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STABILITY INDICATING RP-HPLC METHOD FOR DETERMINATION OF DROTAVERINE HYDROCHLORIDE IN BULK AND ITS FORMULATION

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ABSTRACT

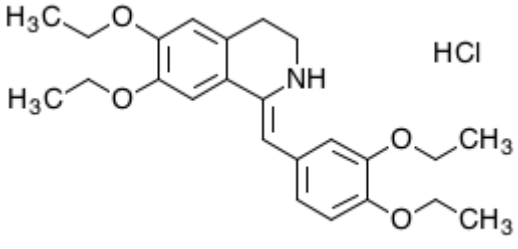
A simple, rapid and accurate stability indicating RP-HPLC method was developed for the determination of Drotaverine Hydrochloride in pure and tablet form. The method was carried out using Phenomenex ODS C-18 column (250 x 4.6 mm, packed with 5 micron) using Acetonitrile and Water (50:50) with 0.05% glacial acetic acid as the mobile phase with detection at 357 nm and a flow rate of 0.5 ml/min. The retention time was found to be 4.260 min. The responses were linear in concentrations range of 10-120 µg/ml with correlation coefficient of 0.999. The percentage recovery was found to be between 98.01-101.69% and %RSD from recovery studies was found to be less than 1. The drug was subjected to acid, alkali and neutral hydrolysis, oxidation, dry heat and photolytic degradation. The degradation studies indicated the drug to be susceptible to acid and alkali hydrolysis. The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision, ruggedness and robustness and can be successfully applied for determination determination of these drugs in commercial tablets.

KEY WORDS

Drotaverine Hydrochloride, RP-HPLC, Validation, Stability, Degradation.

INTRODUCTION:

Drotaverine Hydrochloride is chemically 1-[(3,4-diethoxyphenyl)-methylene]-6,7-diethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (Fig. 1) [8]. It is is an antispasmodic drug and inhibits phosphodiesterases hydrolyzing cAMP, thereby increasing cAMP concentration. It is structurally related to papaverine and it is a selective inhibitor of phosphodiesterase 4. In addition, it has no anticholinergic effects.



Drotaverine HCl

It is not official in any of the pharmacopoeia but is listed in the Merck Index and Martindale: The complete drug reference. Literature survey revealed the estimation of Drotaverine Hydrochloride by several techniques such

as simultaneous estimation by HPLC[1-3], determination of Drotaverine Hydrochloride in human plasma by HPLC[4], by RP-HPLC techniques[5], quantitation of Drotaverine by HPLC[6] and stability study by HPLC[7]. The focus of present study was to develop and validate a rapid, stable and economic RP-HPLC method for the estimation of Drotaverine Hydrochloride in bulk and its formulation.

MATERIALS AND METHODS

Chemicals & Reagents:

Analytically pure Drotaverine Hydrochloride was obtained as a gift sample from Khandelwal Remedies, Mumbai, India. Commercial tablet formulations were purchased from the local market. All chemicals and reagents used were of AR/HPLC grade, obtained from Merck.

Instrument:

A High-Performance Liquid Chromatographic system, with Spinchrom data handling system (Shimadzu-LC 2010) with Analytical Column- Phenomenex ODS C18 (250 X 4.6 mm, 5 µ particle size), equipped with quaternary gradient pump, 2010C UV-VIS detector in isocratic mode was used for the analysis. Calibrated electronic single pan balance (Sigma 200/A Super), pH Meter (LABINDIA), PCi (3.5L) Ultrasonicator were also used during the analysis.

Chromatographic Conditions:

The mobile phase constituted of degassed mixture of Acetonitrile and Water (50:50) with 0.05% glacial acetic acid. The injected volume was 20 µl with a flow rate of 0.5 ml/min. Detection was carried out at 357 nm with a run time set at 10 minutes.

Preparation of Mobile Phase and Standard Stock Solution:

The mobile phase was prepared by mixing 500 ml of acetonitrile with 500 ml of water to get the proportion of 50:50 v/v and finally 0.05% v/v glacial acetic acid. The solution was sonicated for 15 minutes and filtered using 0.45-micron membrane filter. Standard stock solution of Drotaverine Hydrochloride was prepared by dissolving 10 mg of Drotaverine Hydrochloride in 10 ml of mobile phase to get a concentration of 1000 mg/ml. It was further diluted to get a concentration of 100 µg/ml.

Calibration Curve for Drotaverine Hydrochloride:

Appropriate aliquots of standard stock solution were taken in different 10 ml volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of 10-120 µg/ml of Drotaverine Hydrochloride respectively. The solutions were injected using a 20 µl fixed loop system and chromatograms were recorded.

