths with a Camag 100L sample syringe (Hamilton, Switzerland) on a silica gel G60 F_{254} pre-coated HPTLC aluminium plate with a thickness of 250 µm using a Camag® Linomat-IV applicator. The application rate was 0.4 L/s, with a 6 mm separation between the two bands. Linear ascending development was carried out in a 20 cm × 10 cm twin trough glass chamber, which was then saturated with the mobile phase (Toluene: Methanol; 7:3 v/v).The method was found to be linear over the 200-1200 ng/band range. The stability-indicating method demonstrated desired accuracy, higher precision, better robustness, significantly good LOD and LOQ values, and optimized systems suitability parameters.DRO showed degradation in acidic, alkaline, thermal and oxidative conditions. However, the extent of degradation was different. The method for quantifying DRO has been developed to be quick, precise, accurate, and selective and can be utilized to determine bulk medicines and pharmaceutical dosage forms quickly and easily.

INTRODUCTION: Drotaverine (DRO) **Fig. 1** is an anti-spasmodic medication that acts by inhibiting phosphodiesterase-4 (PDE_4), an enzyme that breaks down cyclic adenosine monophosphate (cAMP). PDE_4 inhibition causes an increase in cAMP, which causes smooth muscle relaxation [1].

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It is a benzylisoquinoline derivative that's chemically identical to papaverine; however, it has stronger antispasmodic properties than papaverine. DRO was used to manage gastrointestinal illnesses, biliary dyskinesia, and vasomotor diseases linked with smooth muscle spasms, among other spastic ailments.

It is also being studied for dysmenorrhea, abortion, and labour augmentation. DRO has become popular in treating benign prostatic hyperplasia, para-influenza and avian influenza viruses in recent years [2]. The FDA, the European Medicines Agency and Health Canada have all rejected DRO.

It is legal to use it as oral pills or intramuscular injections in Thailand [3]. *In-vitro*, DRO acts like voltage-dependent L-type calcium channel blockers, suggesting that it may have minimal allosteric calcium channel blocking characteristics. DRO is used to treat cholecystitis, gallbladder diseases, and gastrointestinal and genitourinary smooth muscle spasms. It improves cervical dilatation and relieves visceral spasms [4]. In recent studies, low levels of cAMP have been linked to brain carcinogenesis, prompting the development of PDE_4 inhibitors as possible anticancer treatments. DRO inhibited the growth of numerous human tumour cell lines and non-malignant murine fibroblasts *in-vitro* [5].

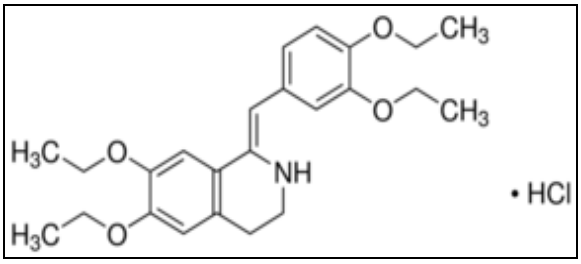


FIG. 1: STRUCTURE OF DROTAVERINE HYDROCHLORIDE

All of the foregoing, especially the HPTLC technique, where Rf values were found to be extremely variable and no specialized degradation has yet been evaluated under moisture, sunlight, oxidative stress, acidic environment, and alkaline settings. The current research focuses only on a significantly improved HPTLC-based exact, accurate and specific approach for DRO identification in the presence of degradation constituents employing densitometric detection.

MATERIALS AND METHODS:

Chemicals: DRO of pharmaceutical quality (100.85% w/w pure) was acquired as a gracious gift sample from Yarrow Pharma Chem Pvt. Ltd., Mumbai, Maharashtra, India. Merck Specialties Pvt. Ltd., Mumbai, Maharashtra, India, provided analytical quality reagents, solvents and chemicals.

