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Method Development and Stability indicating RP-HPLC Method for Drotaverine Hydrochloride for Bulk and Pharmaceutical Dosage Form

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Abstract: Drotaverine hydrochloride (DRO) is a well-accepted non pharmacopoeial drug used widely as antispasmodic**.** Present work describes a precise, accurate, reproducible and stability indicating high performance liquid chromatographic (RP-HPLC) method for the analysis of DRO both as a bulk drug and in formulations. The formulation was subjected to ICH recommended stress conditions. Chromatography was carried out on C8 YMC (150 × 4.6 mm i.d., 5 µm) column using mobile phase of 0.2% formic acid (v/v): methanol (55:45) at a flow rate of 1.0 ml/min with UV detector at 300nm. The retention time of the DRO was about 5.2 mins. The detector response is linear from 40-180 µg/ml of test concentration with a correlation coefficient of 0.9998 of DRO. The limit of detection and Limit of quantification were 0.02µg/ml and 0.06µg/ml. The method was validated with specificity, linearity, LOD & LOQ, precision, accuracy, stability of solution and robustness. Accuracy (recovery) was between 99.05 and 101.47%. The specificity of the method was determined by assessing interference from the placebo and by stress testing of the drug (forced degradation).Intraday and interday system and method precision was determined. The proposed method is simple, fast, sensitive, linear, accurate, rugged and precise and hence can be applied for routine quality control of DRO in bulk and in tablet dosage form.
Keywords: Drotaverine Hydrochloride, Stability indicating assay, HPLC with UV detector, Method Validation.

INTRODUCTION

Pain is an ill-defined, unpleasant sensation, usually evoked by an external or internal noxious stimulus [1]. Excessive pain may produces other effects like sinking sensation,apprehension, sweating, nausea, palpitation, rise or fall in BP or tachypnoea, analgesics relieve pain as a symptoms without affecting its cause [2,3]. Pain may be acute or chronic, renal colic pain and labour pain is most common and chronic pain generally not controlled by Non Steroidal Anti-inflammatory Drugs (NSAIDS) and more potent drug needed to control pain. Drotaverine hydrochloride (DRO) is Chemically 1-[(3,4-[diethoxyphenyl) methylene]-6,7-Diethoxy-1, 2,3,4-tetrahydroisoquinoline. It is crystalline powder with pale yellow color. Its molecular formula is

($C_{24}H_{31}NO_4$.HCl) and its molecular weight is 433.97 g/mole. DRO is soluble in ethanol (96%) and chloroform and moderately soluble in water. Melting point and pH are 210 ºC and 4.14 respectively.

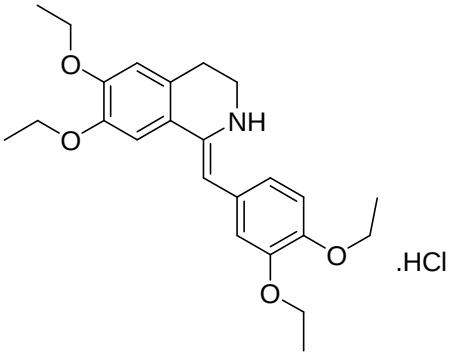

Figure 1 Chemical Structure of Drotaverine Hydrochloride

DRO is a benzylisoquinoline derivative and is an analogue of papaverine(opium alkaloid) which is an antispasmodic drug acts by phosphodiesterase-IV inhibition to increases the intracellular level of cyclic adenosine monophosphate (cAMP) which causes relaxation of smooth muscle that suppress the pain associated with spasm caused by smooth muscle contraction, especially in tubular organs like stomach, intestine, ureter, urinary bladder, cholecystalgia, chronic cholecystitis, neck of uterus spasm during delivery and also used as a cerebral and coronary vasodialator in subarachnoid haemorrhage, coronary artery bypass surgery and microsurgery [4-7].

Several methods are reported for the determination of DRO. Most of these methods are for the determination of separately, or in combination with other drugs in pharmaceutical formulations or biological fluid are HPLC [8-10], TLC [11], spectrophotometric [12]. Our aim of this study is to develop simple and fast stability indicating HPLC-UV assay method then other methods which have been reported earlier.