Assay of marketed Formulations:

Twenty tablets of Drotin (manufactured by Martin & Harris Lab.) were accurately weighed and finely powdered and mixed. The powder equivalent to the average weight of one tablet was transferred into a 100 ml volumetric flask. The drug was extracted four times by adding solvent in portion, 20 ml each and then volume was made up to the mark using the same solvent. After appropriate dilution (within their linearity range), peak area of the sample solutions were recorded. The amount of Drotaverine Hydrochloride per tablet was calculated using the calibration curve.

Method Validation: [9]

1. Linearity: Various working standard solutions were prepared and the linearity range was calculated from the observation.

2. Accuracy: The accuracy of the proposed method was tested by recovery studies at 80%, 100%, and 120% by adding a known amount of pure drug to the pre-analyzed formulation of concentration 30 µg/ml.

3. Precision: The precision of the proposed method was ascertained by actual determination of 6 replicates of a fixed concentration of the drug (30 µg/ml) within the Beer's range and finding out the average peak area by the proposed method.

4. Sensitivity: The sensitivity of the method was determined with respect to LOD and LOQ. The LOD and LOQ were separately determined based on the standard calibration curve using the formula LOD = 3 x S.D /S and LOQ = 10 x S.D/S, where, S.D is the standard deviation of the y- intercept of regression line and S is the average slope of the calibration curve.

5. Intraday Assay and Interday Assay: The intraday assay and interday assay of the proposed method were ascertained by actual determination of 8 replicates of a fixed concentration of the drug (30 µg/ml and 40 µg/ml) and finding out the average peak area by the proposed method at 3 different time period of the same day and on three different days respectively.

Degradation Studies: [10]

Degradation in Acidic Condition: Ten micrograms of the Drotaverine Hydrochloride was weighed accurately and transferred in to cleaned 10 ml volumetric flask. The content of the volumetric flask was dissolved in 10 ml of

1N HCl. Then the volumetric flask was subjected to heat on water bath at 100°C. Samples were prepared for different time intervals, such as 0 hr, 30 min., 1 hr, 2 hr, 4 hr and 6 hr. The sample solutions were then diluted to prepare 50 µg/ml with solvent acetonitrile and water (50:50) with 0.05% glacial acetic acid and sonicated for 10 min. These were then filtered through 0.22 µm filter and 20 µl were injected into HPLC. The obtained chromatograms were observed for any degradation undergone during the time.

Degradation in Alkaline Condition: Ten micrograms of Drotaverine Hydrochloride was weighed accurately and transferred into cleaned 10 ml volumetric flask. The content of the volumetric flask was dissolved in 10 ml of 1N NaOH. Then the volumetric flask was subjected to heat on water bath at 100°C. Samples were prepared for different time intervals, such as 0 hr, 30 min, 1 hr, 2 hr, 4 hr and 6 hr. The sample solutions were then diluted to prepare 50 µg/ml with solvent acetonitrile and water (50:50) with 0.05% glacial acetic acid and sonicated for 10 min. These were then filtered through 0.22 µm filter and 20 µl were injected into HPLC. The obtained chromatograms were observed for any degradation undergone during the time.

Degradation in Neutral Condition: Ten micrograms of Drotaverine Hydrochloride was weighed accurately and transferred in to cleaned 10 ml volumetric flask. The content of the volumetric flask was dissolved in 10 ml of distilled water. Then the volumetric flask was subjected to heat on water bath at 100°C. Samples were prepared for different time intervals, such as 0 hr, 30 min., 1 hr, 2 hr, 4 hr and 6 hr. At different time interval, different sample solutions were taken out. The sample solutions were then diluted to prepare 50 µg/ml with acetonitrile and water (50:50) with 0.05% glacial acetic acid and sonicated for 5 min. These were then filtered through 0.22 µm filter and 20 µl was injected into HPLC. The obtained chromatograms were observed for any degradation undergone during the time.

Thermolytic Degradation: For thermal degradation, 100 mg of Drotaverine Hydrochloride was weighed accurately and transferred into cleaned petridish. Then the petridish was placed in the incubator at 70°C. Samples were drawn at different time intervals. From these stock solutions of 1000 µg/ml were prepared with solvent acetonitrile and water (50:50) with 0.05% glacial acetic acid, from which 50 µg/ml of working solutions were prepared. These were sonicated and filtered through 0.22 µm filter and 20 µl of the samples were injected into HPLC. The reports were analyzed for any degradation that has under gone due to heat.

Oxidative Degradation: For oxidation, 10 mg of Drotaverine Hydrochloride was weighed accurately and transferred in to cleaned 10ml volumetric flask. The content of the volumetric flask was dissolved in 10ml H_2O_2 (6%). Then the volumetric flask was subjected to heat on water bath at 100°C. Samples were prepared for different time intervals, such as 0 hr, 30 min., 1 hr, 2 hr, 4 hr, 6 hr and 12 hr. The sample solutions were then diluted to prepare 50 µg/ml with solvent acetonitrile and water (50:50) with 0.05% glacial acetic acid and sonicated for 5 min. These were then filtered through 0.22 µm filter and 20 µl were injected into HPLC. The obtained chromatograms were observed for any degradation undergone during the time.