Instrumentation: Methanol was used to pre-wash HPTLC plates and was activated at 110°C±1°C temperature for 5 min before the chromatographic procedure. The DRO was spotted in 6 mm bandwidths with a Camag 100L sample syringe (Hamilton, Switzerland) on a silica gel G60 F_{254} pre-coated HPTLC aluminium plate with a thickness of 250 µm [E Merck® KG, Germany, provided by Anchrom Enterprises India Pvt. Ltd., Mumbai] using a Camag® Linomat-IV applicator [E Merck® KG, Germany, provided (Switzerland). The application rate was 0.4 L/s, with a 6 mm separation between the two bands. Linear ascending development was carried out in a 20 cm × 10 cm twin trough glass chamber (Camag®, Switzerland), which was then saturated with the mobile phase (Toluene: Methanol; 7:3 v/v). Using the saturation pads, the mobile phase in the chamber needed 20 minutes of saturation at ambient temperature (25°C±2°C). The chromatogram run length was 8 cm. For densitometric scanning in the reflectance absorbance mode, a Camag® TLC Scanner-III with Camag® vision CATS software was used with a slit size of 5 mm 0.45 mm and a scanning speed of 10 mm/s. A deuterium lamp was used to apply UV rays ranging from 200 nm to 400 nm. The DRO was calculated using the intensity of diffused light, and the peak regions were evaluated using linear regression. A Shimadzu® AUW220D balance (Kyoto, Japan) was used to weigh. The pH was measured using a VSI® VSI-1B digital pH metre (Mohali, India). A Transonic Digital S sonicator (Mumbai, India) was utilized for sonication.

Preparation of Standard Stock Solution: Accurately weighed quantity (10 mg) of DRO was transferred to 10mL volumetric flask and the volume was made with methanol to produce a concentration of 1000µg/mL.

Preparation of Sample Solution: 20 tablets were weighed and crushed to obtain fine powder and the average weight of tablets was calculated. Tablet powder equivalent to 10mg of DRO was accurately weighed and transferred to the 10mL volumetric flask. The above content was dissolved in 5mL methanol by properly sonicating for 10min duration. Further volume was made up to the mark with methanol. The solution was filtered through membrane filter of 0.45 µm pore size.

Selection of Mobile Phase: Aliquot portions of standard stock solution 0.4µL, was applied on TLC plates in the form of band (band size: 6 mm) and the plates were run in different solvent systems. In order to achieve the desired Rf value range with a compact band, several trials were conducted using

different solvent systems containing non-polar and relatively polar solvents at various concentration levels [8:2 v/v (Trial 1) and 7:3 v/v (Trial 2)] to determine the best conditions for effective DRO separation.

Preparation of Diluent: In this study, methanol: toluene (50: 50 v/v) was produced and utilized as a diluent.

Selection of Analytical Wavelength for Densitometric Evaluation: Using a CAMAG LINOMAT-V automated sample applicator, a standard stock solution of 0.4 g/mL was applied to a TLC plate and then chromatographed for 10 minutes in a twin-through glass chamber saturated with the mobile phase. The plate was removed and air-dried after chromatographic development. The bands on the TLC plate were scanned in the 200 nm to 400 nm wavelength range.

Analysis of Marketed Formulation: Two bands of standard stock solution and four bands of sample solution, each 0.4 L, were applied to the TLC plate, and the plate was developed and scanned under optimal chromatographic conditions. After scanning, the peak obtained for standard and sample bands were integrated. The amount of DRO (mg/tablet) was calculated by comparing mean peak area of sample bands with that of standard bands using the following equation:

Content of drug in sample = PA_{Spl} / PA_{Std} × Weight of Standard (mg) / d_{f1} × d_{f2} / Weight of powder taken (mg) × A

Where, PA_{Spl} (Peak area of sample), PA_{Std} (Peak area of standard), d_{f1} (Dilution factor for standard), d_{f2} (Dilution factor for sample) and A (Average weight of tablet in mg).

Analysis of Standard Laboratory Mixture: Accurately weighed 10 mg of DRO were transferred to 10 mL volumetric flask, volume was then made up to the mark with methanol. On the TLC plate, six bands of sample solution, 0.4 µL each, were applied and the plate was developed. The content was scanned under the optimum chromatographic condition and the densitogram was recorded. Measured mean peak area, standard deviation (SD) and % RSD were expressed.

Method Validation: The developed HPTLC method was properly validated in agreement with the Q2A guideline and Q2B guideline of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH), in assent with the United States Pharmacopoeia (USP), and with the guidance of the United States Food and Drugs Administration (USFDA).

Linearity and Range: DRO (10 mg) was dissolved in methanol after being put in a volumetric flask (10 mL capacity). The solvent was poured to the mark, sonicated for 5 minutes, then filtered again to reach a concentration of 1000 µg/mL. Then, in a similar manner, a concentration of 100 µg/mL was created. Furthermore, dilutions in the 10-100 ng/mL range were prepared by diluting the standard solution by 1 mL to 10 mL. The chromatography was carried out using a band of 10 µL content applied to the HPTLC plate. The calibration curve was produced using the average peak area versus concentration. The regression equation was created afterwards [6].

