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RESEARCH ARTICLE

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR THE DETERMINATION OF DROTAVERINE HYDROCHLORIDE

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Abstract:
A simple accurate and reproducible stability indicating RP-HPLC method with UV-visible spectrophotometer was developed for the analysis of Drotaverine hydrochloride. Efficient chromatographic separation was achieved on SHISEIDO CAP CELL PACK C_{18} column 250mm × 4.6 mm id, 5µm using 0.2% v/v formic acid and methanol (55:45) as mobile phase at floe rate 1 ml/min with column temperature 25º C. The method showed linearity over the range 40-180 µg/ml with correlation coefficient 0.9998.The drotaverine hydrochloride drug was subjected to acidic, alkaline, neutral, oxide, thermal and photolytic stress conditions. Drotaverine was found to be degrading significantly in alkaline, acid, oxide and photolytic stress conditions. Drotaverine hydrochloride was stable in neutral and thermal stress conditions. The developed method was validated according to ICH guidelines with respect to linearity, specificity, accurate, precise and robustness.

Keywords: Drotaverine hydrochloride, Anti spasmodic drug, RP-HPLC, Stability indicating study, ICH guide lines.

Introduction

There are many methods for thedevelopment and validation studies of drotaverine hydrochlorideas a drug substance as well as in pharmaceutical dosage forms can be determined by bioanalytical study but very few methods are available describing the forced degradation. Furthermore these methods are not impressionable to achieve the high throughput study which can be possible by optimizing the method. Hence, it can be maximum utilized for the analysis of formulation development and stability testing as well as at qualitycontrol laboratory for routine use.

The aim and scope of the proposed work are as under.

1. To develop rapid RP-HPLC method for quantification of the drug substance with highest selectivity, precision and accuracy.
2. Forced Degradation Study to confirm the stability of the drug substance.
3. Perform analytical method validation for the proposed method as per ICH Q2 (R1) guideline.

1.1 DRUG PROFILE[1]

Drotaverine hydrochloride is a crystalline powder with pale yellow colour, having molecular formula $C_{24}H_{31}NO_4.HCl$ and molecular weight of Avg. wt.397.5072 Monoisotopic 397.225308485. It is soluble in ethanol (96%), easily soluble in Chloroform, moderately soluble in Water. Drotaverine hydrochloride (DRO) causes smooth muscle relaxation by increasing intercellular levels of cyclic adenosine monophosphate (cAMP) secondary to inhibition platelet aggregation in a dose dependent manner. Drotaverine is a selective inhibitor of phosphodiesterase 4, and has no anti cholinergic effects. Drotaverine has been shown to possess dose-dependent analgesic effects in animal models. Hence it is used as an antispasmodic, prescribed for pain and dysfunction caused by smooth muscle spasm.

2. MATERIALS AND METHODS[2]

High-performance liquid chromatography (HPLC; formerly referred to as high-pressure liquid chromatography), is a technique in analytical chemistry used to separate the components in a mixture, to identify each component, and to quantify each component. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out the column. The chemicals and instruments used for the quantification of drug are mentioned below:

3. OPTIMISED CHROMATOGRAPHIC CONDITIONS: [3, 4, 5]

On the basis of literature survey, previous experience and several exploratory efforts, the chromatographic compatibility was achieved using Formic acid in water (0.2%v/v): Methanol (55:45) as an isocratic elution. This gives the best results as a mobile phase. The flow rate of 1 ml/min was selected as it gave good result, system suitability parameters and reasonable retention time. The retention time of Drotaverine hydrochloride was observed 10 min at 280 nm.

Selection of Detector and Wavelength: UV detector was selected, as it is reliable and

Wavelength was set to 280nm.

In this study buffer is not used because 0.2% formic acid and methanol (55:45) v/v gives sharp peak with good retention time. Usage of buffer is based on functional groups of the drug.

3.2 Mobile Phase Preparation

The mobile phase consist of Formic acid in water, 0.2%v/v: Methanol (55:45) was prepared by dissolving 2.0mL of formic acid was taken in 1000 ml of HPLC grade water which further mixed with methanol (55:45) ratio and filtered through 0.45µm filter followed by degassing in ultra sonic bath for 20 min.