MATERIAL AND METHODS

Chemical and Reagents

DRO was obtained as a gift sample from Emcure pharmaceutical Ltd. Pune, India. HPLC grade methanol (spectrochem) and formic acid (Merck) was used. Milli-Q water(HPLC grade) was used in mobile phase preparation. Commercially available DRO (Drotikind 80, Windlas Biotech Limited, Dehradun, India) were purchased from local market.

Instrumentation

Chromatography was performed under, ambient conditions, with HPLC equipment (Waters Corporation, Milford, USA) consisted comprising quaternary 600 pump, a variable-wavelength programmable 2489 UV-Visible detector and YMC C8 (150mm × 4.6mm id, 5 µm particle size) column was used. A Rheodyne injector with a 20µl loop was used for the injection of the sample. The HPLC system was equipped with Empower software for data processing.

Chromatographic Condition

Stability indicating HPLC assay method was developed on YMC C8 (150mm × 4.6mm id, 5 µm particle size) column, using a mobile phase containing a Formic acid in water (0.2%v/v): Methanol (55:45) at ambient temperature. The flow rate was maintained 1.0 ml/ min throughout analysis. Drug shows good absorbance at 300nm, which was selected as wavelength for further analysis. All determinations were performed at constant column temperature (25˚C) in column oven.

Preparation of Standard Solution

Stock solution (500µg/ml) of DRO working standard was prepared by transferring accurately weighed 25 mg into a 50 ml volumetric flask and adding 20 ml water- methanol (50:50, v/v). The mixture was sonicated for 1 min to dissolve the DRO and the solution was then diluted up to the mark with the same diluent. Standard Solution (100µg/ml) was prepared by diluting 10 ml standard stock solution to 50 ml, in a volumetric flask, with the same diluent.

Preparation of Test Solutions

To prepare Test stock solution for assay, 20 tablets were weighed and mixed. An aliquot of powder equivalent to the weight of 1 tablet was accurately weighed and transferred to 100 ml volumetric flask and 60 mL diluent (water- methanol 50:50, v/v) was added to the flask and the mixture was sonicated for 30 min with normal hand shaking. The contents of the flask were then left to return to room temperature and diluted up to the mark with diluent. This solution was filtered through a 0.45-µm nylon syringe filter.

To prepare test solution (100µg/ml) for assay 12.5 ml test stock solution was transferred to 100 ml volumetric flask and diluted up to the mark with water-methanol (50:50, v/v).

Method Validation

The analytical method was validated with respect to parameters such as linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, specificity, recovery and robustness/ruggedness.

Forced degradation Studies

To perform the forced degradation study 50 mg drug was subjected to acidic, alkaline, oxidizing, thermal and photolytic conditions. For acidic degradation the drug was heated under reflux with 1M HCl at 80 °C for 2 h and then the mixture was neutralized with 0.5M NaOH. For alkaline degradation the drug was treated with 1M NaOH at 80°C for 2 h and the mixture was neutralized with 0.5M HCl. For degradation under oxidizing conditions the drug was heated under reflux with (6%, v/v) H_2O_2 at 80 °C for 2 h. For thermal degradation the powdered drug was exposed at 70 °C for 72 h. For photolytic degradation the powdered drug was exposed to sunlight for 72 h (8h per day). The placebo was also subjected to the same stress conditions to determine whether any peaks arose from the declared excipients. After completion of the treatments the solutions were left to return to room temperature and diluted with water-methanol (50:50, v/v) to furnish 100 µg/ml solutions. The purity of the drug peak obtained from the stressed sample was measured using UV detector.

Linearity

Eight solutions were prepared containing 40, 60, 80, 100, 120, 140, 160 and 180 µg/ml DRO, concentrations which corresponded to 40, 60, 80, 100, 120, 140, 160 and 180% respectively, of the standard solution concentration. Each solution was injected in duplicate. Linearity was evaluated by linear-regression analysis.

Precision

System precision was evaluated by analyzing the standard solution five times and method precision (repeatability) was evaluated by assaying six sets of test samples prepared for assay determination, all on the same day (intraday precision). System precision and method precision were also determined by performing the same procedures on a different day (interday precision), and by another person under the same experimental conditions (intermediate precision).

Accuracy

Accuracy was assessed by determination of the recovery of the method at three different concentrations (corresponding to 50, 100 and 150% of test solution concentration) by addition of known amounts of standard solution to placebo solution. For each concentration, three sets were prepared and injected in duplicate.