Accuracy: The accuracy (or recovery parameter) of this HPTLC method was assessed by using a standard drug solution at concentrations of 80 percent, 100 percent, and 120 percent of the target concentration (standard addition method). Based on the precise concentrations, the procedure was completed in triplicate, and the obtained mean results were articulated in the form of a percent recovery confidence interval with calculated percent relative error [7].

Precision: The precision (variability) of this HPTLC method was determined by applying the standard drug solution at concentrations of 50 percent of the target concentration, 100 percent of the target concentration, and 120 percent of the target concentration three times in a single day (intra-day variability), and three times on three different days (standard addition method) (inter-day variability). The gathered relative standard deviation (RSD) values were used to determine the degree of accuracy [8].

Robustness: The system suitability parameters, such as mobile phase composition (by ±0.1 mL) and chamber saturation time (by ±5 min), were altered with all other features (factors) being constant and uniform. The new method's capacity

to identify drug content even after modifying essential parameters was tested [9].

Systems Suitability Parameters: By applying the standard solution five times and evaluating metrics such as peak area, retention duration, tailing factor, and theoretical plates, the reproducibility characteristics of the new HPTLC technique were fully determined. The concentrations (400 ng/band) were investigated in triplicate, with the effects on the peak area and Rf value reported [10].

Limit of Detection: The limit of detection (LOD) is the least concentration that can be detected by this HPTLC method without needing to be quantified to an exact number [11]. LOD was estimated from formula:

$$LOD = 3.3 (\sigma/S)$$

This signifies the calibration curve slope determined from the analyte calibration curve and σ referred to standard deviation of response.

Limit of Quantification: The limit of quantification (LOQ) is the lowest concentration that can be reliably determined with high precision and accuracy [12]. LOQ was estimated from formula:

$$LOQ = 10 (\sigma/S)$$

Where, S signifies the calibration curve slope determined from the analyte calibration curve and σ referred to standard deviation of response.

Forced Degradation Studies:

Alkali Degradation Studies: In a volumetric flask (100 mL), 10 mg equivalent of DRO was placed and diluent (methanol) was poured to precisely half the capacity, sonication was performed for 15 minutes, and the volume was then filled to the appropriate level. The aforementioned ingredients were thoroughly combined using a magnetic stirrer for 30 minutes, followed by 5 minutes of centrifugation at 3,000 rpm. 5 mL of the aforesaid content was placed in a volumetric flask, to which 10 mL of 0.1 N NaOH solution was added, and the mixture was heated at 80°C ± 1°C for 8 hours in the dark. The content was neutralised with the same amount of HCl and the mobile phase was used to reach the necessary volume. With a pore size of 0.45 µm nylon membrane, the final content was filtered. 4 bands of test solution and 2 bands of reference drug solution of 0.4 µL each were put to the HPTLC plate, the plate was developed, and the plate was scanned under optimal chromatographic conditions. After scanning, the bands' peaks were added together [13].

Acid Degradation Studies: In a volumetric flask (100 mL), 10 mg equivalent of DRO was placed and diluent (methanol) was added to precisely half the capacity; sonication was performed for 15 minutes, and the required volume was obtained. The contents were agitated for 30 minutes using a magnetic stirrer, followed by 5 minutes of centrifugation at 3,000 rpm. 5 mL of the aforesaid content was placed in a volumetric flask with 10 mL of 0.1 N HCl solution was added. The mixture was heated at 80°C ± 1°C for 8 hours in the dark. The content was neutralized using equal NaOH, and the mobile phase was used to produce the necessary volume. The final content was filtered with a pore size of 0.45 µm nylon membrane. 4 bands of test solution and 2 bands of reference drug solution of 0.4 µL each were put to the HPTLC plate, the plate was developed, and the plate was scanned under optimal chromatographic conditions. After scanning, the bands' peaks were added together [14].

Neutral Degradation Studies: In a volumetric flask (100 mL), 10 mg equivalent of DRO was placed and diluent (methanol) was added to precisely half the capacity; sonication was performed for 15 minutes, and the required volume was obtained. The contents were refluxed for 6 hours before being centrifuged for 5 minutes at 3,000 rpm. The final content was filtered with a pore size of 0.45 µm nylon membrane. 4 bands of test solution and 2 bands of reference drug solution of 0.4 µL each were put to the HPTLC plate, the plate was developed, and the plate was scanned under optimal chromatographic conditions. After scanning, the bands' peaks were added together [15].

