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METHOD DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF DROTAVERINE HYDROCHLORIDE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A simple, economical, rapid, accurate, precise spectrophotometric method was developed and validated for the anti-spasmodic agent, Drotaverine hydrochloride in active pharmaceutical ingredients (API) and in tablet dosage forms. The absorption maxima of Drotaverine, was found to be at about 234 nm wave length using water as solvent. The method was found to be linear and obeys Beer's law in the concentration range of 1-12µg/ml, with the correlation coefficient being more than 0.998. The relative standard deviation was found to be <2%. The percent recovery was within the range of 99% - 101%. The developed method was validated according to ICH guidelines and was found to be accurate and precise. The method can be applied for the routine analysis of Drotaverine hydrochloride in API and pharmaceutical dosage forms.

KEY WORDS

Drotaverine hydrochloride, UV Spectrophotometry and ICH guidelines.

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INTRODUCTION

Drotaverine Hydrochloride (DCH) is [1-(3, 4-diethoxybenzylidene)-6, 7-diethoxy-1, 2, 3, 4-tetrahydroiso-quinoline]. Drotaverine has antispasmodic effect medicated via inhibition of phosphodiesterase-IV, specific for smooth muscle. It has a rapid and direct action on the smooth muscle. It acts to correct cyclic AMP and Ca imbalance at the spastic site. Thereby relieving smooth muscle spasm and pain. It has a molecular formula of $C_{24}H_{31}NO_4$.Hcl and a molecular weight

of 433.97 g / mol and its structure is given in Figure No.1.

Literature survey revealed that studies had been carried out on HPLC[1-4], Differential spectroscopy[5-6],Potentiometric method[7-9], Polarographic method[10], Spectrophotometric method[11-15], Thin layer chromatography[16], Voltammetric method[17]. Chromatographic techniques are time consuming, costly and require expertise. A simple and accurate UV-spectrophotometric method can be highly useful for routine analysis of bulk and tablet dosage forms. The present work aims to present a new, simple, rapid, cost effective and sensitive method for the determination of Drotaverine Hydrochloride in pure form and tablet dosage forms.

MATERIALS AND METHOD

Experimental procedures

Materials

Authentic drug sample of Drotaverine Hydrochloride was obtained as a gift sample from Aurobindo Pharma Ltd., Hyderabad. Drotaverine Hydrochloride tablets were procured from local market. Distilled water was used for the preparation of solutions.

Instruments

Lab India - 3000+ UV -Visible double beam Spectrophotometer with a fixed slit width (2 nm) and 10 millimeter quartz cell was used to obtain spectrum and absorbance measurement.

Selection of solvent

Distilled water was selected as solvent for developing spectral characteristics of Drotaverine Hydrochloride. The selection was made after evaluating the solubility of Drotaverine Hydrochloride in different solvents.

Preparation of stock solution

100 mg of standard Drotaverine Hydrochloride drug was weighed, transferred to a 100 ml volumetric flask and dissolved in distilled water. The flask was shaken and volume was made up to the mark with distilled water to give a solution containing 1000 µg / ml. From this stock solution, 10 ml of solution was pipetted out and placed into 100ml volumetric flask. The volume was made up to mark with distilled water to give a solution containing 100 µg / ml.

Determination of absorbance maxima

1 ml of standard stock solution was taken in 10 ml standard volumetric flask diluted to 10 ml with water to get the concentration of 10µg/ml. The absorbance of resulting solution was measured against respective blank solution (water) in the UV region of 200-400 nm, which shows maximum absorbance at 234 nm and was given in Figure No.2.

Selection of Analytical concentration ranges

From the standard stock solution of Drotaverine Hydrochloride, appropriate aliquots were pipetted out in to 10ml volumetric flasks and dilutions were made with distilled water to obtain working standard solutions of concentrations from 1-12 µg / ml. Nine different concentrations were prepared in the range of 1-12µg/mL and the absorbance were measured at 234 nm against solvent (water) blank and the absorbance values were shown in Table No.1. The obtained absorbance values are plotted against the concentration of Drotaverine hydrochloride to get the calibration graph and were represented as Figure No.3.