Photolytic Degradation: For Photolysis, 100 mg of Drotaverine Hydrochloride was weighed accurately and transferred into cleaned petridish. Then the petridish was placed under direct sun light at day time keeping the cover of petridish closed and left for degradation. At different time of interval 10 mg of sample was taken. From these stock solutions of 1000 µg/ml were prepared and were sonicated for 5 min and diluted to prepare 50 µg/ml with solvent acetonitrile and water (50:50) having 0.05% glacial acetic acid. Then filtered through 0.22 µm filter and injected into HPLC. The obtained chromatograms were observed for any degradation undergone during the time.

UV Degradation: For UV degradation, 100 mg of Drotaverine Hydrochloride was weighed accurately and transferred into cleaned petridish. Then the petridish was placed under UV chamber keeping the petridish at 30 cm distance from the UV lamp. The covers of the petridish was removed and left for degradation. At different time of intervals, the UV lamp was switched off and 10 mg of sample was taken. From these stock solutions of 1000 µg/ml were prepared with solvent acetonitrile and water (50:50) with 0.05% glacial acetic acid, from which 50 µg/ml of working solution were prepared. Then these were sonicated and filtered through 0.22 µm filter and 20 µl of the samples were injected into HPLC. The reports were analyzed for any degradation that has under gone due to UV light.

RESULTS AND DISCUSSION

Calibration Curve:

The peak areas for the different concentrations (10-120 µg/ml) were recorded at 357 nm. The calibration curve (Fig. 2) data, and the HPLC spectra (Fig. 3) is shown in Table 1 and the assay results of the marketed formulations is shown in Table 2.

