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Stability-indicating HPTLC method for simultaneous determination of Drotaverine and Aceclofenac in tablet formulation

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ABSTRACT

A sensitive, selective, precise and stability indicating high-performance thin-layer chromatographic method for quantification of Drotaverine and Aceclofenac in pharmaceutical dosage form has been established and validated. The method employed TLC aluminium plates precoated with silica gel 60F_{254} as stationary phase and the solvent system consisted of Ethyl acetate: Benzene: Methanol: Glacial acetic acid (0.5:7:2:0.5 v/v/v/v) as mobile phase. Densitometric analysis of drugs was carried out in absorbance mode at 242 nm. The Rf values was found to be 0.24 for DRT and 0.76 for ACE. The linear regression analysis data for the calibration plots showed good linear relationship over the concentration range of 300–1800 ng per band (correlation coefficient r^2=0.9955) for aceclofenac and (correlation coefficient r^2=0.9930) for drotaverine, respectively. The method was validated for accuracy, precision, ruggedness, and robustness. The limits of detection and quantification were 15.00 and 45.46 ng per band, respectively, for aceclofenac and 222.70 and 674.84 ng per band, respectively, for drotaverine. The method was validated as per ICH guidelines. Stability studies were performed by forced degradation of tablet sample with acid and base hydrolysis, oxidation, dry heat and UV-induced degradation. Peaks of the degraded products were well resolved from the pure drugs. As the method could effectively separate the drugs from their degradation products, it can be used as a stability indicating method.

Key Words: High Performance Thin Layer Chromatography, Drotaverine, Aceclofenac.

INTRODUCTION

Drotaverine (DRT), Chemically, is 1-[(3,4-diethoxyphenyl)methylene]-6,7-diethoxy-1, 2, 3, 4-tetrahydro isoquinoline. It is analog of papaver and is used as antispasmodic agent and to reduce excessive labour pain[1]. Aceclofenac (ACE), Chemically it is [[[2-[(2, 6-Dichlorophenyl)

amino]phenyl] acetyl] oxy] acetic acid. It is a non steroidal anti-inflammatory drug with good analgesic and anti-rheumatic properties and is used in various pain conditions like rheumatoid arthritis, osteoarthritis[8]. Both the drugs are marketed as combined dose tablet formulation (80:100 mg DRT: ACE). Literature survey reveals that drotaverine can be estimated by spectrophotometry[6] ,HPLC[1-3]and by HPTLC[4] methods. Aceclofenac is reported to be estimated by spectrophotometry[7] , HPTLC[14] and HPLC[8-13] .The reported methods are applicable for the estimation of either DRT or ACE individually or in combination with other drugs from pharmaceutical dosage forms or biological fluids. Since no studies have described simultaneous determination of aceclofenac and drotaverine in pharmaceutical dosage form, this study attempts to describe a selective, precise, accurate and reproducible high performance thin layer chromatography.

MATERIALS AND METHODS

Sample, Reagents and Chemicals:

Drotaverine and Aceclofenac were received as gift samples from Sanofi-Aventis Ltd. Mumbai and Glennmark Pharmaceuticals Ltd, Nashik respectively. Test samples (ESNIL, containing100 mg ACL and 80 mg DRT; manufactured by Dewcare Concept, Ahmedabad India) were purchased from local market. All reagents were of analytical reagent-grade purchased from Qualigens, Mumbai, India.

HPTLC Instrumentation and Chromatographic conditions:

The samples were spotted in the form of bands of width 6 mm with a Camag 100 microlitre sample (Hamilton, Bonaduz, Switzerland) on 10 cm x 10 cm aluminum-backed HPTLC plates coated with silica gel $60F_{254}$ (E. Merck, Darmstadt, Germany; supplied by Merck India, Mumbai, India) using Camag Linomat V (Muttenz, Switzerland). The plates were prewashed with methanol and activated at 100^{o}C for 30 min prior to chromatography.