3.3 Standard solution preparation

A Drotaverine hydrochloride standard solution containing 100µg/ml was prepared in a 100 ml volumetric flask by dissolving 10 mg Drotaverine Hydrochloride in 25 ml water- methanol (50:50, v/v) and then diluting to volume with water- methanol (50:50, v/v) to the mark. The sample was filtered through a 0.45 µm membrane filter.

3.4 Sample Solution Preparation

Twenty commercially available 80.0mg label claimed tablets of Drotaverine hydrochloride have weighed, and the average weight of a tablet was determined. From these, 5 tablets were weighed and transferred into a 500 ml volumetric flask and added 50 ml water- methanol (50:50, v/v) followed by sonication of minimum 30 min with intermittent shaking. Then the solution has brought back to room temperature and diluted to volume with water- methanol (50:50, v/v). 1.25 ml of above solution was pipette out and transferred into 10 ml volumetric flask followed by diluted to volume with diluents. The sample has filtered through a 0.45 µm membrane filter.

3.5 System Suitability Study

A system suitability test for the chromatographic system was performed before each validation experiment. Five replicate injections of standard preparation were injected and asymmetry, theoretical plate and % RSD of peak area were determined for same. Only after the system suitability results were in acceptance criteria the experiments were precede further.

4. Validation of the method[6, 7]

Method for the determination of Drotaverine Hydrochloride in bulk drug as well as in pharmaceutical dosage form is further validated as per ICH Q2 (R1) guideline. To prove this method, force degradation study was also being performed and also included. Validation of analytical method was performed using commercially available Drotaverine Hydrochloride (Drotikind 80) formulation as well as bulk drug substance equivalent to the formulation.

4.1. Specificity: It is the ability to assess the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

The evaluation of the specificity of the method was determined against placebo and stress (forced degradation) application. The interference of the excipients of the claimed placebo present in the

pharmaceutical dosage form was derived from placebo solution.

4.1.1. Standard Stock solution Preparation: 25.00 mg of Drotaverine hydrochloride working standard was accurately taken. The concentration of 1000 µg/ml Drotaverine hydrochloride was prepared and further diluted to get 100 µg/ml of analyte.

4.1.2 Test stock solution Preparation: 10 Tablets were accurately weighed (1896.00 mg) and transferred into 100 ml volumetric flask and the concentration of 8000 µg/ml was prepared. From which further concentration of 100 µg/ml was diluted. These solutions were prepared according to ICH guidelines.
Placebo stock solutions were also prepared.

4.2 Linearity Standard Solution Preparation:
From Standard Stock Solution of accurately 1000 µg/ml pipette out exact 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6 and 1.8 ml and dilute it up to 10 ml each with diluents to achieve 40-180 µg/ml concentration range.

A stock solution of the drug (1mg/ml) was prepared in diluents andfrom this eight concentrations of the drug were prepared in diluents within the concentration range of 40-180 µg/ml.
The linearity plot was prepared with 8 concentration levels (40, 60, 80, 100, 120, 140, 160 and 180 µg/ml of Drotaverine hydrochloride). The peak areas vs. concentration data were evaluated by linear regression analysis.

4.3 Precision
Precision study was established by evaluating method precision and intermediate precision study. Method precision of the analytical method was determined by analyzing six sets of sample solution preparation and mean % assay value, standard deviation and % relative standard deviation for the same was calculated.

4.3.1 Standard Solution Preparation for Precision Study:
Standard Stock Solution prepared from 1000 µg/ml, to accomplish 100 µg/ml concentrations.
Test stock solution was prepared through ICH guidelines only and also prepared six Test preparation Sets. Same approach was applied for the intermediate precision study on the second day with different analyst and different raw materials.

4.4 Accuracy
This Experiment can be performed by the recovery test. Recovery of the method was evaluated at 3 different concentration levels (Generally corresponding to 50, 100 and 150% of test solution concentration) by addition of known amounts of standard to placebo preparation. For each concentration level, 3 sets were prepared and injected in duplicate.

4.4.1 Standard preparation:
Stock solution and standard solution has been prepared as per ICH guidelines.

4.5 Robustness Study
Robustness of the method was evaluated by assaying test solutions under slight but deliberate changes in analytical conditions, such as change in flow rate and change in proportions of Buffer-Methanol (57:43 and 53:47, v/v)

4.6 Flow Rate change: In this experiment the test samples were analyzed at the flow rate of 0.9 ml/min and 1.1 ml/min each and the results were observed in terms of assay value and chromatographic compatibility (System Suitability Test).Blank, Standards and Sample solutions were prepared as per the same procedure mentioned followed by ICH guidelines.