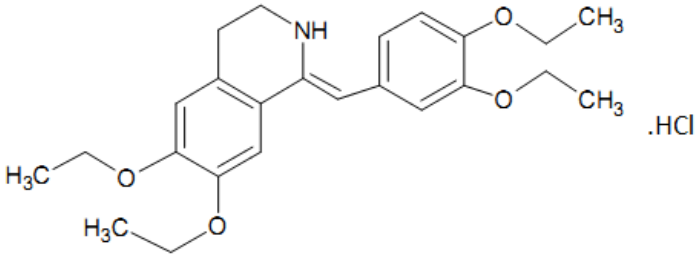


Figure 1: Structure of Drotaverine Hydrochloride

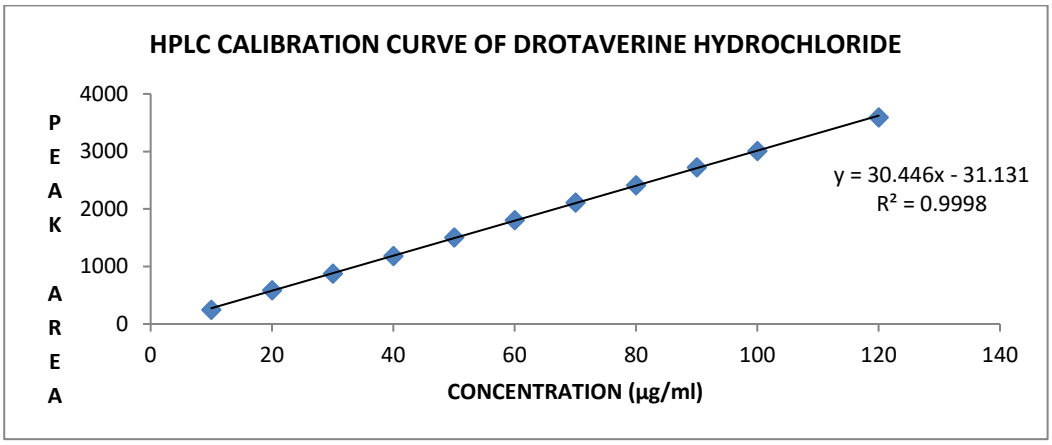


Figure 2: Calibration Curve of Drotaverine Hydrochloride

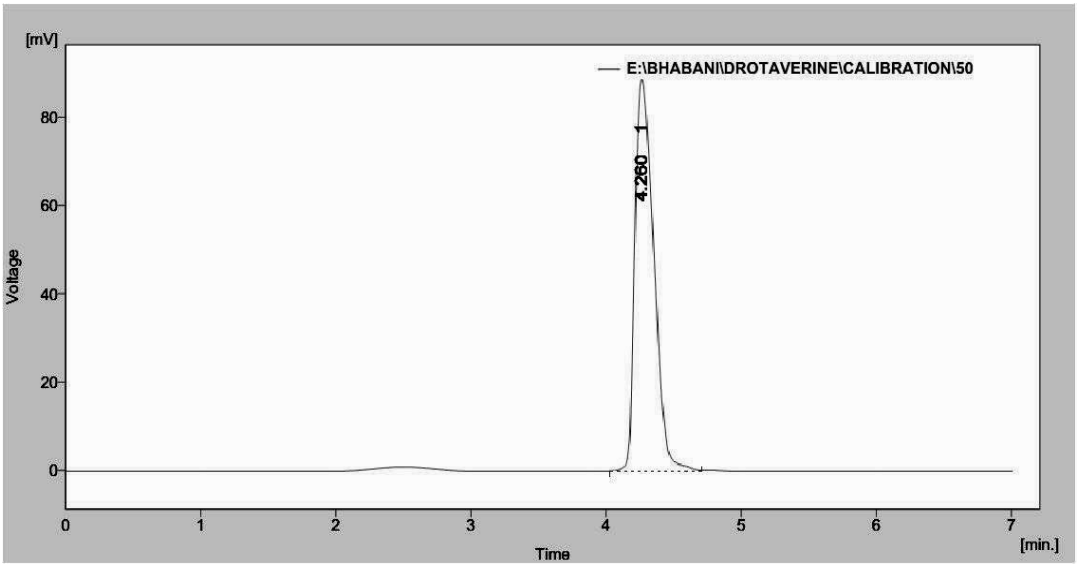


Figure 3: RP-HPLC Spectra of Drotaverine Hydrochloride

Table 1: Calibration Curve Table of Drotaverine Hydrochloride

Conc. (mcg/ml)	Mean Peak Area (mV.s) (n=4)	Statistical Analysis
10	245.257	
20	587.166	
30	874.355	
40	1185.015	
50	1503.933	Slope= 30.44
60	1807.474	Intercept= 31.13
70	2114.216	r^2= 0.999
80	2413.260	
90	2722.394	
100	3009.565	
120	3593.410	

Table 2: Assay Result of The Marketed Formulation by The Proposed Method

Formulation	Label claimed	Observed Amount	% Recovery
Drotin	50 mg	49.79 mg	99.58

Accuracy:

The percentage recovery was found to be in the range of 98.01% and 101.69% as shown in Table 3.

Table 3: Accuracy Readings Of Drotaverine Hydrochloride By RP-HPLC

Sample ID	Concentration(µg/ml)		% Recovery of Pure Drug	Statistical Analysis
	Pure Drug	Formulation		
S_1: 80%	24	30	98.18	**Mean=** 98.50
S_2: 80%	24	30	99.32	**SD=** 0.712
S_2: 80%	24	30	98.01	**%RSD=** 0.723
S_4: 100%	30	30	99.54	**Mean=** 99.78
S_5: 100%	30	30	100.22	**SD=** 0.376
S_6: 100%	30	30	99.60	**%RSD=** 0.377
S_7: 120%	36	30	97.83	**Mean=** 99.80
S_8: 120%	36	30	99.47	**SD=** 1.164
S_9: 120%	36	30	101.69	**%RSD=** 1.167

Precision:

From Table 4, the %RSD for precision was found to be 0.602.

Table 4: Precision Results for Drotaverine Hydrochloride By RP-HPLC

Concentration (µg/ml)	Injection	Area (mV.s)	Cal. Amount (µg/ml)	Statistical Analysis
30	1	874.355	29.75	
30	2	880.863	29.96	**Mean=** 29.94
30	3	875.315	29.78	