Limit of Detection and Limit of Quantification:

The limit of detection (LOD) and quantification (LOQ) were established by evaluating the minimum level at which the analyte could be readily detected and quantified with accuracy. As per the ICH guideline the signal-to-noise ratio should be more than 3.3 and 10.0 for LOD and LOQ respectively. LOD and LOQ were calculated using the formulae: LOD = 3.3σ/S and LOQ = 10σ/S, where σ = Residual Standard Deviation of regression line and S = Slope of regression line.

Robustness

The Robustness of the method was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions. The factor chosen for this study were the flow rate (±0.1 ml/min), mobile phase composition [0.2% formic acid (v/v): methanol (57:43 and 53:47)] and at different column oven temperature (±2˚C).

Solution Stability

Solution Stability was evaluated for the standard solution and the test solution. The solutions were stored at 5° C and at ambient temperature without protection of light and tested after 12, 24, 36 and 48 h. The responses for the aged solution were evaluated by comparison with freshly prepared solutions.

System Suitability

The suitability of the chromatographic system was tested before each stage of validation. Five replicate injections of standard solution were injected, and asymmetry, number of theoretical plates, and RSD (%) of peak area were determined.

RESULTS AND DISCUSSION

In this work an analytical HPLC method for assay of DRO in a tablet formulation was developed and validated. The basic chromatographic conditions were designed to be simple and easy to use and reproduce and were selected after testing the different conditions that affect HPLC analysis, for example column, aqueous and organic components of the mobile phase, proportion of mobile phase components, detection wavelength, diluents, concentration of analyte, etc. The YMC Pack C8 column was used because of its advantages of high resolving capacity, better reproducibility, low-back pressure, and low tailing. To develop a precise, accurate, specific and suitable stability indicating RP-HPLC method of the estimation of the DRO different mobile phases were employed and proposed chromatographic condition was found for the quantitative determination in presence of degradation products. The proportion of the mobile phase components was optimized to reduce retention times and enable good resolution of DRO from the degradation products. A detection wavelength of 300 nm was selected after scanning the standard solution over the range 190-370 nm by use of the UV detector. Detection at 300 nm resulted in good response and good linearity.

We have targeted degradation more than 10% for the establishing stability indicating nature of the assay method, as even intermediate degradation products should not interfere with any stage of drug analysis. Though conditions used for forced degradation were attenuated to achieve degradation in the range of 10-80%, this could not be achieved in some cases even after exposure of prolonged duration. The drug substance was easily extracted from the pharmaceutical dosage form by use of Methanol: Water (50:50, v/v). The tablet dispersed readily in water and the drug substance was easily soluble in Methanol. Standard solution and Test solution were found to be stable in this solvent mixture. After development of the analytical method, it was validated in accordance with ICH guideline [13]. This furnished evidence the method was suitable for its intended purpose. The specificity of the method was evaluated by checking the peak purity of the analyte peak during the forced degradation study. The peak purity of the DRO peak under different stress conditions was 1.0000, which is satisfactory and indicates there was no interference with the analyte peak from degradation products. Major degradation (up to 27%) occurred under alkali conditions (Figure 4). Under acidic conditions the drug was degraded by approximately 17% (Figure 5). The drug was approximately 17% degraded under oxidizing conditions (Figure 6). The drug was degraded 7% under thermal condition (Figure 7) and 13% degradation occurred under photolytic conditions (Figure 8).