Analysis of marketed formulation

20 tablets of Drotaverine hydrochloride were weighed, pulverized and the powder equivalent to 10 mg of Drotaverine was weighed accurately and transferred into a 100 ml standard volumetric flask. The contents were dissolved in water. This solution was filtered through Whatmann filter paper number 40. 1 ml of above was diluted to 10 ml with water to obtain a solution of 100 µg / ml. Again 1 ml of above test solution was diluted to 10 ml with water in 10 ml standard volumetric flask to produce the concentration 10 µg/ ml. The concentration of Drotaverine hydrochloride in marketed formulation was determined in Table No.2.

Method validation

Linearity

Nine different concentrations were prepared in the range of 1-12µg/mL and the absorbances were measured at 234 nm. The regression equation and

correlation coefficient were determined and are given in Table No.3.

Accuracy

Accuracy is the closeness to the true value. To study the accuracy, 20 tablets of Drotaverine hydrochloride were taken, and the powder was used to carry out the analysis. Recovery studies were carried out by addition of standard drug solution (80%, 100% and 120% µg/ml) to the sample at 3 different concentration levels and results were presented in Table No.4.

Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. To check the degree of repeatability of methods, suitable statistical evaluation was carried out. Repeatability was performed for six times with tablets formulation. The standard deviation and coefficient of variance was calculated.

Intermediate Precision (Interday and Intraday precision)

The experiments were repeated three times in a day to determine intraday precision and on three different days to determine interday precision. The results of the same are presented in Table No. 5.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of Drotaverine hydrochloride by proposed methods were determined using calibration standards. LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively, where S is the slope of the calibration curve and Y=MX+C is the standard deviation of response. The results of the same are shown in Table No.6.

RESULTS AND DISCUSSION

The absorption spectral analysis shows the λ_{max} of Drotaverine hydrochloride at 234 nm. The calibration curve was obtained for a series of concentration in the range of 1 - 12 µg/ml. It was found to be linear and hence, suitable for the estimation of the drug. The slope, intercept, correlation coefficient and optical characteristics are summarized in Table No.3. Regression analysis of Beer's law plot revealed a good correlation. The effects of various excipients generally present in the tablet dosage form of Drotaverine hydrochloride were investigated. The results indicated that they did not interfere in the assay in amounts far in excess of their normal occurrence in it. The proposed method was validated as per the ICH guidelines. The precision was measured in terms of repeatability, which was determined by sufficient number of aliquots of a homogenous sample and results are shown in Table No.5. The % RSD was found to be within the limits. This showed that the precision of the method is satisfactory. The recovery technique was performed to study the accuracy and reproducibility of the proposed method. For this, known quantities of the Drotaverine hydrochloride solution was mixed with definite amounts of pre-analyzed formulation and the mixtures were analyzed. The total amount of Drotaverine hydrochloride was determined by using the proposed methods and the amount of added drug was calculated by the difference. The % RSD was less than ± 2.0. This showed that the recoveries of Drotaverine hydrochloride by the proposed methods are satisfactory and the results are shown in Table No.4. Limit of detection (LOD) and Limit of Quantitation (LOQ) were determined by the proposed method and results are shown in Table No.6.

Table No.1: Results of Calibration Curve at 234 nm for Drotaverine Hydrochloride

S.No	Concentration (µg / ml)	Absorbance at 234nm
1	1	0.041
2	2	0.073
3	3	0.106
4	4	0.153
5	5	0.186
6	6	0.221
7	8	0.298
8	10	0.388
9	12	0.463

Table No.2: Assay of Drotaverine hydrochloride formulation

S.No	Formulation	Label claimed (mg/tab)	Amount found (mg) (n=3) Mean ± SD	Assay	%RSD
1	Droverin	40	40.003±0.43	98.67%	1.042

Table No.3: Regression analysis of calibration curve

S.No	Parameters	Results
1	Beer's law limits (µg/ml)	1-12
2	Regression equation (Y)	Y = 0.038X+0.005
3	Correlation coefficient(R^2)	0.998
4	Slope (b)	0.038
5	Intercept (a)	+0.005

Table No.4: Determination of Accuracy results for Drotaverine hydrochloride at 234 nm

S.No	Brand name	Amount of sample (mcg/ml)	Amount of drug added (mcg/ml)	Amount Recovered	% Recovery ± SD**
1	Droverin	10	8	17.95	99.72 ± 0.26
2	Droverin	10	10	19.93	99.65 ± 0.48
3	Droverin	10	12	21.96	99.81 ± 0.34