4.7 Mobile Phase Proportion Change: In this experiment the test samples were analyzed at the mobile phase proportion of 57:43 and 53:47, v/v (Buffer: Methanol) each and the results were observed in terms of assay value and chromatographic compatibility (System Suitability Test).

Blank, Standards and Sample solutions were prepared as per the ICH guidelines.

4.8 Forced degradation study [8-22]
Forced degradation studies were performed to provide an indication of the stability indicating property of the drug. Drug at a concentration of 1mg /ml was used in all degradation studies. The degradation samples were prepared and employed for acidic, alkaline, neutral, oxidant, thermal and photolytic conditions. After the degradation treatments were completed, the stress content solutions were diluted with diluents to attain about 100µg/ml concentration.

4.9 Preparation of samples for HPLC analyses:
For hydrolysis study during 0.1N HCl, 0.1 N NaOH and oxidative study during 3% H_2O_2 the samples were diluted 100 times with water to a concentration of 100 µg /ml. The solution was diluted with water to get a concentration of 100 µg/ml for the above mentioned studies.

Degradation behavior of Drotaverine Hydrochloride:
HPLC studies of sample obtained on Stress testing of drotaverine hydrochloride under different stress conditions using 0.2% formic acid and methanol (55:45) as the mobile phase system.
Suggested following degradation behavior:

A. Acidic degradation studies:
It was observed that the drug gets slowly degraded about 17.21% in strongly acidic conditions over a 24 h at 100°C. As shown in the chromatogram No.17 the degradation of the drug resulted in five degraded products (DP1-5) withretention time at 5.009 R_t. This indicates that the drug was hydrolyzed under higher acidic conditions.

B. Alkaline degradation studies:
In alkali, the drug was found to decompose almost 27.24% after refluxing for 24 h at 100°C in 1N NaOH. As shown in below chromatograph No.18 the degradation of the drug resulted in five degraded products (DP1-5) with respect to the peak of drotaverine hydrochloride at 5.020 min R_t.

C. Neutral degradation studies:
In neutral condition mild degradation was occurred. 9.82% degradation was seen after reflux for 24 h at 100°C in water. As shown in chromatograph no.19 the drug degradation resulted in five degraded products (DP1-5) but there was no significant rise in the height of the peak with time.

D.Oxidative degradation studies:
The drug was found to be stable 3% H_2O_2 at room temperature. However about 16.84% drugs degradation was observed on exposure to elevated temperature 100° C. As shown in chromatograph No.20 several peaks are resulted in that eight degraded products (DP1-8).This signifies that the drug was degraded in oxidative conditions.

E.Thermal degradation studies:
The drotaverine hydrochloride was found to be degraded in very negligible amount as 6.82% drug was degraded on heating drug at 100°C for 48 h in hot air oven. As shown in chromatograph No.21 the degraded products (DP1-5) were observed with respect to the peak of drotaverine hydrochloride (DRT) at 5.310 R_t. Hence drug was stable in thermal Stress conditions.

F.Photolytic degradation studies:
Photolytic degradation studies were carried out in dry form. Here the drug was directly exposed to the sun light for 48 h on a hot sunny day. Total 13.16% of drug was degraded in this study. As shown in chromatograph No.22 seven degraded products are appeared but there was no significant rise in the height of the peak with the time.

Chromatogram no.5-X axis = Concentration (µg/ml) Y axis = Peak Area
Correlation coefficient of the linearity study was found to R2 = 0.9998 with linear regression equation y = 25174x - 16560, which proves the method is linear over the working range 40 – 180 µg/ml.

All the results of LOD and LOQ data were within the acceptance criteria, hence it can concludethat the LOD and LOQ of the method was 0.15 µg/ml and 0.6 µg/ml respectively which correspond to 0.6% and 0.15% of working concentration.

Overall the data for the precision study suggest % Assay value for each Test Preparation is between 98 – 102% which is under the acceptance criteria while % RSD of all results are less than 2%. Hence from all the observation it can conclude that the proposed method is highly precise.