Oxidation Degradation Studies: DRO's oxidative stress was evaluated by dissolving 10 mg equivalent weight in H_2O_2 (3 percent v/v) (10 mL volume) in a volumetric flask and boiling for 1 hour. To begin the decomposition process, the aforementioned substance was held at room temperature. The material was then appropriately diluted, sonicated and the necessary volume was

reached. The final content was filtered with a pore size of 0.45 µm nylon membrane. 4 bands of test solution and 2 bands of reference drug solution of 0.4 µL each were put to the HPTLC plate, the plate was developed, and the plate was scanned under optimal chromatographic conditions. After scanning, the bands' peaks were added together [16].

Photostability Studies: In photostability investigations, the compound absorbs photons, which causes the absorption bands to overlap, causing the valence electron to attain the excited state. For a single day, 10 mg equivalent of DRO was placed in a petridish and exposed to sunlight for 12 hours. The exposed material was then put in a volumetric flask (100 mL), the mobile phase was added, and 15 minutes of sonication was done to attain the necessary volume. The final content was filtered with a pore size of 0.45 µm nylon membrane. The degradation process was started using 254 nm wavelength radiations, and the principal degradation products were identified using HPTLC. 4 bands of test solution and 2 bands of reference drug solution of 0.4 µL each were put to the HPTLC plate, the plate was developed, and the plate was scanned under optimal chromatographic conditions. After scanning, the bands' peaks were added together [17].

Thermal Studies: In a 100 mL volumetric flask, 10 mg of the corresponding quantity of medication combination was placed and heated to 80±1°C for 1 hour. The content was diluted with diluent and swirled for 30 minutes before being sonicated and increased to a volume of 100 mL. The contents were centrifuged at 3000 rpm for 5 minutes and with a pore size of 0.45 µm nylon membrane, the final content was filtered. 4 bands of test solution and 2 bands of reference drug solution of 0.4 µL each were put to the HPTLC plate, the plate was developed, and the plate was scanned under optimal chromatographic conditions. After scanning, the bands' peaks were added together [18].

RESULT AND DISCUSSION:

Optimisation of Mobile Phase: In the case of mobile phase composition Toluene: Methanol (8:2 v/v), the peak was not sharp, and the Rf value was not optimized. In contrast, the mobile phase composition Toluene: Methanol (7:3 v/v) demonstrated a sharp peak and optimized Rf value **Fig. 2**.

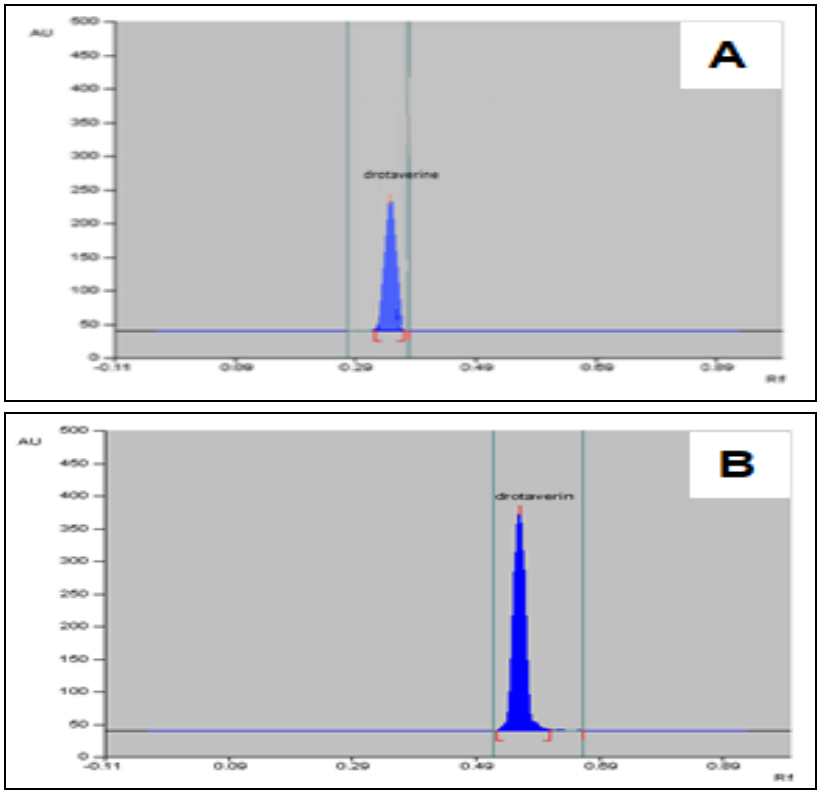


FIG. 2: TRIALS FOR MOBILE PHASE: (A) TOLUENE: METHANOL (8:2 V/V) AND (B) TOLUENE: METHANOL (7:3 V/V)

Detection of Wavelength: The λmax was found to be 241 nm, along with two other peaks at 303 nm, and 359 nm, respectively **Fig. 3**. The UV-Vis spectra of standard drotaverine hydrochloride closely matched with the marketed preparation.