**Average of six determinations.

Table No.5: Determination of Precision Results for Drotaverine at 234 nm

S.No	Concentration µg / ml	Inter-day Absorbance Mean ± SD**	% RSD	Intra-day Absorbance Mean ± SD**	% RSD
1	LQC (2µg/ml)	0.073 ± 0.000133	1.41	0.066 ± 0.001045	1.58
2	MQC (5µg/ml)	0.186 ± 0.001414	0.76	0.183 ± 0.000156	0.08
3	HQC (8µg/ml)	0.388 ± 0.002828	0.72	0.380 ± 0.002080	0.54

**Average of six determinations.

Table No.6: Summary of Validation Parameters

S.No	Parameters	Values
1	λ_{max} (nm)	234
2	Beer's law limits (µg/ml)	1-12
3	Regression equation (Y*)	Y = 0.038X+0.005
4	Slope (b)	0.038
5	Intercept (a)	+0.005
6	Correlation coefficient(R^2)	0.998
7	% RSD**	< 2%
8	Limit Of Detection (µg/ml)	0.0897
9	Limit Of Quantitation (µg/ml)	0.2718

*Y= mX + c, where X is the concentration of Drotaverine hydrochloride in µg/ml
Y is the absorbance at the respective $\lambda_{max.}$, c = Intercept.
**Average of six determinations

