Research Article

Development of New Analytical Methods and Their Validation for the Determination of Drotaverine Hydrocloride in Bulk and Marketed Formulations

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ABSTRACT

Drotaverine Hydrochloride in presence of acidic medium reacts with excess amount of ceric ammonium sulphate and unreacted ceric ammonium sulphate react with malachite green to produce green colour.The final stock solution was made to produce 100µg/ml with methanol. The $\lambda_{max}$was found to be 605nm for assay. The linearity was found in concentration range of 5-30µg/ml. The correlation coefficient was found 0.9987. The regression equation was found as Y=0.0221x+0.0074. The method was validated according to ICH Guidelines.

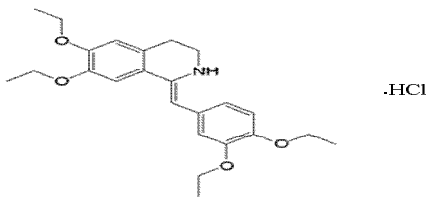
Keywords: Drotaverine Hydrochloride, malachite green, ceric ammonium sulphate, H_2SO_4.

INTRODUCTION

A study of the interaction of light (or other electromagnetic radiation) with matter is an important and versatile tool for the chemist. Indeed, much of our knowledge of chemical substances comes from their specific absorption or emission of light. In this experiment, we are interested in analytical procedures based on the amount of light absorbed (or transmitted) as it passes through a sample.[1]

Drotaverine Hydrochloride is an antispasmodic drug, structurally related to papaverine. Drotaverine is a selective inhibitor of phosphodiesterase 4, and has no anticholinergic effects. Drotaverine has been shown to possess dose-dependent analgesic effects in animal models. One small study has shown drotaverine to be eliminated mainly non-renally[2].

Drotaverine inhibits phosphodiesterases hydrolysingcAMP, thereby increasing cAMPconcentration, decreasing Ca uptake of the cells and changing the distribution of calcium among the cells. It may also have minor allosteric calcium channel blocking properties.[2]



Drotaverine (DRV), [(1 - (3, 4 – diethoxybenzylidene) - 6, 7 – diethoxy - 1, 2, 3, 4 tetrahydroisoquinoline) hydrochloride], a benzylisoquinoline derivative.

It is soluble in freely soluble in acetone, soluble in alcoholup to now there is HPLC and spectrophotometric method developed on Drotaverine Hydrochloride.[3-12]

The validation defines eight steps and that are Accuracy, Precision, Specificity, Limit of detection, which are described Limit of quantitation, Linearity and range, Ruggedness' Robustness and that are describe in validation part.

EXPERIMENTAL

Drotaverine Hydrochloride was determined spectrophotometrically in bulk and marketed formulation by using Malachite green indicator and ceric ammonium sulphate as a strong oxidizing agent.

Reagent and chemicals

1. Working standard stock solution (100µg/ml)
2. 0.01M Ceric ammonium sulphate solution
3. 4M H_2SO_4
4. 0.05% Malachite green

Preparation of standard stock solution of Drotaverine Hydrochloride

Standard stock solution prepared by accurately weighing 100 mg of Drotaverine Hydrochloride in 100 ml calibrated volumetric flask and made up the volume with Methanol up to 100 ml.

Preparation of working standard solution of Drotaverine Hydrochloride
Working standard was prepared by transferring of 10 ml standard stock solution into 100 ml calibrated volumetric flask and made up the volume with Methanol for getting concentration of 100µg/ml.

Preparation of 0.01 M Ceric ammonium sulphate solution
650mg of CAS was weighed accurately and transferred into 100ml volumetric flask. Add 25ml of water and few ml of concentrated H_2SO_4 and make up the volume up to 100ml with water.

Preparation of 4M H_2SO_4
216 ml of concentrated $H_2SO_4$was accuratelytransferred into 1000 ml volumetric flask and made up the volume with distilled water.

Preparation of Malachite Green
50mg of Malachite green was weighed accurately and diluted up to 100 ml with distilled water.

Preparation of Standard curve
Standard curve was prepared by using pure Drotaverine Hydrochloride in the concentration range of 5-30µg/ml by this method and selecting absorbance maximum at 605nm.