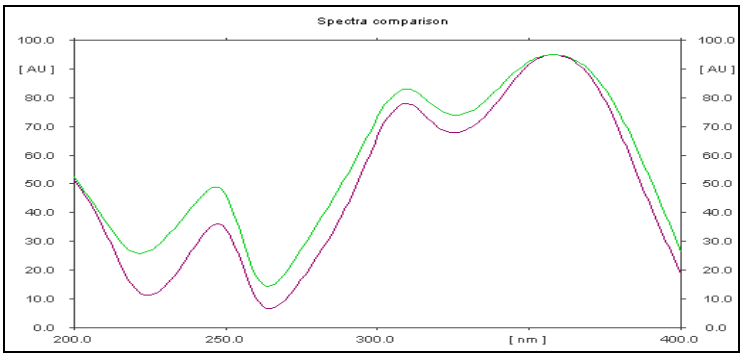


FIG. 3: UV-VIS SPECTRA OF STANDARD DROTAVERINE HYDROCHLORIDE (MAGENTA) AND MARKETED PREPARATION (GREEN)

Chromatographic Parameters: The optimum mobile phase was toluene: methanol (7:3 v/v) which resolved the degraded elements from the standard peak. In the existence of degradation components, the method pleasingly resolute the drug material with R_f value of 0.61 **Fig. 4**. The detection UV was selected at 241 nm as DRO was appreciably absorbed at this wavelength. As the developed analytical HPTLC method completely separated the API from the other excipients, it was considered quite specific.

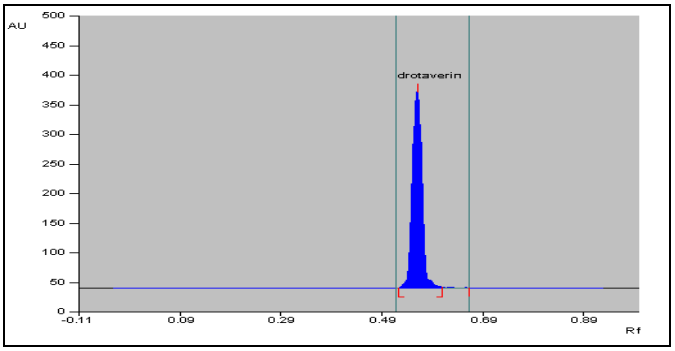


FIG. 4: REPRESENTATIVE DENSITOGRAM OF STANDARD SOLUTION OF DROTAVERINE HYDROCHLORIDE

Method Validation:

Linearity: High linearity was detected between the concentration and peak area throughout the 200-1200 ng/band range for DRO. The linear regression equation was found to be *y = 10.21x + 890.4* with a regression coefficient value of 0.994, indicating a high degree of linearity (**Fig. 5**.

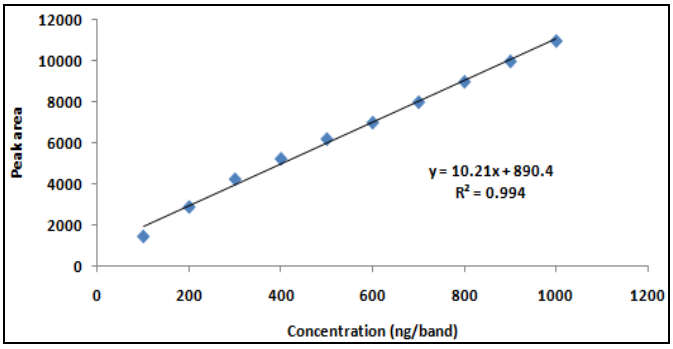


FIG. 5: CALIBRATION CURVE FOR DROTAVERINE HYDROCHLORIDE

Accuracy: The Y-intercept and slope of the graph played a critical part in determining the % recovery characteristic of the suggested technique for simultaneous estimation by using the calibration curve. The established % RSD values for DRO was found to be 0.404, 1.496 and 0.311 at 80%, 100%, and 120% levels. All of these were under the US Pharmacopeia acceptability standard of <2% **Table 1**. Overall, the technique indicated that the recovered data was accurate.