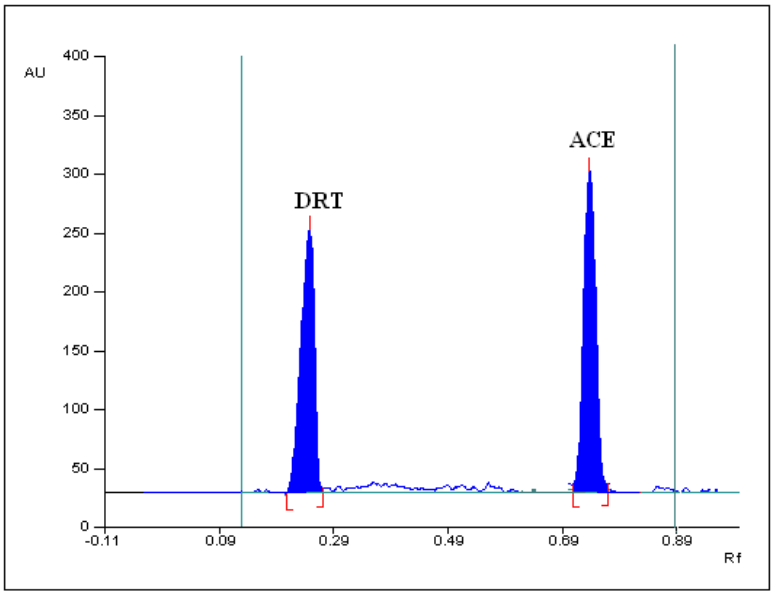

Fig.1. Typical Densitogram of Drotaverine and Aceclofenac

A constant application rate of 150 nL s^{-1} was employed and space between two bands was 6 mm.the slit dimension was kept at 5 x 0.45 mm and 20 mm/s scanning speed was employed. The mobile phase consisted of ethyl acetate: Benzene: Methanol: Glacial acetic acid in the ratio 0.5:7:2:0.5 v/v/v/v and 10mL of mobile phase was used per chromatography. Linear ascending development was carried out in 10 x 10 cm twin-trough glass chamber (Camag, Muttenz, Switzerland). The optimized chamber saturation time for mobile phase was 30 m at room temperature (25°C). The length of chromatogram was 8 cm and approximately about 30 min. subsequent to the development. TLC plates were dried in a current of air dryer in wooden chamber eith adequate ventilation. Densitometric scanning was performed at 242 nm with a Camag TLC scanner III operated in reflectance-absorbance mode and controlled by WinCATS software (Version 1.4.3.6336).

Preparation of Standard Stock Solutions (Linearity):
Standard stock solution containing DRT and ACE was prepared by dissolving 25 mg of each drug in 25.0 ml methanol. Aliquots of standard stock solution were appropriately diluted with methanol to obtain concentration of 300 µg/ml for each drug. The standard stock solution (1, 2, 3, 4, 5 and 6 µl) was applied to a TLC plate to furnish final amounts of 300-1800 ng per band. The plate was chromatographed and scanned under the above mentioned chromatographic conditions. Sample application and chromatographic development was performed on six plates individually. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

Table 1: Linear regression data for calibration plots for analysis of DRT and ACE by HPTLC (n = 6)

Drug	Linear range(ng/per band)	r^2 ± SD	Slope ± SD	Intercept ± SD
DRT	300-1800	0.9955± 0.0019	2.697±0.0223	285.47±12.2598
ACE	300-1800	0.9930±0.0029	3.223±0.0867	2340.17±217.5219

Analysis of tablet formulation:
Twenty tablets were weighed and average weight was calculated. Tablets were then crushed to obtain fine powder.

