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Stability Indicating RP-HPLC Method Development for Drotaverine Hydrochloride Using PDA Detection

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A sensitive, selective, precise and stability-indicating HPLC method for analysis of drotaverine hydrochloride both as bulk drug and in formulation was developed and validated. The drug was subjected to acid, base hydrolysis (0.1 M HCl and 0.1 M NaOH) and oxidative decomposition (2.5 % v/v H_2O_2). The study was carried out from room temperature to 60 ºC and observed for 10 % decomposition. The degradation products were separated on a C_8 (phenomenax, 250 mm × 4.6 mm) chromatographic column with mobile phase comprising of methanol: 0.05 M KH_2PO_4 (pH 3.5, 55:45 v/v) pumped at 1 mL/min. The drug was estimated at 241 nm. Retention time of drotaverine hydrochloride was 8.705 min, whereas retention time of base degradation products were 3.696, 4.001 and 6.532 min. The drug was relatively stable to acidic and oxidative degradation conditions, where the degradation occurred in a tune of 10-15 %. The method was validated in accordance with ICH guidelines. The developed method is stability indicating and can be used in drug development and testing laboratory for the quality control.

Key Words: Drotaverine hydrochloride, Hydrolytic degradation, Oxidative degradation.

INTRODUCTION

Drotaverine hydrochloride, (DH; 1-[(3,4-Diethoxy phenyl) methylene]-6,7-diethoxy-1,2,3,4-tetra hydro isoquinoline (Fig. 1) is an analogue of papaverine[1]. It acts as an antispasmodic agent by inhibiting phosphodiesterase(IV) enzyme, specific for smooth muscle spasm and pain, used to reduce excessive labour pain[2]. Drotaverine hydrochloride is official in pharmacopoeia of Poland[3]. A few UV spectrophotometric[4-9] and HPLC[10-14] methods are reported. Till date, there is no reported method which describes the hydrolytic and oxidative degradation for drotaverine hydrochloride. So, it was thought worthwhile to develop a RP-HPLC method for the separation of the hydrolytic and oxidative degradation products.

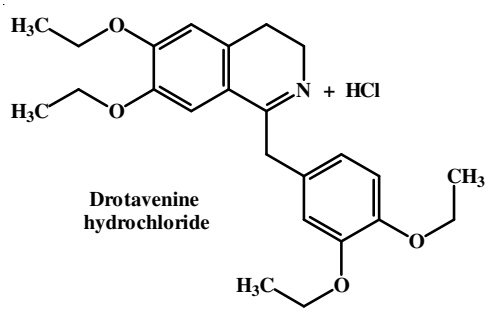

Fig. 1. Structure of the analyte

EXPERIMENTAL

All solvents were of HPLC grade (E-Merck, Mumbai). Drotaverine hydrochloride (99.5 % pure) was procured from Glen-Mark Pharmaceuticals, Nasik, India. HPLC grade water was obtained by double distillation in glass and purification through RO water purification system (Canpex, Mumbai). Water was filtered through 0.22 mm filters.

Preparation of stock solution: Drotaverine hydrochloride (100 mg) was accurately weighed and transferred to a 100 mL volumetric flask; dissolved in methanol to obtain a stock solution of 100 µg/mL. From this stock solution, working standard solutions of drugs were prepared by appropriate dilutions. Linearity was set by injecting standard solutions of 04-24 µg/mL on C_8 chromatographic column.

Stress conditions for degradation study

Protocol for stress testing: There were four samples prepared in each testing procedure; (a) Blank solution stored under normal condition; (b) Blank solution subjected to stress condition similar to that of drug; (c) Zero time sample containing the drug which is stored under normal condition (control) and (d) Drug solution subjected to stress condition.

Hydrolytic study of drotaverine hydrochloride (HCl / NaOH): Each mL of stock solution was subjected to 0.1 M HCl and 0.1 M NaOH, separately. The solutions analyzed periodically at different time intervals by exposing up to 70 ºC.

Oxidative degradation study: 1 mL of stock solution was subjected to 2.5 % v/v H_2O_2. The study was carried out over a temperature range of room temperature to 60 ºC.