TABLE 1: PERCENTAGE RECOVERY STUDY

% Level	Conc. ng/band	Area of spiked sample	Area of Sample	Area of Std.	% Recovery	S.D.	% R.S.D.
80	720	9320	4670	4650	100.4	0.405	0.404
		9345	4665	4680	99.67		
		9302	4659	4643	100.34		
100	800	10326	5210	5116	101	1.505	1.496
		10328	5214	5114	101.95		

		10439	5200	5239	99		
120	880	11490	5752	5738	100.24	0.3121	0.311
		11509	5764	5745	100.33		
		11464	5725	5739	99.75		

Precision: The technique was shown to be very accurate throughout the tested ranges for DRO in intra-day study and inter-day variability study for precision data. In all instances, the peak area of the sample solution matched that of the standard solution, with a % RSD of less than 2%. The % RSDs were observed to be in the range of 0.164-0.499 for intra-day studies and 0.181-0.736 for inter-day studies, which indicates high precision and minimal variation **Table 2**.

TABLE 2: PRECISION DATA

Parameter	Conc. (ng/band)	Peak area	Mean peak area	S.D.	% R.S.D.
Intraday Precision	100	1243, 1245, 1238	1242	3.6055	0.290
	500	6120, 6100, 6108	6109	10.066	0.164
	1000	10811, 10890, 10915	10872	54.286	0.499
Interday Precision	100	1420, 1432, 1440	1431	10.535	0.736
	500	6250, 6271, 6268	6263	11.357	0.181
	1000	11098, 11101, 11045	11082	32.14032	0.290

Robustness: A significant shift in the chromatogram for DRO was found with the deliberate modification of one of the most important chromatographic parameters; chamber saturation time and mobile phase composition. When the mobile phase composition and chamber saturation time were increased by 0.1 level as well as decreased by 0.1 level, a marginal change (<5%) in peak area, retention time, and tailing factor was detected in **Table 3**. The recovery was slightly increased in +0.1 level, whereas it decreased slightly in -0.1 level under both conditions. The intentional modifications in the parameters of the proposed method concluded that the method had resilient properties.

TABLE 3: ROBUSTNESS STUDY

Change in Mobile Phase Composition						
Mobile phase composition	**Level**	**Mean Peak Area**	**R_f value**	**% Recovery**	**S.D.**	**% R.S.D.**
7.1: 2.9	+ 0.1	5194	0.63	99.02	0.076	0.077
7: 3	0	5182	0.58	98.80	1.0666	1.0795
6.9: 3.1	- 0.1	5058	0.62	96.54	0.8031	0.8318
Change in Chamber Saturation Time						
Duration for chamber saturation	**Level**	**Mean Peak Area**	**R_f value**	**% Recovery**	**S.D.**	**% R.S.D.**
5 min	- 5	4063.80	0.45	99.23	0.087	0.088
10 min	0	4716.75	0.49	98.91	1.176	1.081
15 min	+ 5	4865.40	0.47	97.34	0.914	0.942

Systems Suitability Parameters: The proposed method's system suitability characteristics showed high repeatability and may be used for regular medication combination analysis. The proposed technique for DRO demonstrated an average retention factor (Rf) of 0.60, a mean peak area of 5173.833 and a % RSD value of 0.924. **Table 4**. % RSD value of<2% is an imperative pharmacopeia parameter. A tailing value of less than 2% showed no specific tailing in any case. Both symmetric and asymmetric components are similar in an ideal Gaussian peak with excellent peak symmetry (asymmetric factor = 1). The notion that the suggested method met the minimum standards of US Pharmacopoeia monograph (minimum theoretical plates of 2000 and tailing factor of less than 2%), implies that it has good resolution, isolation, column efficacy, and reproducibility. The separation factor (α) and resolution factor (Rs) were significantly elevated than the ICH limits and necessary guidelines of 1 and 1.5, respectively, denoting that the recommended analysis method generates larger isolation of both peaks with much less tailing and greater resolution. The method may be utilized for regular analysis because of its high precision, reproducibility, and accuracy.