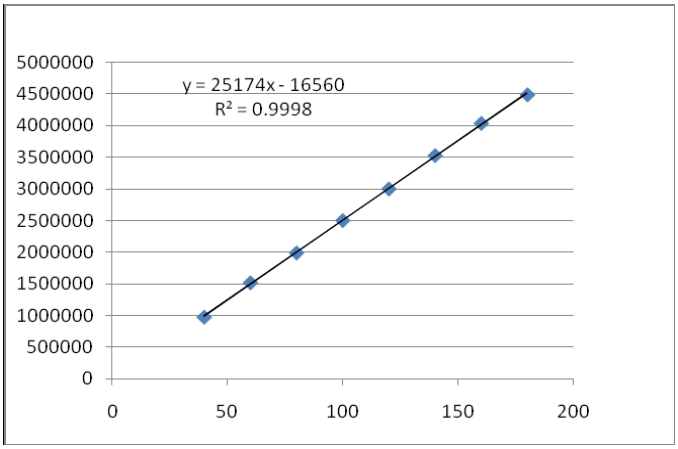

Figure 2 Linearity Graph

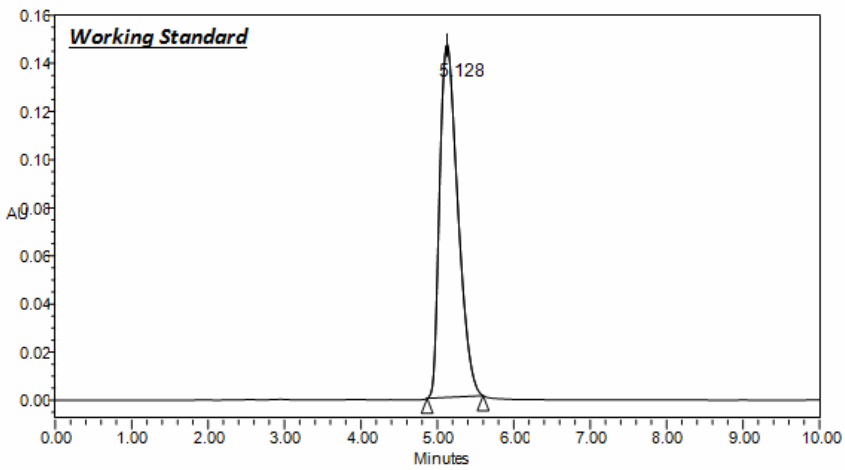

Figure 3. Chromatogram of Drotaverine Hydrochloride (100 µg/ml)

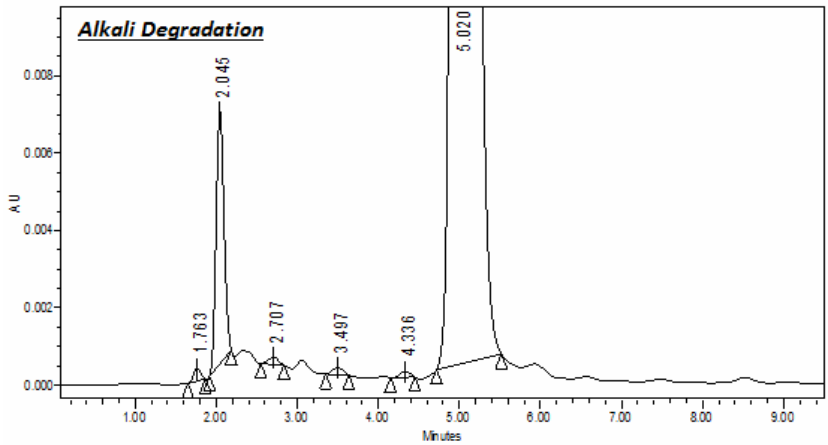

Figure 4. Chromatogram of alkali forced degradation study.

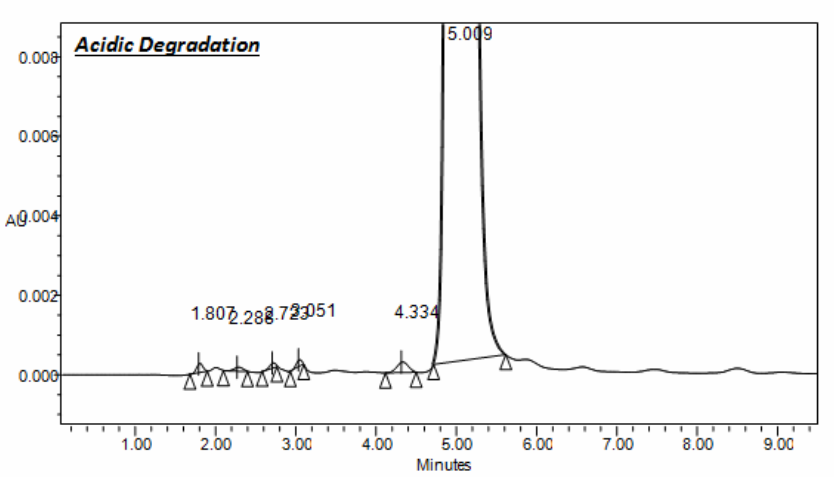

Figure 5. Chromatogram of acidic forced degradation study.