Accurately weighed quantity of tablet powder equivalent to about 20 mg DRT and 25 mg ACE was transferred to 25.0 mL volumetric flasks, added 15 mL of methanol, sonicated for 10 min and volume made up to the mark with methanol. Solution was mixed and filtered through Whatmann filter paper No.42. From the filtrate, 2.0 mL was transferred to 10.0 mL volumetric flask and diluted to the mark with methanol (Concentration 160 µg/ml DRT and 200 µg/ml ACE). The diluted tablet sample solution was applied on the TLC plate (5 µl, 4 bands) along with the standard solution containing 160 µg/ml DRT and 200 µg/ml ACE (5 µl, 2 bands) followed by chromatographic development with above stated chromatograohic conditions. Content of DRT and ACE was calculated by comparing peak area of sample with that of the

standard. Six samples were prepared and analyzed in similar manner. The possibility of interference in the analysis by excipients was studied.

Table 2: Results of Analysis of Tablet Formulation

Component	Amount present (mg)	Amount found (mg/tab)	% Amount found	Standard deviation	Relative Standard Deviation
DRT	80	79.91	99.75	±0.7621	0.7640
ACE	100	99.75	99.89	± 1.0310	1.0320

**Average of six determinations, SD-Standard Deviation, CV- Coefficient of Variation.*

Method Validation:

Accuracy: To ascertain the accuracy of the proposed method recovery studies were carried out by standard addition method, adding known amount of each drug to the preanalysed tablet powder, at three levels 80 %, 100 % and 120 % of the label claim. Recovery studies were carried out in triplicate at each level. The results of recovery studies were expressed as percent recovery and are shown in Table No. 3

Table 3: Result of recovery studies

Level of Recovery	Component	Amt. of pure drug added (mg)	Amount of drug recovered (mg)	% recovery* (mg)	S.D.	C.V.
80 %	DRT	64	64.16	100.24	±1.69	1.68
	ACE	80	80.12	100.65	±1.50	1.49
100 %	DRT	80	80.48	100.28	±0.91	0.90
	ACE	100	99.86	99.86	±0.89	0.89
120 %	DRT	96	96.12	100.13	±0.98	0.97
	ACE	120	120.72	100.60	±1.15	1.14

**denotes average of three observations.*

Table 4: Result of Precision studies.

Parameters	Component	% Estimation*	S.D.	C.V.
Intra-day	DRT	100.53	± 0.6331	0.6266
	ACE	100.01	± 1.3820	1.3818
Inter-day	DRT	99.50	± 0.7204	0.6266
	ACE	99.60	± 0.9165	0.9201

Precision: Intra-day precision was determined by analyzing the tablet samples at three different time intervals on the same day and for inter-day precision tablet samples were analyzed on three different days. Standard deviation for intra-day and inter-day assay precision was calculated. Results of precision studies are shown in Table No. 4.

Limit of detection (LOD) and Limit of quantitation (LOQ): LOD and LOQ for both the drugs were calculated by using the values of slopes and intercepts of the calibration curves for both the drugs. Following formulas were used for calculation for LOD & LOQ:

$$\text{Limit of quantitation} = \frac{3.3 \times \text{S.D.(Intecept)}}{\text{Slope (Mean)}}$$

$$\text{Limit of detection} = \frac{3.3 \times \text{S.D.(Intecept)}}{\text{Slope (Mean)}}$$

Table No. 5: Results of Robustness Studies

Chromatographic Changes			
Factor	**Level**	**R_f Value**	
Mobile phase composition (± 0.1 mL)		**DRT**	**ACE**
0.4:6.9:2.1:0.6	– 0.1	0.20	0.75
0.5:7:2:0.5	0	0.24	0.76
0.6:7.1:1.9:0.4	+ 0.1	0.22	0.80
Amount of Mobile Phase (v/v)(± 1mL)		**DRT**	**ACE**
9	– 1.0	0.20	0.78
10	0	0.24	0.76
11	+1.0	0.21	0.80
Duration for chamber saturation (± 2 min)		**DRT**	**ACE**
28 min	- 2	0.20	0.77
30 min	0	0.24	0.76
32 min	+ 2	0.22	0.81

Robustness

Robustness studies were performed by making small but deliberate variations in chromatographic conditions viz., mobile phase composition, mobile phase volume and duration of chamber saturation with mobile phase. The effect of these variations on Rf valus of components was studied. The composition of mobile phase was changed slightly (± 0.1 mL). Chromatograms were run with mobile phases of composition, Ethyl acetate: Benzene:

Methanol: Glacial acetic acid (0.6:7.1:1.9:0.4 v/v/v/v and 0.4:6.9:2.1:0.6 v/v/v/v). Mobile phase volume was varied at 10 ± 1 mL (9 and 11 mL) and 10 % variation in camber saturation time at 30 ± 3 min. (27 and 33 min.).

Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The bands for DRO and ACE in sample were confirmed by comparing the Rf and spectra of the bands with that of standard. The peak purity for DRO and ACE were assessed by comparing the bands at three different levels, i.e., peak start (S), peak apex (M) and peak end (E) positions of the band. The spectrum for DRO & ACE are shown in Fig No.2 & 3.

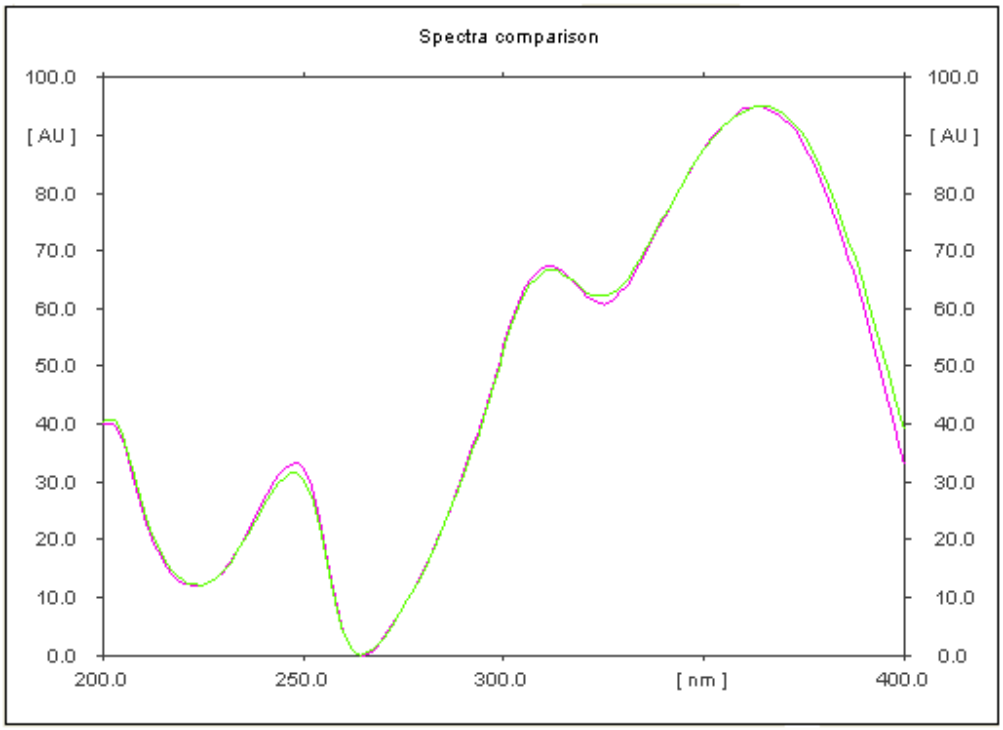

Figure 2: Spectrum of DRO standard and sample measured from 200 to 400 nm

Forced Degradation study of DRT and ACE

Amount of tablet powder equivalent to 20 mg DRT and 25 mg ACE was separately transferred to five 25 mL volumetric flasks (Flask No. 1, 2, 3, 4 and 5), added 5 mL of 0.1 M HCl, 0.1 M NaOH and 3 % H_2O_2 to Flask No. 1, 2 and 3, respectively. Flask No. 1, 2, and 3 were then kept in reflux at 80^0C for 3 h. Flask No. 4 containing tablet powder was also kept at 60^0C for 24 h to study the effect of heat on tablet sample (heat degradation). The forced degradation was performed in the dark to exclude the possible degradative effect of light. Flask No. 5 was exposed to ultraviolet radiations at 254 nm for 24 h in a UV-chamber. All the flasks were removed, the tablet samples were treated in similar manner as described under analysis of tablet formulation and chromatography was performed as described.