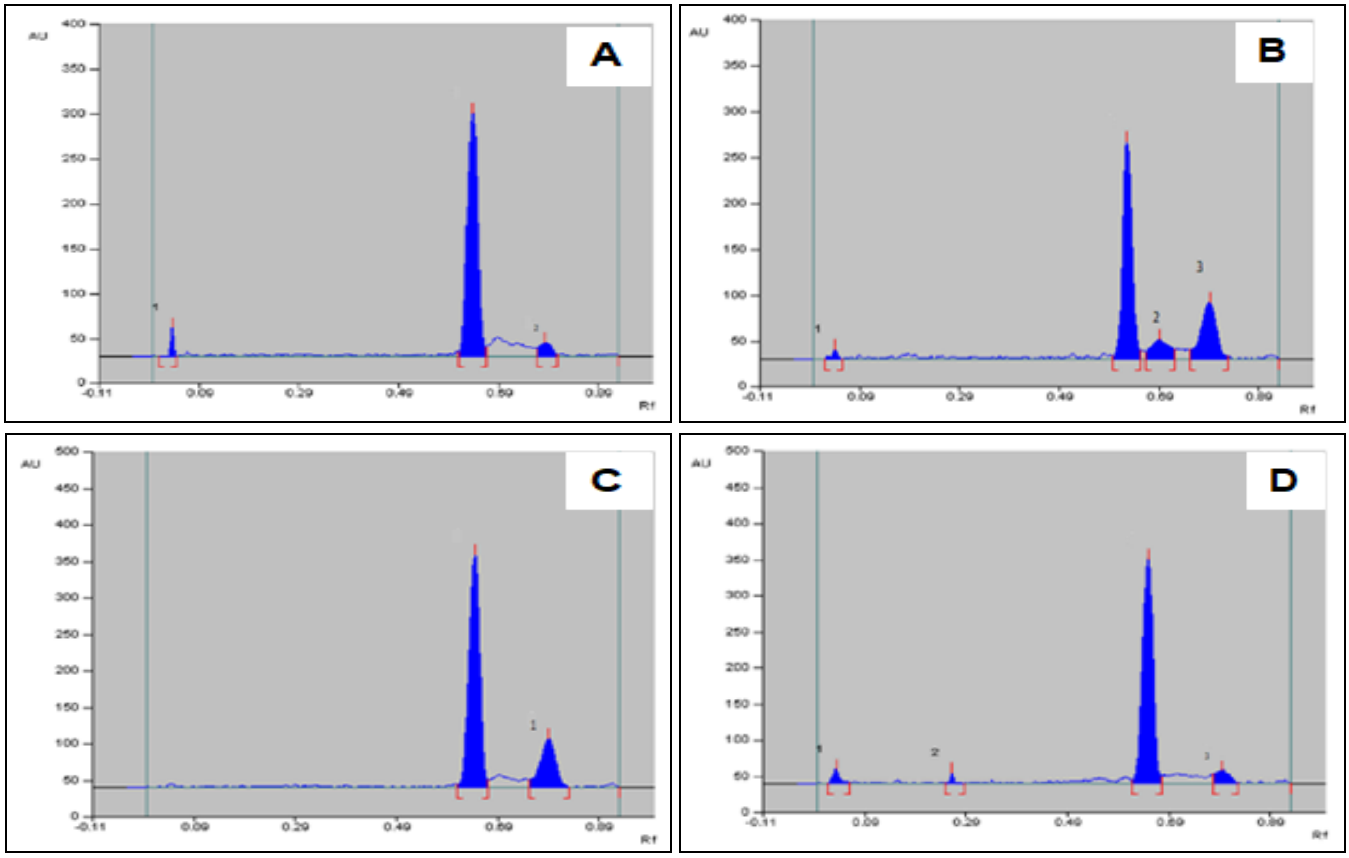
TABLE 4: SYSTEM SUITABILITY PARAMETERS

S. no.	Concentration of std. drug solution (ng/band)	Peak area	Rf value
1	400	5110	0.60
2	400	5184	0.61
3	400	5202	0.62
4	400	5120	0.60
5	400	5199	0.60
6	400	5228	0.62
	Mean	5173.833	0.60
	SD		47.82642
	%RSD		0.92439

Limit of Detection and Quantification: The LOD and LOQ for DRO were observed to be 0.000602 ng/band and 0.00182 ng/band, respectively, which indicate the method's tremendous detection ability for the lowest possible concentration of the solute in formulation (either singly or in combination).

Forced Degradation Studies: In acidic degradation, two degradants were found at Rf 0.04 and 0.78. The % recovery and % degradation was found to be 90.65% and 9.35% **Table 5**, respectively. In alkaline degradation, 3 degradants were observed at Rf values of 0.04, 0.69, and 0.79 with % recovery and % degradation of 85.85% and 14.2%, respectively. In neutral degradation, only one degradant was view in densitogram at Rf value of 0.78. The % recovery and % degradation was observed to be 87.90% and 12.1%, respectively. In oxidative degradation, 3 degradants was detected at Rf values of 0.03, 0.26, and 0.79. The % recovery and % degradation was detected to be 86.20% and 13.8%, respectively.