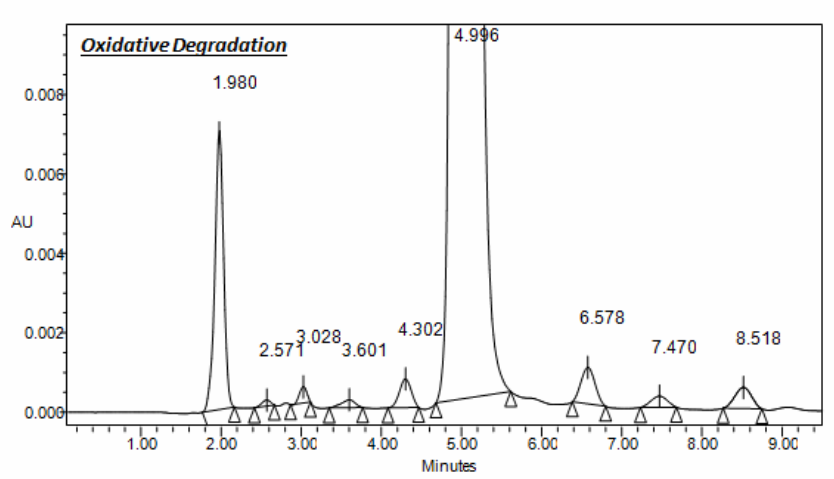

Figure 6. Chromatogram of oxidative forced degradation study.

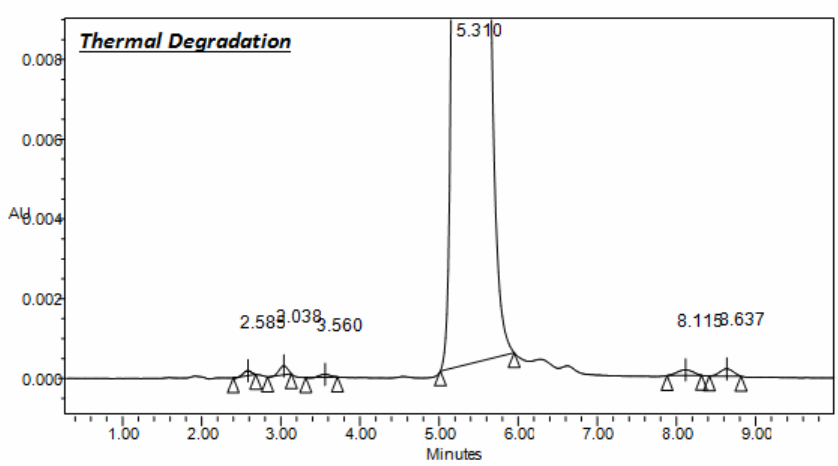

Figure 7. Chromatogram of thermal forced degradation study.

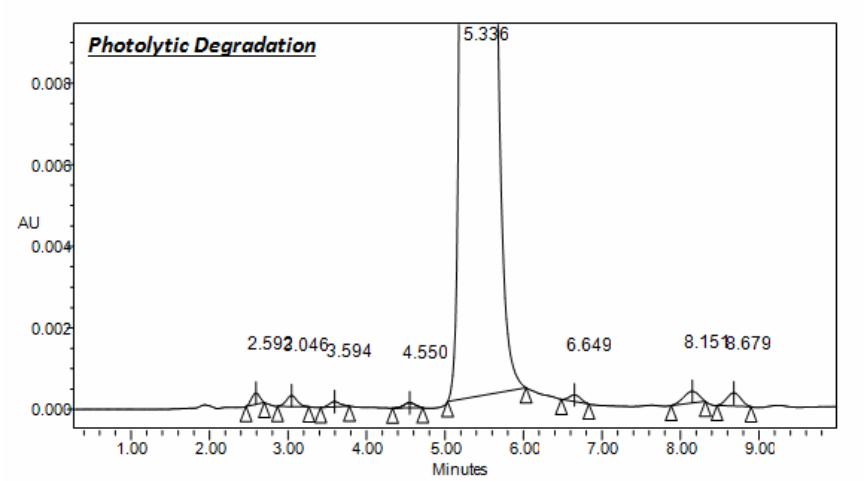

Figure 8. Chromatogram of photolytic forced degradation study.