30	4	879.560	2992	**SD=** 0.180
30	5	889.934	30.26	
30	6	884.557	30.08	**%RSD=** 0.602
30	7	874.691	29.76	
30	8	882.992	30.03	

Sensitivity:

The LOD was found to be 1.31 µg/ml and the LOQ was found to be 4.53 µg/ml at 357 nm, respectively.

Intraday and Interday Assay:

The %RSD for Intraday and Interday Assay were found to be 0.488 and 0.333 respectively. Low values of %RSD

indicate that the proposed method is accurate. The data is shown in Table 5 and 6.

Table 5: Intraday Precision Reading of Drotaverine Hydrochloride By RP-HPLC

Concentration (µg/ml)	Injection	Area 1 (mV.s)	Area 2 (mV.s)	Area 3 (mV.s)	Mean %RSD
30	1	874.355	877.612	883.277	0.488
30	2	885.415	874.357	879.634	
30	3	873.681	876.560	884.993	
30	4	882.865	886.339	880.325	
30	5	877.452	883.657	878.390	
30	6	879.571	875.982	886.572	
30	7	876.212	878.691	875.261	
30	8	871.931	885.215	876.122	
Avg. Cal. Amount (µg/ml)		29.85	29.93	29.95	
% RSD		0.514	0.504	0.447	

Table 6: Interday Precision Reading of Drotaverine Hydrochloride By RP-HPLC

Concentration (µg/ml)	Injection	Day 1 (Area)	Day 2 (Area)	Day 3 (Area)	Average %RSD
40	1	1185.085	1182.046	1185.846	0.333
40	2	1188.323	1180.551	1180.337	
40	3	1181.89	1179.438	1184.693	
40	4	1187.609	1186.320	1179.214	
40	5	1178.728	1184.879	1187.078	
40	6	1185.376	1190.567	1190.585	
40	7	1182.982	1188.488	1181.409	
40	8	1190.399	1183.600	1191.106	
Avg. Cal. Amount (µg/ml)		39.95	39.93	39.95	
%RSD		0.311	0.317	0.370	

Robustness:

To evaluate robustness of the developed method, deliberate variations were made in the method parameters such as the flow rate of the mobile phase and ratio of mobile phase. The results are presented in Table 7.

