A VALIDATED RP-HPLC METHOD FOR ESTIMATION OF DROTAVERINE HCL IN PHARMACEUTICAL TABLET DOSAGE FORM

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ABSTRACT

An accurate, sensitive, precise and robust reverse phase HPLC method was developed for the determination of the Drotaverine HCl in pharmaceutical dosage form and validated. Chromatographic separation was conducted on an Hypersil C18 (150 × 4.6 mm), 5µ column from Shimadzu in isocratic mode, with mobile phase pH 3.0 Potassium dihydrogen phosphate and Di potassium hydrogen phosphate buffer and Acetonitrile. The flow rate was 1.0ml/ min and effluent was monitored at 210 nm. The retention time was 3.140min. The method was found to be linear in the range of 10-60µg/ml with Correlation Coefficient of 0.9992. As per ICH guide lines the method was validated over the range of 80-120 ug/ml for the three analytes, and is accurate (average accuracies of three different concentrations ranged from 98 to 102% for Drotaverine HCL) and precise. The LOD and LOQ values of Drotaverine were 2.80 and 4.77 respectively. The proposed method is simple, selective, reproducible, sensitive and accurate with good precision.

Keywords: Drotaverine HCl, RP-HPLC method, Formulations, Validation.

INTRODUCTION

Drotaverine is chemically 2, 3, 4-Tetrahydro-6, 7-diethoxy-1 3, 4-diethoxyphenyl) methylene)-isoquinoline hydrochloride. Aits empirical formula is $C_{24}H_{31}NO_4 \cdot HCl$; $C_{24}H_{32}ClNO_4$, having a molecular weight of 433.97. . It acts as an antispasmodic agent by inhibiting phosphodiesterase IV enzyme, specific for smooth muscle spasm and pain, used to reduce excessive labor pain[1]. Drotaverine hydrochloride is official in Polish Pharmacopoeia[2]. Its chemical structure was given in Fig 1.
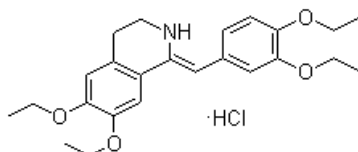

Fig. 1: Structure of Drotaverine HCl

Drotaverine hydrochloride is highly potent spasmolytic agent[3]. Several analytical methods that have been reported for estimation of drotaverine hydrochloride are spectrophotometry [4, 5, 6], HPLC[7, 8, 9], thin layer chromatography [10,11]and voltametry[12]. Only very few HPLC estimations have been reported in the literature for the determination of Drotaverine hydrochloride present in biological fluids. There are no reported methods for the determination of Drotaverine HCl by HPLC in pharmaceutical dosage forms. Hence an attempt was made to develop a HPLC method for the determination of Drotaverine HCl in pharmaceutical formulations.

MATERIALS AND METHODS

Reference standard of Drotaverine HCl was obtained as gift sample from NATCO Pharmaceuticals, Hyderabad. Marketed product Drotaverine hydrochloride (40mg and 80mg) (DROTIN) Martin and Harris laboratories Ltd. was procured from local pharmacy. Potassium Di Hydrogen Phosphate and Di Potassium Hydrogen Phosphate used were of AR grade, Water was of Milli-Q grade and Acetonitrile was of HPLC grade.

LC108ATvp SHIMAZDU, isocratic pump with UV-VISIBLE detector equipped with SPINCHROM software instrument was used as chromatograph.

Mobile phase

Acetonitrile and Mixed Buffer were mixed in the ratio of 80:20 and sonicated to degas.

Buffer preparation

Weigh accurately 0.295g of Potassium dihydrogen Orthophosphate and 0.108 g of Di Potassium hydrogen phosphate and dissolve in 900ml of Milli-Q water. Adjust the pH to 4.0 with Ortho phosphoric acid and make up the volume to 1000ml with Milli-Q water and then filter through 0.45µm nylon membrane filter and degas.

Chromatographic conditions

Flow rate was 1.0 ml/min. Column used was Hypersil, C_{18}, 150 x 4.6 mm, 5µ with Detector wave length at 210nm. Column temperature was Ambient with Injection volume of 20µl and Retention time was 3.143 mins with Tailing Factor of 1.457. The mobile phase ratio was 80:20 in Acetonitrile and Mixed buffer.

Preparation of Standard solution

Weigh accurately about 25mg of Drotaverine HCL in a 25 ml of standard, dissolve a little amount of mobile phase and make up with the mobile phase. From this pipette out 1ml into 25 ml of standard flask and dilute to volume with mobile phase.