From the all above data it has been proven that the % recovery is within the limit of 98 to 102 % this is in the limit of acceptance criteria and % RSD value of % recovery of replicate set is below 2 % .Hence this suggest that proposed method is highly accurate.

ROBUSTNESS STUDY
A).Flow Rate change: Experimental HPLC sequence for the robustness study -01
Chromatogram of Drotaverine hydrochloride at Flow Rate 1.1 ml/min.
The assay value of test preparation was 101.24% and 99.15% at 0.9 and 1.1 ml/min respectively.

B).Mobile Phase Proportion Change
The assay value of test preparation was 100.64% and 99.39% at 57:43 (Buffer: Methanol, v/v) and 53:47 (Buffer: Methanol, v/v) respectively.
The results of percentage degradation shown by drotaverine hydrochloride in different stressed conditions are given in above Tables.
From the above Table, it can be concluded that, drotaverine hydrochloride stable in thermal and neutral Stress conditions but it is degraded under acidic, basic, neutral oxidative conditions.

CONCLUSION
An accurate and rapid stability indicating assay method was developed for the determination of drotaverine hydrochloride by using RP-HPLC.

The surveillance and outcome obtained from each validation experiment including specificity, linearity and range, LOD and LOQ, precision, accuracy, robustness and system suitability lies well inside the acceptance criteria. Since, all the results are with-in the limit, the developed Analytical method is considered as validated and suitable for anticipated use.

Drotaverine was subjected to hydrolytic (acidic, alkaline and neutral), oxidation, photolytic and thermal stress conditions as per ICH guidelines Q1A (R2).

Table no.1

List of Chemicals and Reagents

Methanol HPLC Grade	Spectrochem Pvt. Ltd.
Formic acid HPLC Grade	Merck Pvt. Ltd.
De-ionized water HPLC Grade	Milli Q
Hydrochloric acid Analytical Grade	Merck Pvt. Ltd.
NaOH Analytical Grade	Merck Pvt. Ltd.
3 % v/v H_2O_2	Merck Pvt. Ltd.

List of Instruments

RP-HPLC	Cyber Lab
Electronic balance	Shimadzu
Ultra sonicator	Spin co
P^H meter	Eli co L127
Cyclone mixture shaker	Spin co
Uv-Vis chamber	Eli co
Melting point apparatus	Eli co L125
Micro pipettes	Cyber Lab

3.1 Drug Material

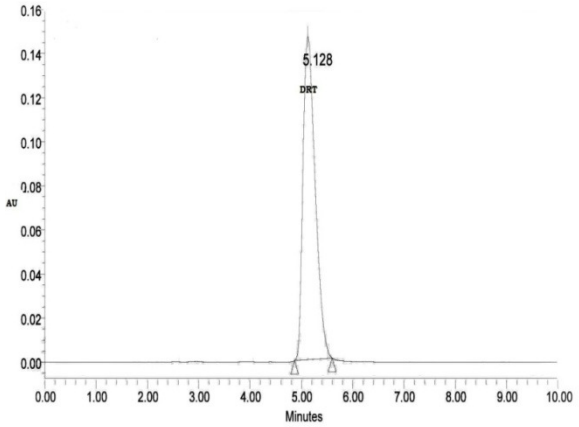
Standard drug	Drotaverinehydrochloride-Emcure
Commercial sample	Drotikind 80 mg – Windlas Biotech limited

Chromatographic Conditions

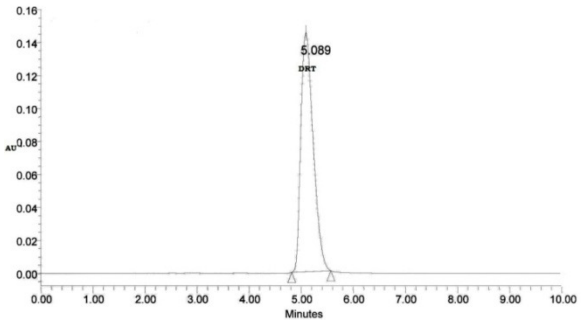
Chromatographic Conditions	Specifications
Column	250 mm × 4.6 mm id,5 µm
Flow rate	1 m l/min
Temperature	Ambient
λ max	280 nm
Injection volume	20 µl
Run time	10 min