To determine linearity a calibration graph was obtained by plotting DRO concentration against peak area. Linearity was good in the concentration range 40-180 µg/ml. The regression equation was y = 25174x - 16560, where x is the concentration in µg/ml and y is the peak area in absorbance units; the correlation coefficient was 0.9998. (Figure 2). The accuracy of the method was assessed by determination of recovery for three concentrations covering the range of the method. Known amounts of DRO (50, 100, and 150 µg/ml) were added to a placebo preparation and the amount of DRO recovered, in the presence of placebo interference, was calculated. The mean recovery of DRO was between 99.6 and 101.7%, which is satisfactory (Table 1). The signal to noise ratio for LOD and LOQ were found 3.4 and 10.3 for the DRO. The Limit of detection and Limit of quantification were found 0.02µg/ml and 0.06 µg/ml respectively for the DRO.

The method was assessed by assaying test solutions under different analytical conditions deliberately changed from the original conditions. For each different analytical condition the standard solution and test solution were prepared separately. The result obtained from assay of the test solution was not affected by varying the conditions and was in accordance with the true value (Table 2). System suitability data were also found to be satisfactory during variation of the analytical conditions. The analytical method therefore remained unaffected by slight but deliberate changes in the analytical conditions. During study of the stability of stored standard solution and test solution for assay determination the solutions were found to be stable for up to 48 h. Assay values obtained after 48h were statistically identical with the initial value without measurable loss (Table 3).

Before each measurement of validation data system suitability was performed by measurement of general characteristics such as peak asymmetry, number of theoretical plates, and RSD (%) of peak area observed for a standard solution. The values obtained were satisfactory and in accordance with in-house limits (< 2.00%). (Table 4).

The intensive approach described in this manuscript was used to develop and validate a liquid chromatographic analytical method that can be used for both assay and determination of DRO in a pharmaceutical dosage form. Degradation products produced as a result of stress did not interfere with detection of DRO and the assay method can thus be regarded as stability indicating.

Table 1: Accuracy Study Data

Level %	No	Amount of drug added (µg/ml)	Amount of drug found (µg/ml)	Recovery (%)	Mean Recovery (%)	RSD[a] (%)
50	1	50.13	49.43	98.60	99.51	0.96
	2	49.21	49.46	100.51		
	3	49.67	49.38	99.42		
100	1	98.21	98.64	101.47	100.23	1.20
	2	99.85	100.00	100.15		
	3	100.98	101.03	99.07		
150	1	149.76	148.66	99.27	99.17	0.11
	2	149.25	148.04	99.19		
	3	150.27	148.84	99.05		

RSD[a] = Relative standard deviation

Table 2: Robustness Study Data

Conditions	Assay%	RT (min)	System Suitability data	
			Theoretical Plates	Asymmetry
0.9 ml/min Flow	101.24	5.95	8568	1.16
1.1 ml/min Flow	99.15	4.91	8132	1.19
0.2%Formic acid :Methanol(57:43)	100.64	6.93	8133	1.08
0.2%Formic acid :Methanol(53:47)	99.39	4.35	8573	1.13
Temperature change (27˚C)	99.84	5.41	8866	1.16
Temperature change (23˚ C)	99.26	5.48	8899	1.11

Table: 3 Solution Stability Data

Time intervals, hrs.	Difference between assays for standard solution (%)		Difference between assays for test solution (%)	
	At 5°C	At room temperature	At 5°C	At room temperature
12	1.46	1.56	0.13	0.20
24	1.31	1.80	0.15	0.16
36	1.52	1.95	1.46	1.67
48	2.12	2.36	2.05	2.18

Table 4: System Suitabality Data

System suitability data In- House limit	RSD[a] (%)NMT[b]2.0	Theoretical Plates NLT[c] 8000	Asymmetry NMT[b]2.0
Specificity	0.74	8532	1.16
Linearity	0.23	8268	1.11
Precision for Assay	0.46	8965	1.09
Intermediate Precision for Assay	0.55	8842	1.08
Accuracy	0.46	8436	1.15
Solution Stability	0.81	8556	1.03
Robustness	0.72	8454	1.14

[a]Relative standard deviation, [b]not more than, [c]not less than

CONCLUSION

This HPLC method for assay of DRO in a tablet formulation was successfully developed and validated for its intended purpose. The method was shown specific, linear, precise, accurate, and robust. As the method separates DRO and all the degradation impurities formed under variety of stress conditions it can be regarded as stability indicating. There is no pharmacopoeial method for assay determination of DRO in pharmaceutical dosage forms, this method can be used in the quality control laboratory of drug content in pharmaceutical preparations.

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