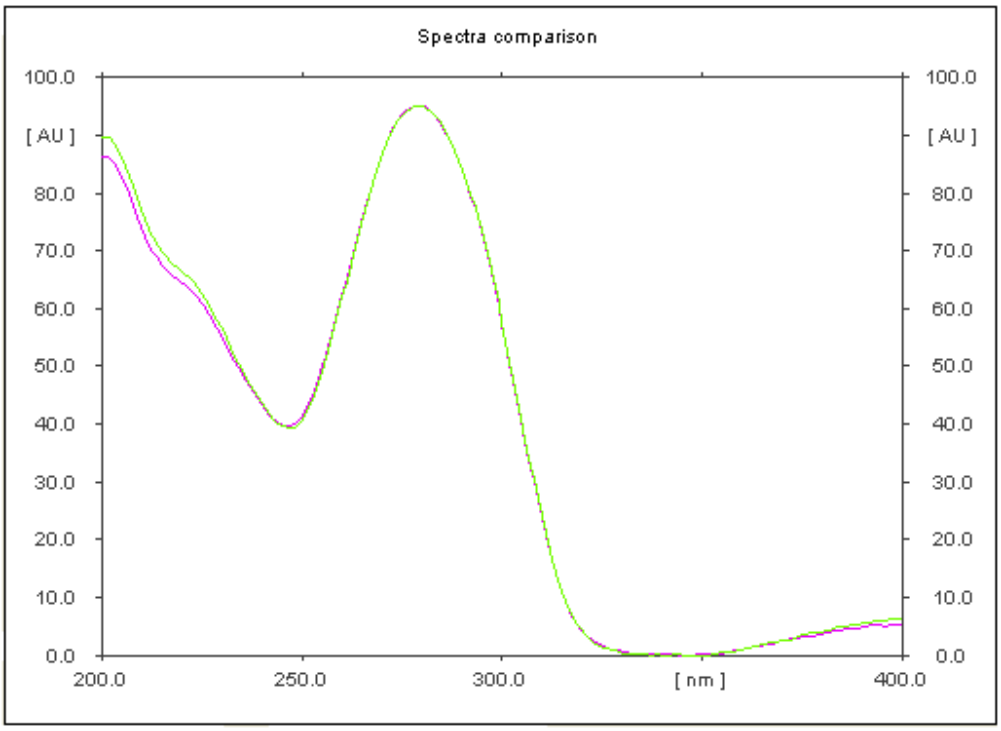

Figure 3: Spectrum of ACE standard and sample measured from 200 to 400 nm

Stability-Indicating Property

Acid-induced degradation

The drug combination was degraded in acidic condition and shows different degradation products at Rf 0.31, 0.55 as shown in Fig No.4.

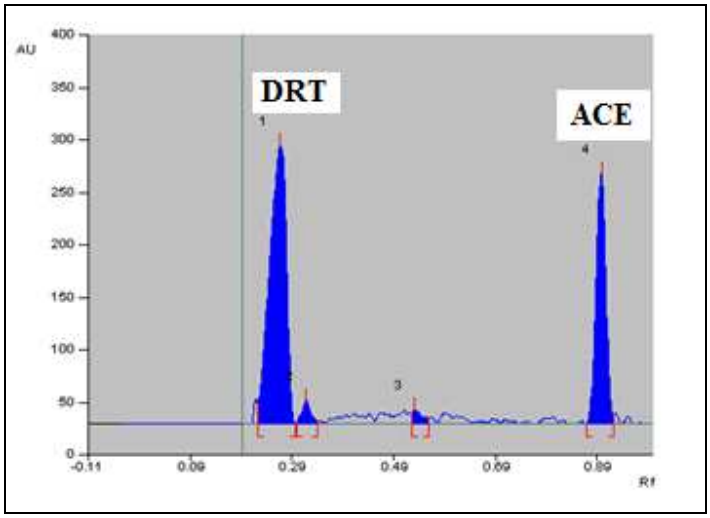

Fig. 4. Chromatogram of HCL treated DRT and ACE

Base-induced degradation

The drug combination was degraded in alkaline condition and shows different degradation products at Rf 0.51, 0.80, as shown in Fig No.5.