Specificity

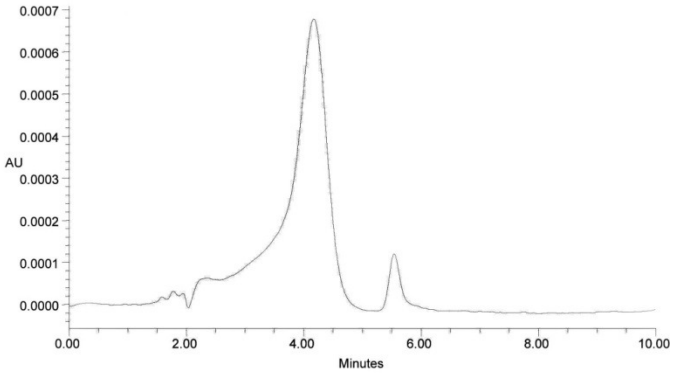
Chromatogram no.1-Chromatograph of working standard



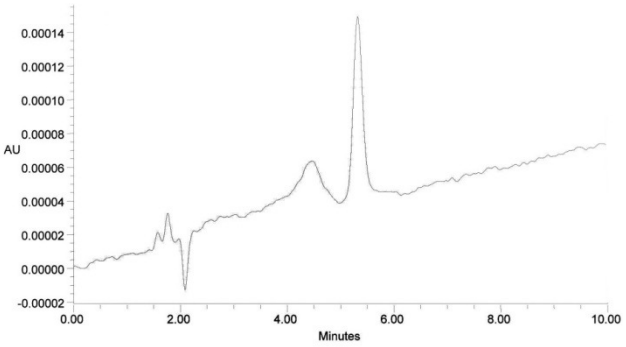
Chromatogram no.2-Chromatograph of Commercial sample



Chromatogram no.3-Chromatogram of Placebo

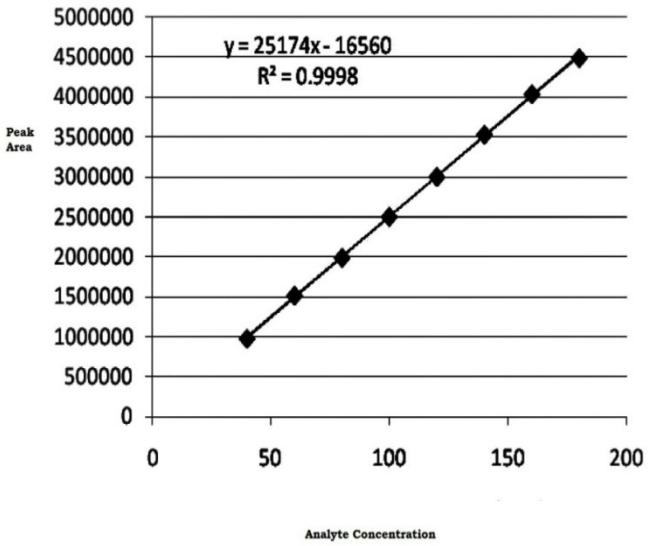


Chromatogram no.4-Chromatogram of Diluents (water and methanol 50:50)



Linearity and Range – Table no.4 Concentration Vs Peak area response data

Linearity Level	Concentration (µg/ml)	Peak Area
1	180	4485622
2	160	4034192
3	140	3525684
4	120	2998577
5	100	2499589
6	80	1988284
7	60	1516129
8	40	972630