Table 7: Robustness of The Proposed Method

Factor	Level	Mean Area (mV.s) (n=5)	Mean Cal. Amount (µg/ml)	%RSD
pH	3.58	1186.405	39.99	0.155
	4.38	1186.100	39.98	0.143
Flow rate (ml/min)	0.4	1187.014	40.01	0.142
	0.6	1186.405	39.99	0.155

Ruggedness:

To evaluate ruggedness of the developed method, deliberate variations were made in the method parameters such as analysts and temperature of the system. The results are presented in Table 8.

Table 8: Ruggedness of The Proposed Method

Factor	Level	Mean Area (mV.s) (n=5)	Mean Cal. Amount (µg/ml)	%RSD
Analyst	1	1184.882	39.94	0.209
	2	1183.969	39.91	0.164
Temperature (^{0}C)	Room Temp.	1185.796	39.97	0.099
	18	1184.578	39.93	0.143

Stability Results:

The results obtained in acidic degradation, alkaline degradation, neutral degradation, thermal degradation, oxidative degradation, photolytic degradation and UV degradation are depicted as chromatograms and given in Figure 4, 5, 6, 7, 8, 9 and 10, respectively and represented in Table 9.

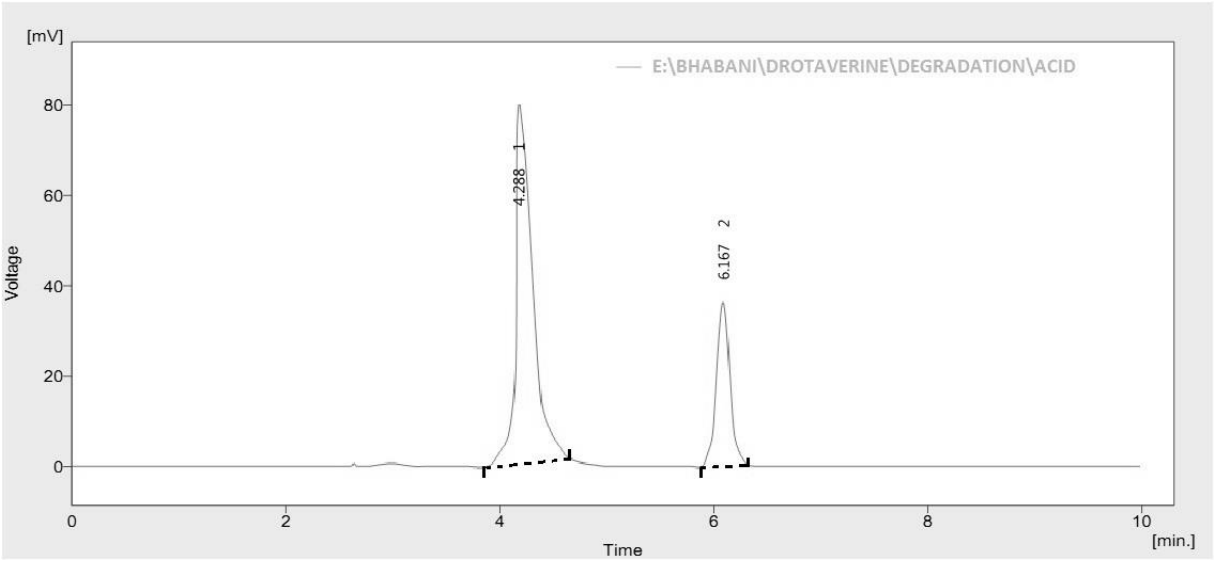


Figure 4: Chromatogram Showing Acidic Degradation of Drotaverine Hydrochloride

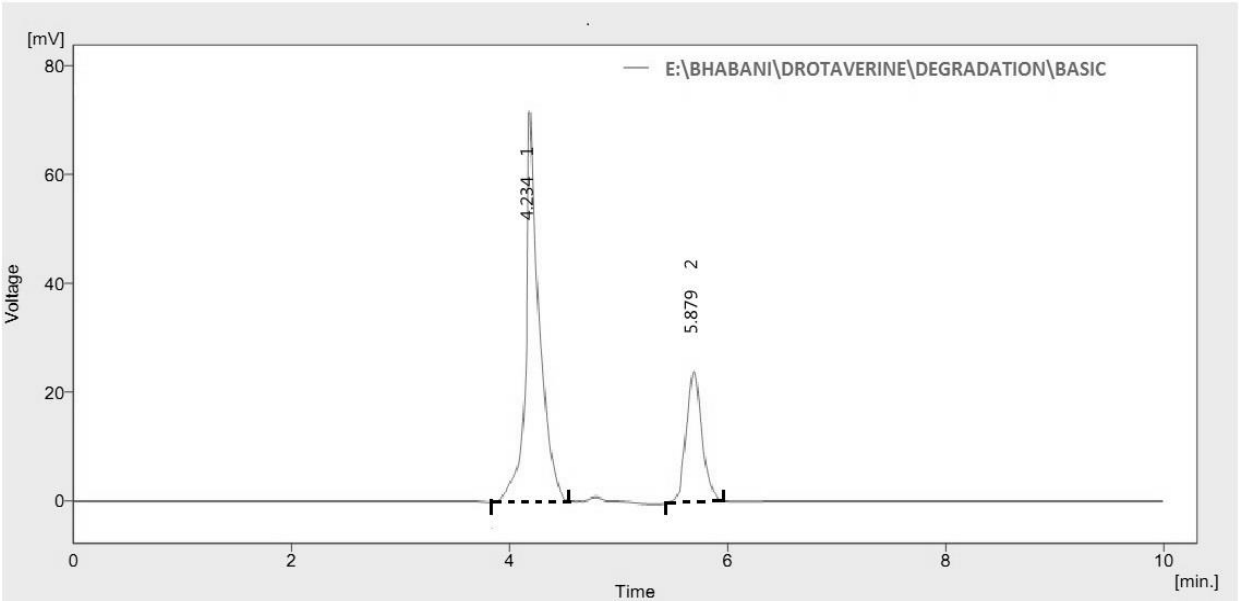


Figure 5: Chromatogram Showing Alkaline Degradation of Drotaverine Hydrochloride

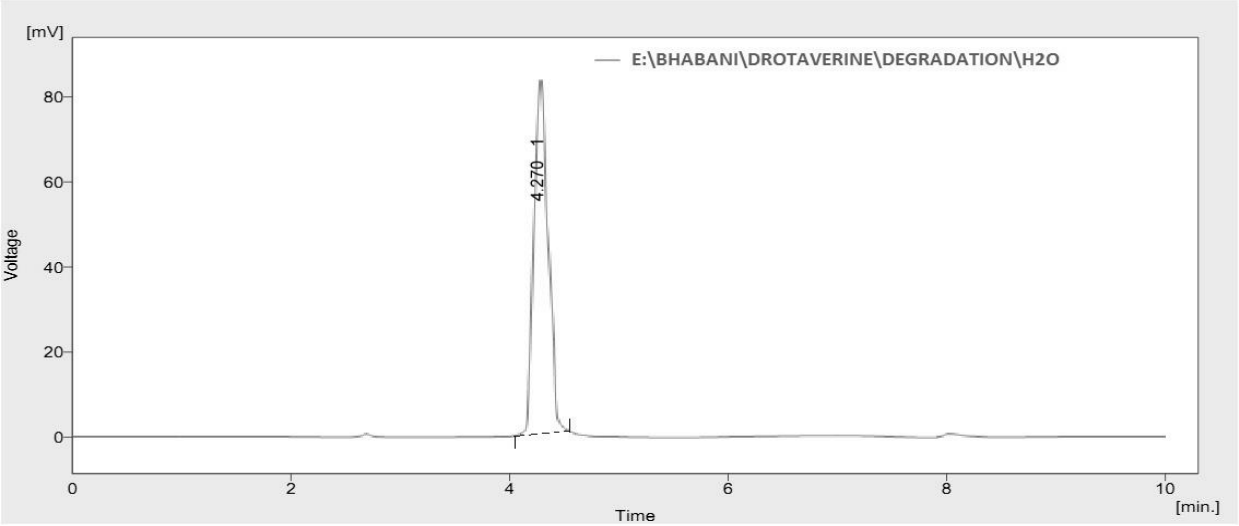


Figure 6: Chromatogram Showing Neutral Degradation of Drotaverine Hydrochloride

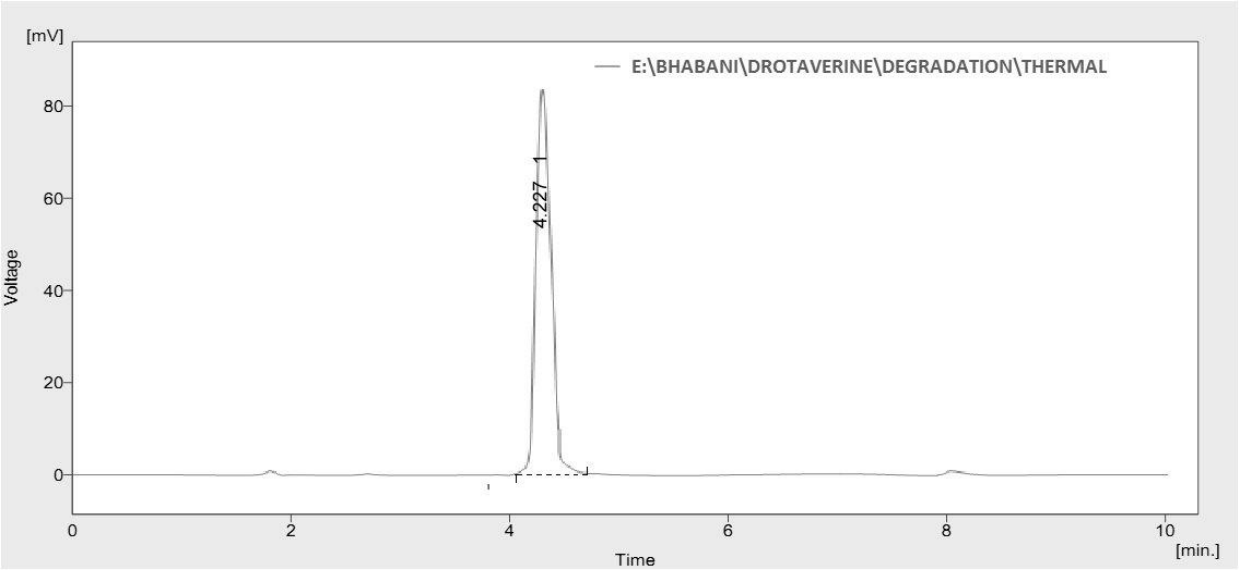


Figure 7: Chromatogram Showing Thermal Degradation of Drotaverine Hydrochloride

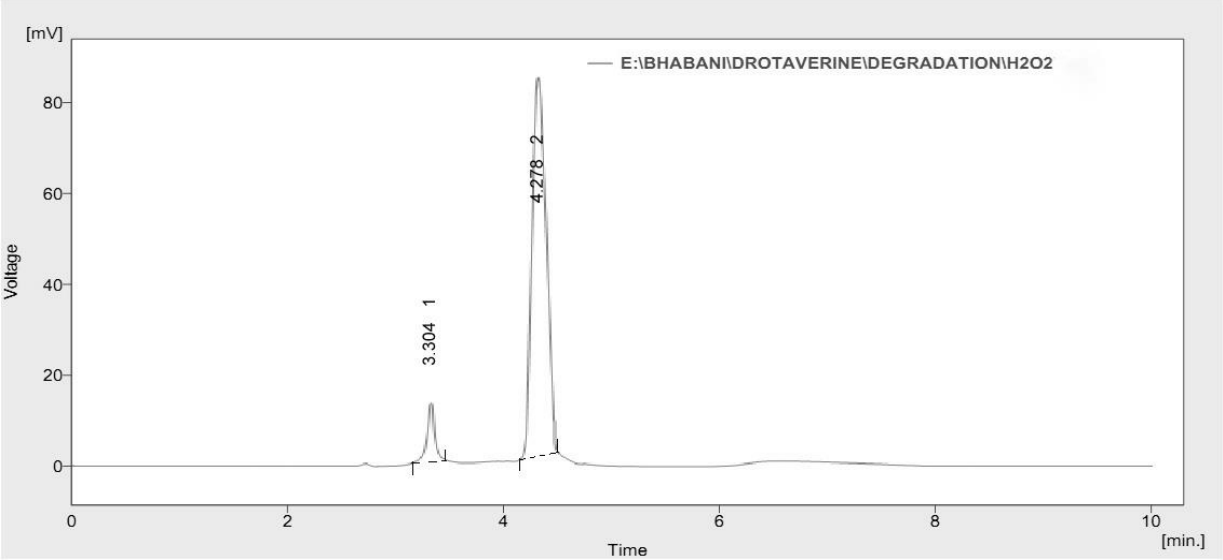


Figure 8: Chromatogram Showing Oxidative Degradation of Drotaverine Hydrochloride