Preparation of sample solution

Weigh 20 Tablets and Powder them Weigh accurately about 100 mg equivalent of Drotaverine HCL in a 100 ml volumetric flask, dissolve in mobile phase and make up the volume with the mobile phase. From this pipette out 10 ml in to 100 ml standard flask and make up the volume with the mobile phase. The solution was filtered through the nylon Millipore filter paper and 20µl of this solution was injected and chromatogram was recorded. Fig.2

The amount of Drotaverine HCL present in each Tablet formulation was calculated by comparing the peak area of the standard. Assay data was given in Table.1

Amount of drug in each capsule of Drotaverine HCL =

Spl. Area × Std.Wt × Spl. Dilution× Std. purity ×100 × Avg.Wt

Std.Area× Spl .wt× Std. dilution × L.C×100

Table 1: Assay of Tablet formulation

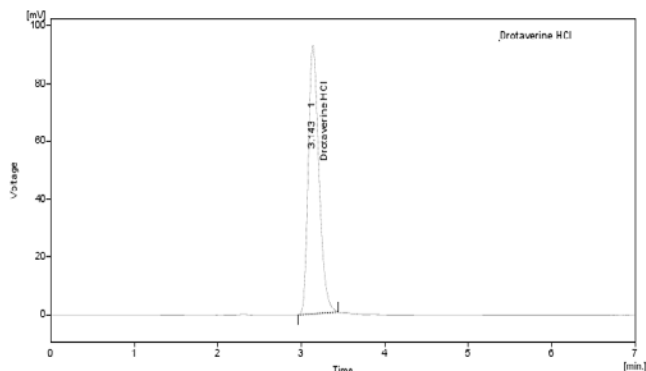| Sample	Label claim	Peak area	Retention time	Percent Purity
Drotaverine HCL	40mg	1418.1105	3.140 min	99.61%

Fig. 2: A Typical Chromatogram of Drotaverine HCl

Validation of Method

The described method has been validated for estimation of Drotaverine HCl using following parameters.

System Suitability

A Standard solution was prepared by using Drotaverine HCL working standards as per test method and was injected ten times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from ten replicate injections for Drotaverine HCL retention times and peak areas. The %RSD for retention times and peak areas were found to be within the limits. The data was given in Table 2.

Table 2: System Suitability Parameters

Parameters	Drotaverine HCl
Retention time(min)	3.140
Peak Area	1415.046
USP Plate count	2886.856
USP Tailing	1.448

Specificity

A study was conducted to demonstrate the effective separation of degradants from Drotaverine HCl, Separate portions of Drug product exposed to following stress conditions to induce degradation.

a) Acid degradation

b) Base degradation

Stressed samples were injected into the HPLC system with UV-VISIBLE detector by following test method conditions. All degradant peaks were resolved from Drotaverine HCl peak in the chromatograms of all samples and placebo did not shown any considerable peaks under the above conditions. The chromatograms of stressed samples were evaluated for peak purity of Drotaverine HCl using SPINCHROM software. For all forced degradation samples the degradants should not interference in quantitating the Drotaverine HCl. Purity angle was found to be less than threshold angle in all forced degradation studies without having signs of purity flags. Thus, this method is considered to be "Stability Indicating". The specificity data was given in Table 3.

Table 3: Specificity study data

Degradation mechanism condition	Observation
Acid degradation 0.1 N HCl Reflux – 30.0 min	No interference at RT of analyte peak
Base degradation 0.01 N NaOH Reflux 30.0min	No interference at RT of analyte peak

Precision

Precision was evaluated by carrying out six independent sample preparation of a formulation. The sample solution was prepared in the same manner as described in sample preparation. Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day (intra day) and day to day (interday) variations, which proves that method is precise. The results for precision are given in Table 4.

Table 4: Precision data

No. of samples	Concentration of Drotaverine HCl (µg/ml)	Peak Areas	Mean	SD	% RSD
1	40	1418.079	1413.821	4.23217	0.299343
2	40	1407.458			
3	40	1415.522			
4	40	1416.282			
5	40	1411.784			

Accuracy (Recovery Study)

A study of Accuracy was conducted. Drug Assay was performed in triplicate as per test method with equivalent amount of Drotaverine HCl into each volumetric flask for each spike level to get the concentration of Drotaverine HCl equivalent to 80%, 100%, and 120% of the labelled amount as per the test method. The average % recovery of Drotaverine HCl was calculated and tabulated in Table 5.The mean % recovery of the Drotaverine HCl at each level should be not less than 98.0% and not more than 102.0%.