Procedure
From the working standard drug solution 0.5, 1.0, 1.5, 2.0, 2.5 and3.0 ml (which gives 5-30µg/ml) drug solution were placed in 6 different 10 ml volumetric flasks. To this 0.5ml of 0.01M CAS and 1ml of 4M H_2SO_4were added and flasks were kept a side for 15 min for the completion of reaction. After 15 minutes, 0.3ml of 0.05% Malachite green was added and volume was made up to 10 ml with Methanol. The blank was also prepared in the same way omitting the drug. The absorbance of the resulting solutions was measured at 605nm against reagent blank. The result was recorded in **table no.1** and graph is given in **figure no.2.**

METHOD VALIDATION
Linearity
Linearity was determined over the range of 5 to 30µg/ml. 6 different 10ml volumetric flasks were taken. To these flasks 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0ml of working standard of Drotaverine Hydrochloride were added. Then 0.5ml of 0.01M CAS and 1ml of 4M H2SO4 were added and flasks were kept a side for 15 min for the completion of reaction. After 15 minutes, 0.3ml of 0.05% Malachite green was added and volume was made up to 10 ml with Methanol. Absorbance was measured against corresponding reagent blank at 605nm.

%Recovery (Accuracy)
The accuracy of the methods was determined by calculating % recovery of Drotaverine Hydrochloride by standard addition method. Known volumes of standard solutions of Drotaverine Hydrochloride were taken for recovery studies in 3 different levels 50, 100, 150% and recovery study was carried out. The results were reported in terms of % Recovery in **table no.3.**

Method precision (% Repeatability)
The precision of the methods was checked by repeated measurement of the absorbance of standard solutions (n = 6) of 6 µg/ml without changing the parameters for the method.The results were reported in **Table no.3.**

Intermediate precision (Reproducibility)
The intraday and interday precision of the proposed methods were performed by analyzing the corresponding responses three times on the same day and on three different days over a period of one week for three different concentrations of standard solutions of Drotaverine Hydrochloride (5, 10, 15 µg/ml). The results were reported in **Table no.3.**

Reproducibility
The absorbance readings of 5µg/ ml were measured at different laboratory using different spectrophotometer by another analyst and the %RSD values obtained to verify their reproducibility. The results were reported in **Table no.3.**

Limit of detection and Limit of quantification
The limit of detection (LOD) and limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using following equations designated by International Conference on Harmonization (ICH) guideline:
LOD = 3.3 X σ/S and LOQ = 10 X σ/S
Where, σ = the standard deviation of the response, S = slope of the calibration curve.
Result is displayed in **table no.2**

Analysis of marketed formulation
Drotaverine Hydrochloride is marketed as Doverine of 40mg tablet manufactured by Intas laboratory Ltd. was taken for analysis.

Preparation of sample solution

Tablet powder equivalent to 100mg was weighed accurately and transferred into 100ml volumetric flask and made up the volume with Methanol to get 1000µg/ml concentration (Stock solution- A). This solution was further diluted to get concentration of 100µg/ml (Stock solution- B).

Recovery experiments

From Stock solution B 1.5ml was pipetted out into a 10ml volumetric flask.To this 0.5ml of 0.01M CAS and 1ml of 4M H_2SO_4were added and flasks were kept a side for 15 min for the completion of reaction. After 15 minutes, 0.3ml of 0.05% Malachite green was added and volume was made up to 10 ml with methanol. The blank was also prepared in the same way omitting the drug. The absorbance of the resulting solution was measured at 605nm against reagent blank. The result was recorded in **Table no.3**

RESULT AND DISCUSSION

Ceric Ammonium Sulphateis strong oxidizing agent. It reacts with Drotaverine Hydrochloride in present of acidic medium. When Ceric Ammonium Sulphate added in excess amount it produces light yellow color complex of Drotaverine Hydrochloride. Unreacted Ceric Ammonium Sulphate now readily reacts with malachite green & malachite green. Remaining molecules of malachite green gives color. So, color of the final solution indirectly indicates the amount of drug present.