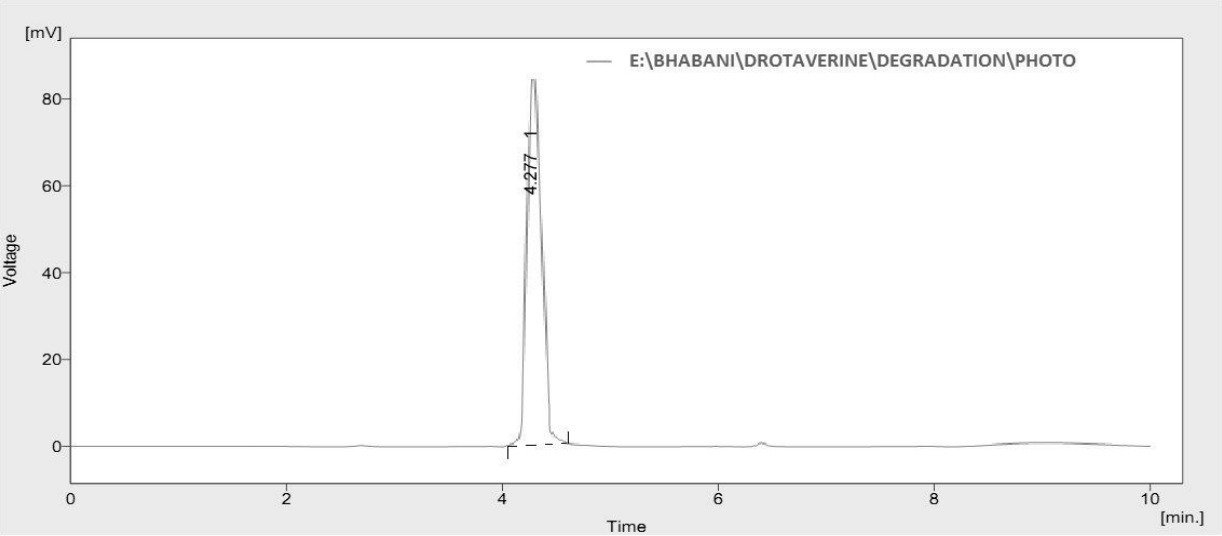


Figure 9: Chromatogram Showing Photolytic Degradation of Drotaverine Hydrochloride

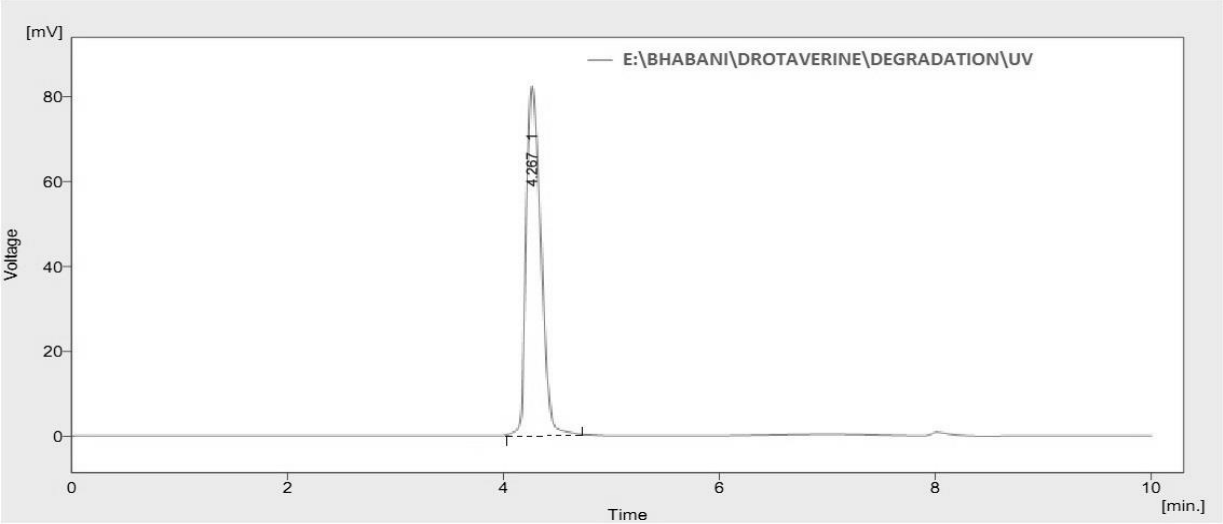


Figure 10: Chromatogram Showing UV Degradation of Drotaverine Hydrochloride

Table 9: Stability Study Results of Drotaverine Hydrochloride

Conditions	Conc. (µg/ml)	Time	Peak Area (mV.s)		
			Before Degradation	After Degradation	% Degraded
Acidic Degradation	50	6 hours	1503.933	1235.654	16.78
Alkaline Degradation	50	6 hours	1503.933	1123.154	24.18
Neutral Degradation	50	6 hours	1503.933	1501.202	0.18
Thermolytic Degradation	50	1 month	1503.933	1496.250	0.51
Oxidative Degradation	50	12 hours	1503.933	1499.449	0.30
Photolytic Degradation	50	1 month	1503.933	1498.815	0.34
UV Degradation	50	48 hours	1503.933	1503.048	0.59

CONCLUSION

The proposed RP-HPLC method enables the determination of Drotaverine Hydrochloride because of good separation of chromatographic peaks. The method can be used successfully for the analysis of Drotaverine Hydrochloride in tablet dosage forms. Moreover, the proposed test procedure does not require any complicated mobile phase and it is simple isocratic method. The proposed method for the estimation of Drotaverine Hydrochloride can be routinely performed

for its accurate and precise quantification even in presence of the degradation as the t_R of all degradants in different conditions differ significantly.

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