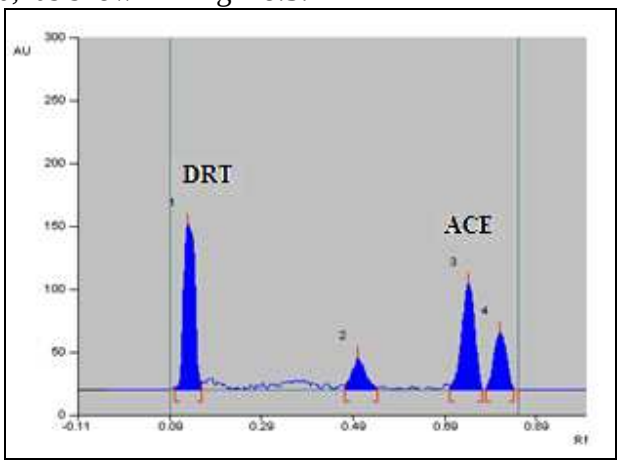

Fig. 5. Chromatogram of NaOH treated DRT and ACE

Hydrogen peroxide-induced degradation

The drug combination was degraded in hydrogen peroxide (3%) at room temperature and shows different degradation products at Rf 0.33, 0.70,0.84 as shown in Fig No.6.

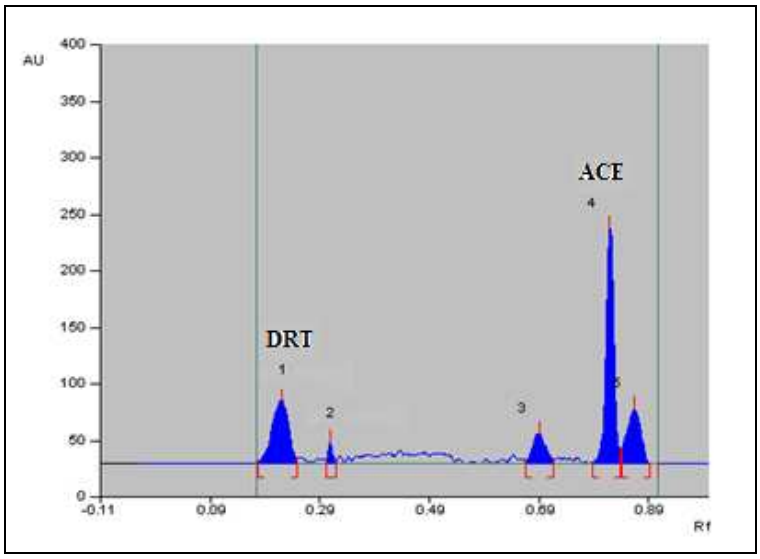

Fig.6. Chromatogram of H_2O_2 treated DRT and ACE

Heat degradation

The drug combination when subjected to heat was degraded and degradation products appeared at Rf 0.005, 0.33, 0.70.0.95 as shown in Fig No.7.