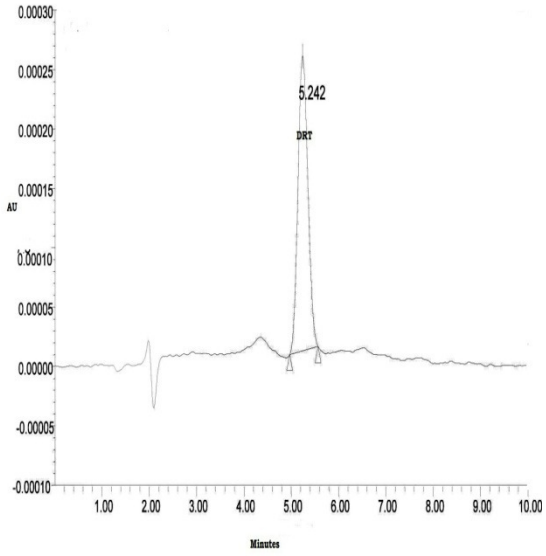


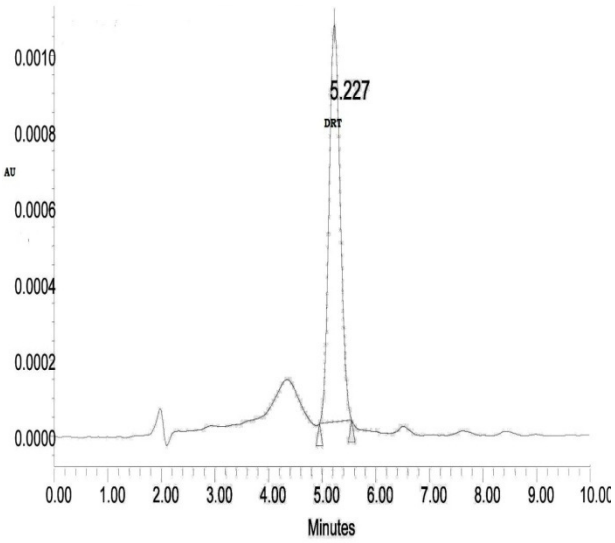
Regression Analysis chart of linearity Study

Tableno.3 Limit of Quantification - Precision data

Injection	Peak Area
1	14439
2	14162
3	13500
4	14374
5	13499
6	14339
Mean	14052
Std. Dev.	437.82
% RSD	3.12

Chromatogram no.3





Limit of Detection (LOD)

Limit of Quantification (LOQ)

All the results of LOD and LOQ data were within the acceptance criteria, hence it can conclude that the LOD and LOQ of the method was 0.15 µg/ml and 0.6 µg/ml respectively which correspond to 0.6% and 0.15% of working concentration.

Table no.4: Method Precision Data

Samples	Replicates	Peak Area	Mean Area	%Assay
Set 01	Injection 01	2483591	2475255	99.50
	Injection 02	2471654		
	Injection 03	2470520		
Set 02	Injection 01	2484772	2492891.3	101.03
	Injection 02	2496635		
	Injection 03	2497267		
Set 03	Injection 01	2494730	2493557.3	99.72
	Injection 02	2493864		
	Injection 03	2492078		
Set 04	Injection 01	2506943	2508589	98.97
	Injection 02	2502602		
	Injection 03	2516222		
Set 05	Injection 01	2497254	2487344.7	101.09
	Injection 02	2485686		
	Injection 03	2479094		
Set 06	Injection 01	2511534	2494333.3	101.05
	Injection 02	2496617		
	Injection 03	2474849		

Table no.5: Intermediate Precision Data

Samples	Replicates	Peak Area	Mean Area	%Assay
Set 01	Injection 01	2390183	2439334	99.08
	Injection 02	2477152		
	Injection 03	2450667		
Set 02	Injection 01	2480432	2461131.67	100.32
	Injection 02	2475165		
	Injection 03	2427798		
Set 03	Injection 01	2454846	2474639	100.80
	Injection 02	2481159		
	Injection 03	2487912		
Set 04	Injection 01	2490325	2470574.33	100.11
	Injection 02	2470837		
	Injection 03	2450561		
Set 05	Injection 01	2470283	2462095	99.79
	Injection 02	2480574		
	Injection 03	2435428		
Set 06	Injection 01	2483442	2463636	99.70
	Injection 02	2450497		
	Injection 03	2456969		

Overall the data for the precision study suggest % Assay value for each Test Preparation is between 98 – 102% which is under the acceptance criteria while % RSD of all results are less than 2%. Hence from all the observation it can conclude that the proposed method is highly precise.