General procedure for stress testing: In every degradation condition, four samples, a, b, c and d were prepared. Since the samples had to be withdrawn at regular intervals a sufficient quantity of stock *i.e*. 100 mL was prepared. The samples were stored under refrigeration at 4 ºC to stop further reaction, whenever necessary.

From each of above stress condition 1 mL of solution was withdrawn periodically and suitably diluted to get 10 µg/mL concentrations using the mobile phase. The diluted samples were injected to chromatographic system to study the % degradation of drotaverine hydrochloride and appearance of any degradation peak. Blanks and control were used to compare the normal concentration of drug with the degradation outcome using peak area. All the solutions were covered with aluminium foil to avoid any light enhanced reaction.

Chromatographic system and conditions: Analysis was performed with a Shimadzu (Japan) chromatograph equipped with an LC-10 AT *vp* solvent-delivery module, an SPD-10A UV-visible PDA detector and a rheodyne model 7725 I injector valve with 20 µL sample loop. Drotaverine hydrochloride and degradation products were separated on a C_8-Phenomenex (250 mm × 4.6 mm i.d., 5-µm particles) under reversed-phase partition chromatographic conditions. The mobile phase was a mixture of methanol, KH_2PO_4 (0.05 mM, pH 3.5 ± 0.1 adjusted with 85 % phosphoric acid) in the ratio 55:45 v/v. With a flow rate of 1.0 mL/min the analyte was monitored at 241 nm. The optimized chromatographic parameters are presented in Table-1.

TABLE-1
COLUMN PERFORMANCE AND PEAK PARAMETERS OF THE HPLC METHOD

Parameter	Drotaverine hydrochloride
Retention Time (min)	8.705
Theoretical plates (*N*)	10714
Tailing Factor (*T*)	1.08

Validation procedure: The method developed was validated according to ICH guidelines for being specific, sensitive, accurate and precise.

System suitability: The system suitability was assessed by six replicate analyses of the drug at a concentration of 10 µg/mL. The acceptance criterion was ± 2 % for the per cent coefficient of variation (% CV) for the peak areas and retention times.

Sensitivity: Limit of detection (LOD) and limit of quantitation (LOQ) were estimated from the signal-to-noise ratio. The detection limit was defined as the lowest concentration level resulting in peak area of three times the baseline noise. The quantitation limit was defined as the lowest concentration level that provided a peak area with a signal-to-noise (S/N) ratio higher than 10, with precision (% CV) and accuracy (% bias) within ± 10 %.

Accuracy and precision: Accuracy of the assay method was determined for both intraday and interday variations using the triplicate analysis of the quality control samples. Precision of the assay was determined by repeatability (intra-day) and intermediate-precision (interday). Repeatability refers to the use of analytical procedure within a laboratory over a short period of time that was evaluated by assaying the quality control samples during the same day. Intermediate precision was assessed by comparing the assay concentrations on different days (3 days). The quality control samples containing 8, 10 and 12 µg/mL of drotaverine hydrochloride were divided into equal portions and each was analyzed (n = 10) as a separate sample.

RESULTS AND DISCUSSION

As per the validation procedure, the method was found to conform ICH guidelines for system suitability, sensitivity, accuracy and precision. The results for validated parameters are presented in Table-2. The samples of degradation study were injected to chromatographic system time to time to observe any additional peaks and % degradation using peak area of drotaverine hydrochloride. Over a period the degradation of drotaverine hydrochloride was considerable in strong conditions. At elevated temperature the degradation was much faster than room temperature. The per cent degradation of drotaverine hydrochloride was found to be 1, 2, 9 and 14 respectively for 30, 40, 50 and 60 ºC after an exposure of 8 h in acid.

TABLE-2
VALIDATION OF CHROMATOGRAPHIC SYSTEM FOR DEGRADATION STUDY

Parameter	Observation
Linearity	04–24 µg/mL
Correlation coefficient	0.9994
LOD	0.6 µg/mL
LOQ	2.0 µg/mL
Precision	
Intra-day (% RSD)	0.49
Inter-day (% RSD)	0.27
Accuracy (± S.D.)	99.83 ± 0.32

In basic condition the per cent degradation of drotaverine hydrochloride after 8 h was 8, 29, 51 and 76 respectively for 30, 40, 50 and 60 ºC. The oxidative hydrolysis with 2.5 % H_2O_2 resulted in 13 % of degradation in 8 h. The time taken to attain a 10 % degradation of drotaverine hydrochloride in hydrolytic and oxidative condition is presented in Table-3.