Table 5: Recovery Study data

Level of Recovery	samples	Amount spiked (µg)	Amount added (µg)	% Recovery	Mean	SD	% RSD
80	1	60	20	98.3	98.21	0.0818	0.0832
	2	60	20	98.19			
	3	60	20	98.14			
100	1	60	40	99.17	99.15	0.0173	0.0174
	2	60	40	99.16			
	3	60	40	99.14			
120	1	60	60	100.22	100.2	0.0173	0.0172
	2	60	60	100.19			
	3	60	60	100.19			

Linearity

A Series of solutions are prepared using Drotaverine HCl working standard at concentration levels from 10% to 60% of target concentration (10%, 20%, 30%, 40%, 50% and 60%).Measure the peak area response of solution at Level 1 and Level 6 six times and Level 2 to Level 5 two times. The correlation coefficient was found to be 0.9992. From the above study it was established that the linearity of test method is from 10% to 60% of the target concentration. The calibration plot was given in Fig 3.
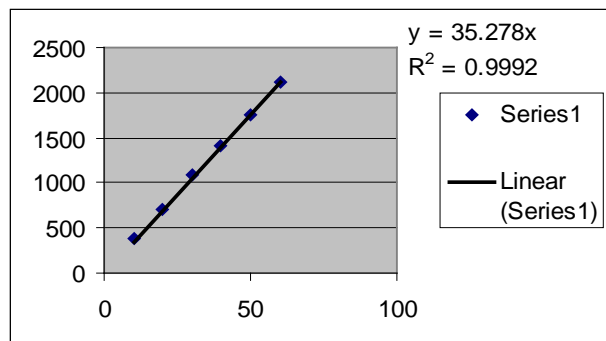

$y = 35.278x$
$R^2 = 0.9992$

Fig. 3: The Calibration Plot of Drotaverine HCl

Table 5: Statistical Parameters of Linearity

Parameters	Drotaverine HCl
Linearity range	10-60µg/ml
Slope	35.278
Coefficient of correlation	0.9992
Intercept	23.33313

Ruggedness

Analyst to Analyst variability study was conducted on different analysts under similar conditions at different times. Two samples were prepared and each was analyzed as per test method. The relative standard deviation for Drotaverine HCl was found to be below 2 % on the Analyst. Comparison of both the results obtained on two different analysts shows that the assay test method is rugged for Analyst to Analyst variability. The results were given in Table .6

Robustness

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 0.9ml/min and 1.1ml/min. The system suitability parameters were evaluated and found to be within the limits for 0.9ml/min and 1.1ml/min flow. Similarly sample solution was chromatographed at 28-30ºC temperature. Drotaverine HCl was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having ambient temperature. A study was conducted to determine the effect of variation in pH. Standard and sample solutions were prepared as per the test method and injected into the HPLC system using pH 2.8 and 3.0. The system suitability parameters were evaluated and found to be within the limits for pH 2.8 and 3.0. The data was given in Table 7.

Limit of Detection and Limit of Quantitation

The limit of Detection (LOD) and limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10).The values of LOD and LOQ were given in Table 8.

Table 6: Ruggedness data

Parameters	Analyst 1	Analyst 2
Retention time	3.137	3.140
Peak area	1414.604	1419.959
Asymmetry	1.469	1.448

Table 7: Robustness study data

Factor		Retention time	Asymmetry
Temperature(°C)	25	3.140	1.491
	27	2.947	1.441
	29	2.827	1.400
pH of mobile phase	2.8	2.827	1.501
	3.0	3.140	1.498
	3.2	2.827	1.521
Flow rate(ml/min)	0.9	2.947	1.501
	1.0	3.140	1.498
	1.1	2.947	1.411

Table 8: LOD and LOQ data

Drotaverine HCl	Limit of Detection (LOD)	Limit of Quantitation (LOQ)
sample	2.80	4.77

RESULTS AND DISCUSSION

In order to develop an effective method for the analysis of the drug in pharmaceutical formulations, preliminary tests were performed in order to select adequate and optimum conditions. Parameters such as detection of wavelength, ideal mobile phase and their proportions, optimum pH and concentration of the standard solution were studied. Several binary or ternary eluents were tested using various proportions of solvents including acetonitrile and water with various proportions. The flow rate of 1.0 ml/min for the mobile phase was selected after these preliminary tests. The goal of this study was to develop a rapid HPLC method for the analysis of Drotaverine in a finished tablet formulation using a commonly employed reverse phase C-18 column with UV detector. The proposed method is simple, rapid and statistically validated for its accuracy. No interfering peaks were found in the chromatograms indicating that the tablet excipients did not interfere in analysis of drugs. The calibration curve showed linearity over a concentration range of 10 to 60 µg/ml for drug and was linear with a correlation coefficient of 0.9992.

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of Drotaverine HCl from tablet dosage form. The mobile phase is simple to prepare and economical. The sample recoveries in formulation were in good agreement with their respective label claims. Hence, it can be easily and conveniently adopted for routine analysis of Drotaverine HCl in tablets.

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