Table no.6

Accuracy			
Replicates	**Area**	**Mean**	**Mean of each level**
Level-1 set 1 inj 01	1213632	1216513	1216267
Level-1 set 1 inj 02	1219394		
Level-1 set 2 inj 01	1210291	1217075	
Level-1 set 2 inj 02	1223858		
Level-1 set 3 inj 01	1216950	1215214	
Level-1 set 3 inj 02	1213478		
Level-2 set 1 inj 01	2377778	2427429	2458153
Level-2 set 1 inj 02	2477079		
Level-2 set 2 inj 01	2453804	2460850	
Level-2 set 2 inj 02	2467895		
Level-2 set 3 inj 01	2484794	2486182	
Level-2 set 3 inj 02	2487570		
Level-3 set 1 inj 01	3698871	3658477	3654836
Level-3 set 1 inj 02	3618082		
Level-3 set 2 inj 01	3621359	3643106	
Level-3 set 2 inj 02	3664853		
Level-3 set 3 inj 01	3671323	3662924	
Level-3 set 3 inj 02	3654525		

Table no.7: Summary of Accuracy Data-02

Accuracy (Recovery) Study							
Accuracy level	**Set no**	**Amount added**(µg/ml)	**Amount found**(µg/ml)	**Recovery (%)**	**Average recovery**	**Std Dev.**	**% RSD**
I (50%)	1	50.13	50.09	99.92	99.9	0.06	0.06
	2	50.10	50.12	100.04			
	3	50.21	50.18	99.94			
II (100%)	1	98.21	98.64	100.44	99.9	0.72	0.72
	2	99.85	100.00	100.15			
	3	100.98	101.03	99.07			
III (150%)	1	149.76	148.66	99.27	99.2	0.11	0.11
	2	149.25	148.04	99.19			
	3	150.27	148.84	99.05			

From the all above data it has been proven that the % recovery is within the limit of 98 to 102 % this is in the limit of acceptance criteria and % RSD value of % recovery of replicate set is below 2 % .Hence this suggest that proposed method is highly accurate.

ROBUSTNESS STUDY

A).Flow Rate change: Experimental HPLC sequence for the robustness study -01
Chromatograms of Drotaverine hydrochloride at Flow Rates 0.9ml/min and 1.1 ml/min were observed.
The assay value of test preparation was 101.24% and 99.15% at 0.9 and 1.1 ml/min respectively.

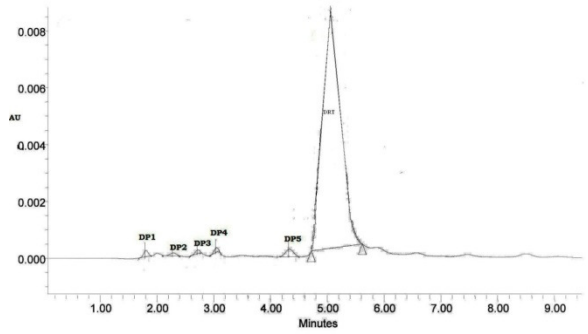
B).Mobile Phase Proportion Change: Experimental HPLC sequence for the robustness study -02- determines the ratios of 57:43 (0.2%formicacid: methanol) and 53:47 (0.2%formicacid: methanol) gives the robustness study**.** The chromatograms and results of Robustness: Mobile Phase Ratio (53: 47) and (57: 43) ratio were compared and observed and the assay value of test preparation was 100.64% and 99.39% at 57:43 (Buffer: Methanol, v/v) and 53:47 (Buffer: Methanol, v/v) respectively.

Summary of robustness study -Table no.8

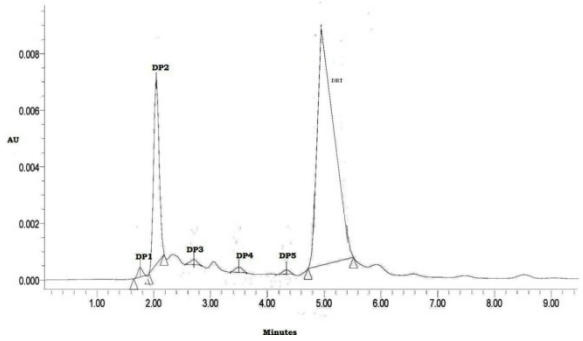
Summary of robustness study				
Robust condition	**% Assay**	**Retention Time (min)**	**System Suitability**	
			Theoretical plates	**Asymmetry**
Flow change 0.9 ml/min	101.24	5.91	10824	1.26
Flow change 1.1 ml/min	99.15	4.57	10773	1.29
MP proportion change 57:43	100.64	6.89	10989	1.28
MP proportion change 53:47	99.39	4.35	11799	1.23