TABLE-3
TIME REQUIRED FOR A 10% DEGRADATION OF DROTAVERINE HYDROCHLORIDE IN HYDROLYTIC AND OXIDATIVE CONDITIONS

Temperature (ºC)	Time (h)		
	Acid	Base	H_2O_2
30	16	9.0	16
40	12	5.5	13
50	9	2.5	10
60	6.5	1.0	7

Retention time of drotaverine hydrochloride was found out to be 8.705 min (Fig. 2a). Degradation of drotaverine hydrochloride under acidic condition did not yield any degradation products, though degradation occurred to an extent of 14 %, (Fig. 2b). Treatment of drotaverine hydrochloride to

basic condition yields three degradation products at 3.696, 4.001 and 6.532 min (Fig. 2c). Under oxidative stress conditions degradation occurred to the tune of 13 % (Fig. 2d).

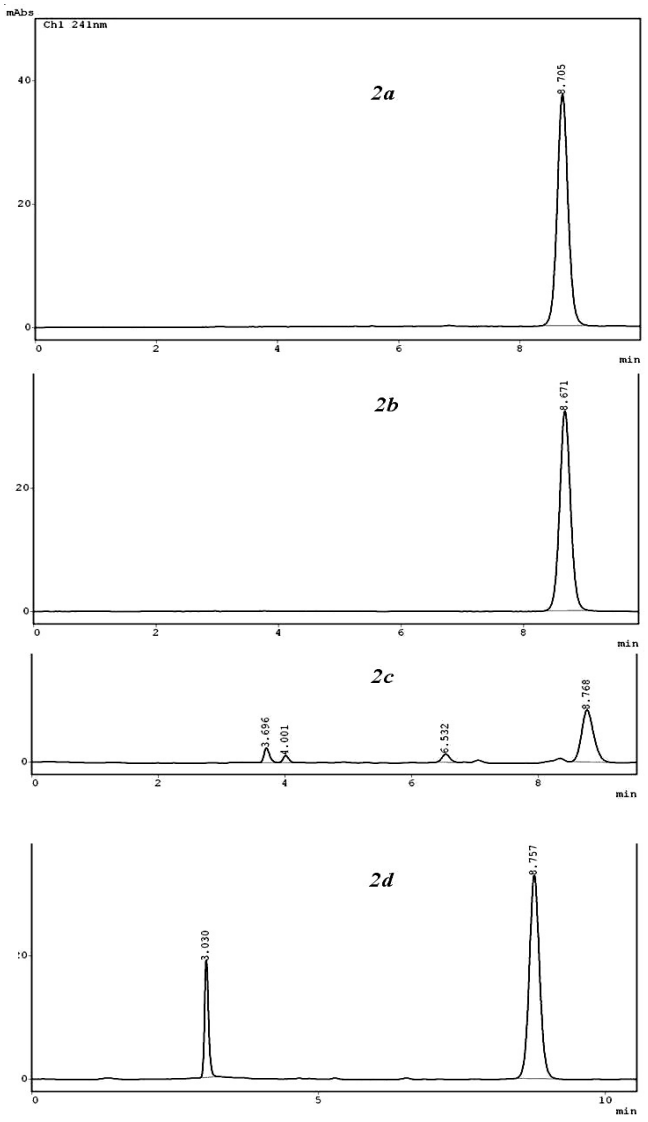

Fig. 2. Chromatograms obtained for various degradation conditions; Fig. 2a. Describes control drug peak; Fig. 2b. Shows acidic degradation condition chromatogram; Fig. 2c. Presents basic degradation condition chromatogram and Fig. 2d. Shows oxidative condition chromatogram of drotaverine hydrochloride

Conclusion

A stability indicating RP-HPLC method is developed for estimation of drotaverine hydrochloride in the presence of hydrolytic and oxidative degradation products. The method is simple, precise, sensitive and accurate. The statistical analysis proved that the method is reproducible. The method is useful in drug development and drug testing laboratory for quality control for drotaverine hydrochloride.

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