In thermal degradation, 3 degradants were predominantly seen at Rf values of 0.01, 0.73 and 0.76. The % recovery and % degradation was found to be 85.83% and 14.17%, respectively. In photolytic degradation, 3 degradants were scrutinized at Rf values of 0.01, 0.03, and 0.73. The % recovery and % degradation were found to be 89.72% and 10.28%, respectively **Fig. 6**.



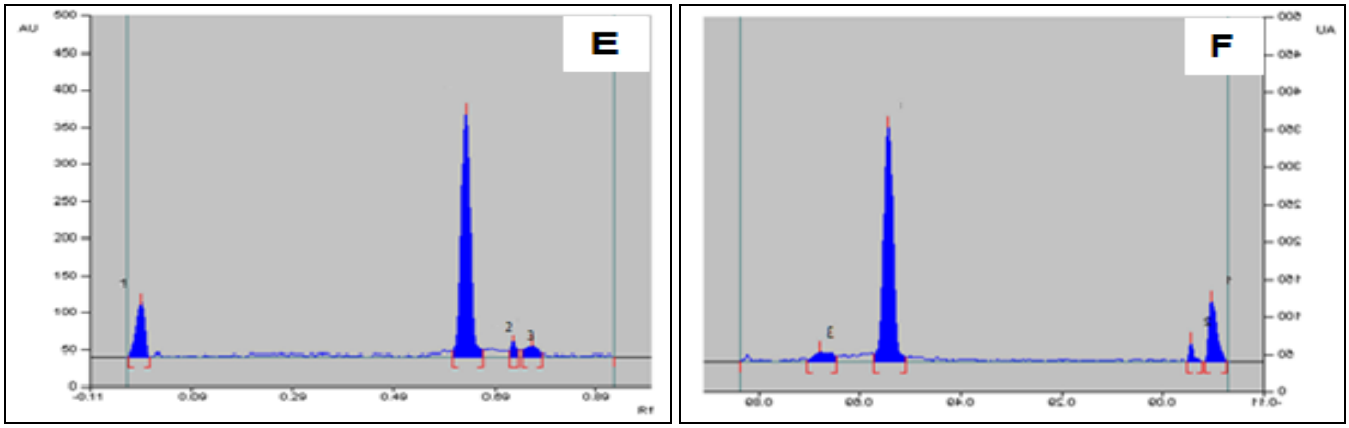


FIG. 6: FORCED DEGRADATION DENSITOGRAMS: (A) ACIDIC HYDROLYSIS, (B) ALKALINE HYDROLYSIS, (C) NEUTRAL HYDROLYSIS, (D) OXIDATIVE DEGRADATION, (E) THERMAL HYDROLYSIS, AND (F) PHOTO-DEGRADATION

TABLE 5: FORCED DEGRADATION STUDIES FOR DROTAVERINE HYDROCHLORIDE

Stress condition	Area	% Degradation	% Recovery
Acid (0.1 N HCl)	4755	9.35	90.65
Alkali (0.1 N NaOH)	4503	14.2	85.85
Oxidative (3% H_2O_2)	4352	13.8	86.20
Neutral Degradation	4438	12.1	87.90
Thermal degradation (80°C)	4502	14.17	85.83
Photolytic degradation (254 nm)	4705	10.28	89.72

Analysis of Marketed Formulation: The evaluation of a commercially available formulation was performed in six replicates. The % assay was found to be 99.25 ±0.75 %w/w Table 6, which was found to be an acceptable limit. Excipients usually found in tablets did not affect the results.

TABLE 6: RESULTS OF ANALYSIS OF MARKETED FORMULATION

S. no.	Amount of drug taken (ng/band)	Peak area	% Assay	Amount of drug estimated (ng/band)
1	400	5228	99.22	396.88
2	400	5211	99.09	396.38
3	400	5216	99.18	396.75
4	400	5222	99.31	397.24
5	400	5235	99.35	398.23
6	400	5214	99.15	396.60
	% Assay			99.25
	S.D.			0.098319
	% R.S.D.			0.099095

CONCLUSION: The present study was concluded to understand the degradation behaviour of DRO under ICH-recommended stress conditions. This study is an example of developing a stability-indicating assay method for DRO where forced degradation was carried out under all stress conditions. DRO showed degradation in acidic, alkaline, thermal and oxidative conditions. However, the extent of degradation was different. The method for quantifying DRO has been developed to be quick, precise, accurate, and selective. The established technique may be utilized to determine DRO in bulk medicines and pharmaceutical dosage forms quickly and easily.

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