Table no.9: Results of System Suitability Test after each Validation Experiment

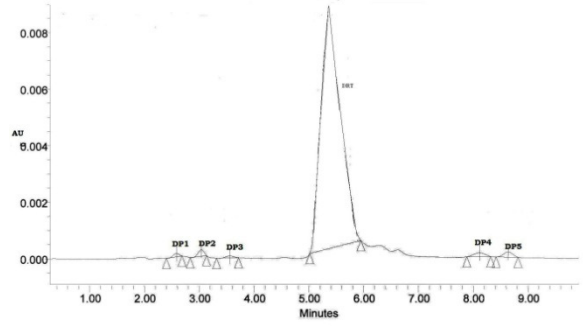
Summary Of System Suitability Study			
Experiment Name	**Theoretical Plates**	**Asymmetry**	**% RSD**
Specificity	10832	1.28	0.67
Linearity and Range	10989	1.22	0.87
LOD and LOQ	10873	1.23	1.30
Method Precision	11593	1.25	1.13
Int.Precision	11138	1.29	0.76
Accuracy	11292	1.23	0.42
Robustness	10850	1.25	0.56



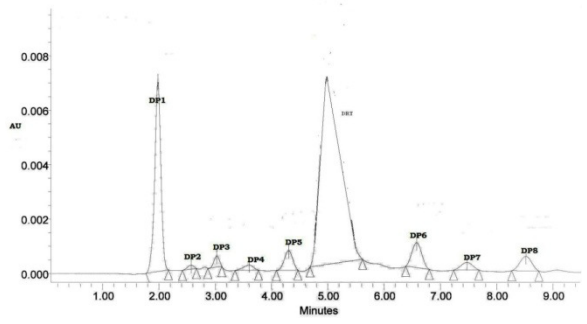
Chromatogram no.4- Acidic Degradation



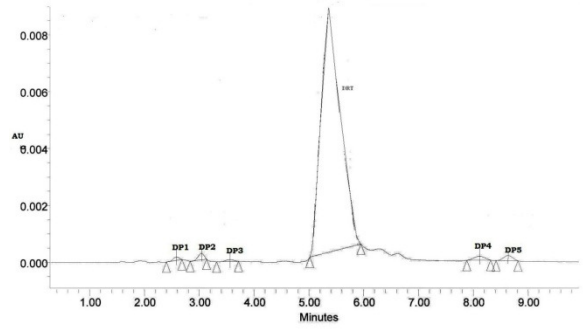
Chromatogram no.5- Alkali Degradation Study



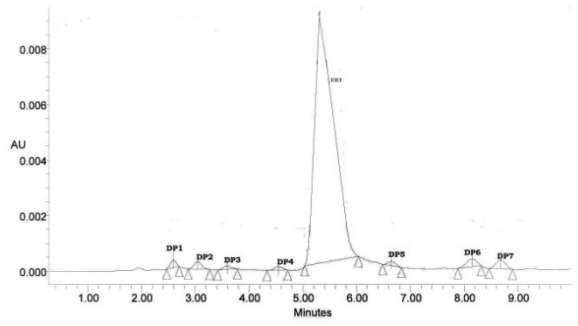
Chromatogram no.6- Neutral degradation



Chromatogram no.7- Oxidative Degradation Study



Chromatogram no.8-Thermal Degradation Study



Chromatogram no.9- Photolytic Degradation Study

Table no.10: Results of stressed degradation studies of drotaverine hydrochloride

Stress degradation studies	Initial	Total peak area	Intact drug found	Degraded drug found
Acidic degradation	643889	579904	82.79	17.21
Alkali degradation	639009	480901	72.76	27.24
Neutral degradation	665787	632244	90.18	9.82
Oxidative degradation	658890	576908	83.16	16.84
Photolytic degradation	665909	550909	86.84	13.16
Thermal degradation	671123	672233	93.18	6.82

From the above Table, it can be concluded that, drotaverine hydrochloride stable in thermal and neutral Stress conditions but it is degraded under acidic, basic, neutral oxidative conditions.

Drotaverine hydrochloride is however susceptible for hydrolysis in all the hydrolytic conditions with order of degradation as Alkaline > Acid > Oxidation > Photolytic > Neutral > Thermal stress conditions.

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