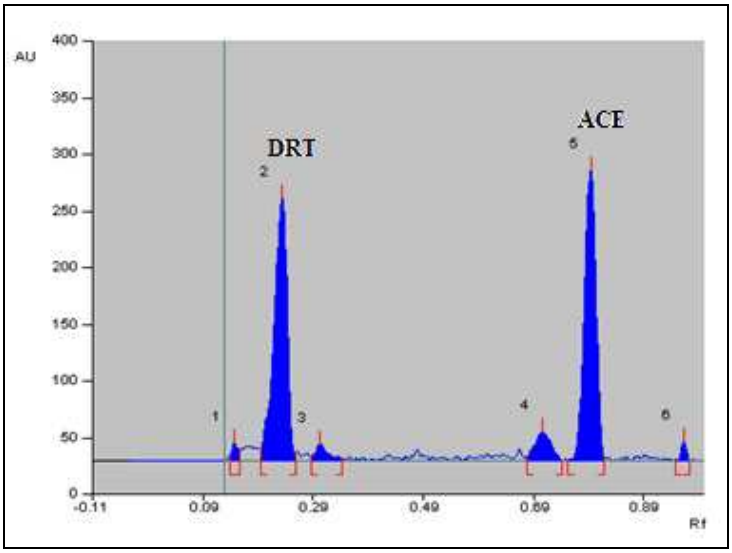

Fig . 7. Chromatogram of dry heat treated DRT and ACE

UV-induced degradation

The drug combination when subjected to photochemical degradation or ultraviolet (UV) light at 254 nm and degradation products appeared at Rf 0.65, 0.70 as shown in Fig No.8.

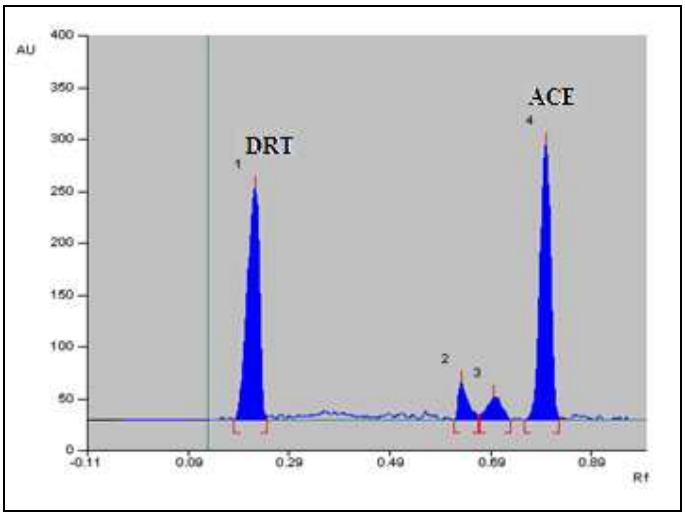

Fig. 8. Chromatogram of UV radiation treated DRT and ACE

RESULTS AND DISCUSSION

The proposed chromatographic conditions were found suitable for effective separation and quantitation of ACE & DRT. The method separates DRT (RF-0.24) and ACE (RF-0.76 min) with good resolution, peak shapes and minimal tailing. The peak areas of the drugs were reproducible as indicated by low coefficient of variance indicating the repeatability of the proposed method. Both the drugs were found to give linear detector response in the

concentration range under study with correlation coefficient of 0.9955 and 0.9930 for DRT and ACE calibration curve, respectively. The sample recoveries from the formulation were in good agreement with their respective label claim indicating that there is no interference from the tablet excipients. The method exhibited good selectivity and sensitivity. Percent recoveries for DRT and ACE were 100.37 % and 100.21 %, respectively indicating accuracy of the proposed method. Percent RSD for tablet analysis, recovery studies and intra-day & inter-day precision studies is less than 2 indicating high degree of precision and reproducibility of the proposed method. LOD and LOQ were found to be 0.1396 & 0.4231 for DRT and 0.2246 & 0.6808 for ACE, respectively. The results of robustness study also indicated that the method is robust and is unaffected by small deliberate variations in the method parameters. Hence, it can be concluded that the assay method is validated and shown to be appropriate for its intended use, and was used to test actual content of drotaverine and aceclofenac in tablet formulation.

CONCLUSION

This stability-indicating HPTLC method is precise, specific, accurate and selective. Statistical analysis confirms the method is repeatable and selective for simultaneous analysis of DRT and ACE as active pharmaceutical ingredients and in pharmaceutical formulations, without interference from excipients.

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