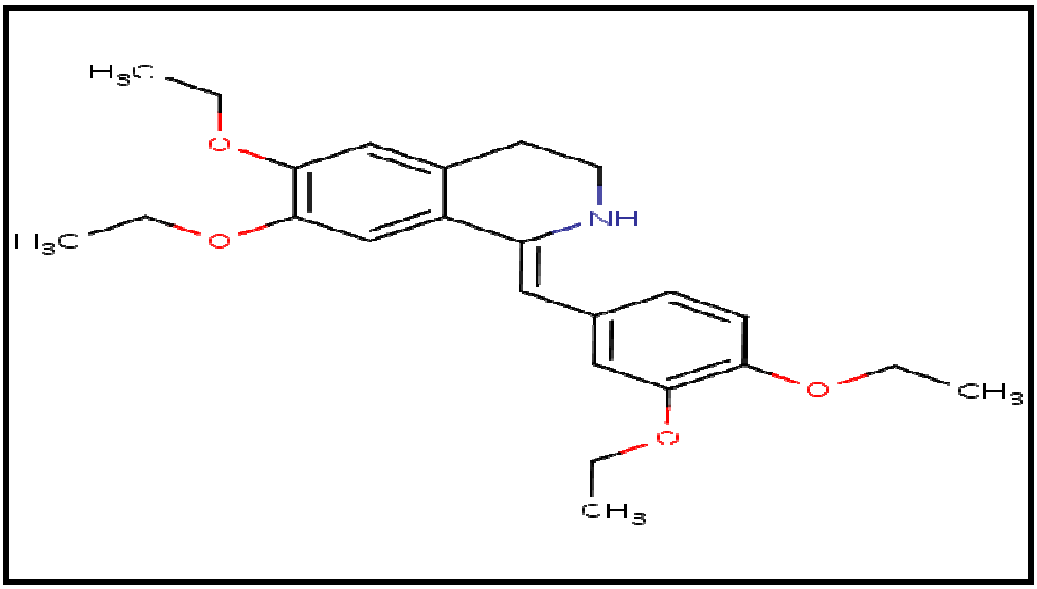

Figure No.1: Chemical Structure of Drotaverine hydrochloride

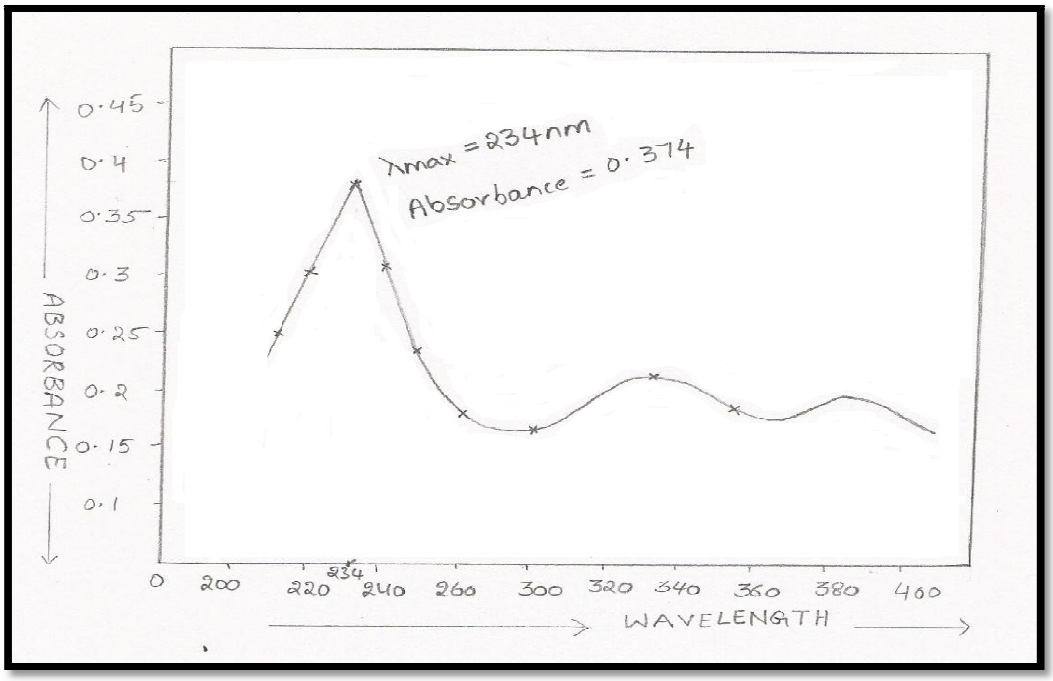

Figure No.2: Spectra of Drotaverine Hydrochloride at 234nm

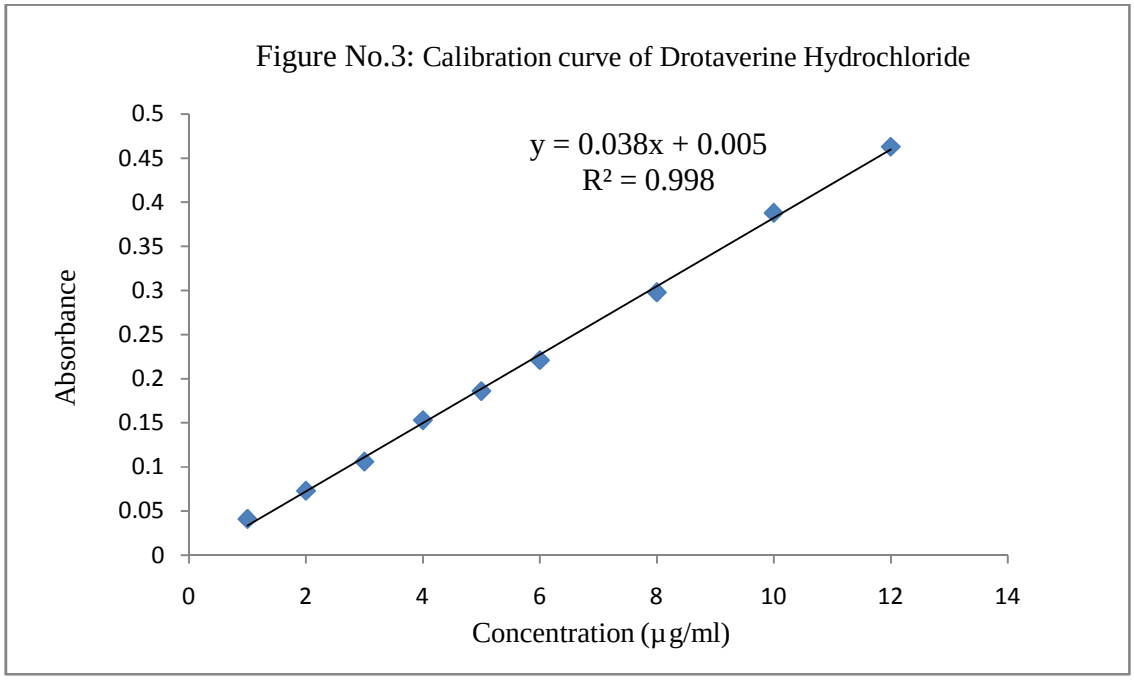

Figure No.3: Linearity Curve for Drotaverine Hydrochloride at 234nm

CONCLUSION

The work was concluded that the methods developed in the present investigation are simple, sensitive, accurate, rapid and precise. Hence, the above said method can be successfully applied for the estimation of Drotaverine hydrochloride in tablet dosage form.

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