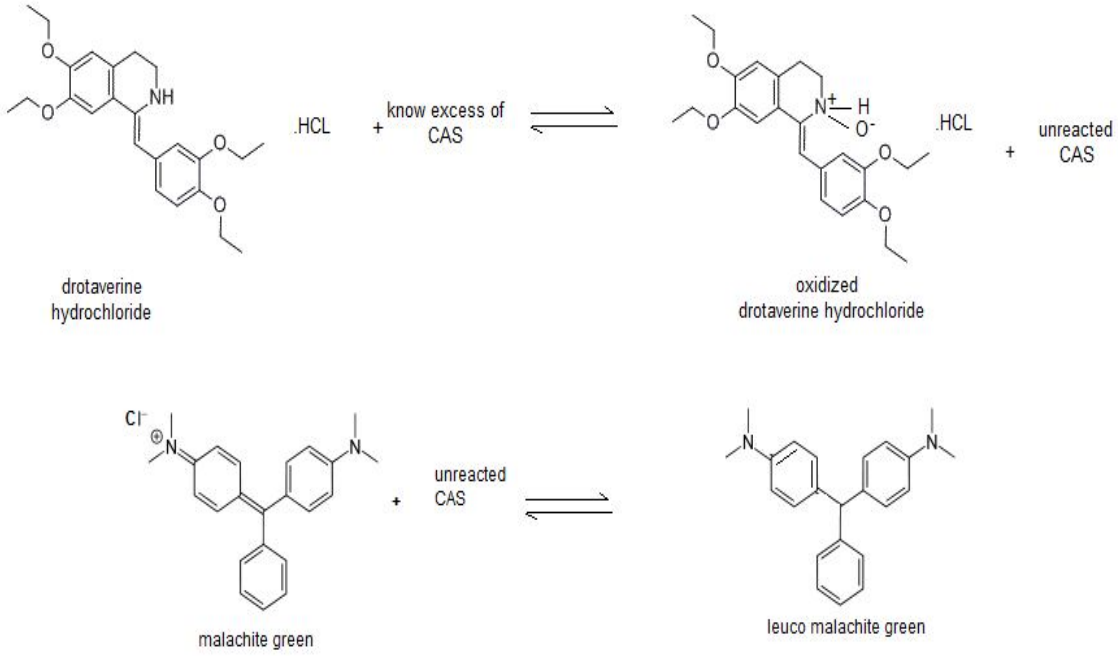


Table 1: Absorbance of different concentration of Drotaverine Hydrochloride at 605 nm

Sr.no.	Volume of working standard of drug (ml)	Concentration of drug (µg/ml)	Absorbance At 605nm
1	0.5	5	0.1283
2	1.0	10	0.2364
3	1.5	15	0.3248
4	2.0	20	0.4506
5	2.5	25	0.5625
6	3.0	30	0.6732

Table 2: Assay Results of Marketed Formulation

Formulation	Actual concentration of Drotaverine hydrochloride(µg/ml)	Amount obtained of Drotaverine hydrochloride (µg/ml)	% Drotaverine hydrochloride
tablet	15µg/ml	14.563 µg/ml	97.07%

Table 3: Statistical data for Drotaverine hydrochloride by colorimetric method

Parameter	Drotaverine Hydrochloride 605nm
Linear Range (µg/ml)	5-30
Regression Equation* (y)	y=bx+a: 0.0221x+0.0074
Slope (b)	0.02216
Intercept (a)	0.00735
Correlation coefficient (R^2)	0.9987
Standard Deviation of Slope	0.0001211
Standard Deviation of Intercept	0.0005787
Limit of Detection (µg/ml)	0.018028
Limit of Quantition(µg/ml)	0.05464
Molar Absorptivity (1/mol.cm)	2.216×10^{-2}
%Recovery	1) At Level-1 (80%)=99.24±0.1835 2) At Level-2 (100%)=98.13±0.2080 3) At Level-3 (120%)=99.55±0.2159
Repeatability Data(%RSD)	0.174-0.817
Reproducibility:- Instrument 1 (%RSD) Instrument 2 (%RSD)	 0.1688 0.1365
Precision(n=3) Intra day precision(%RSD) Inter day Precision(%RSD)	 0.126-0.470 0.277-0.856

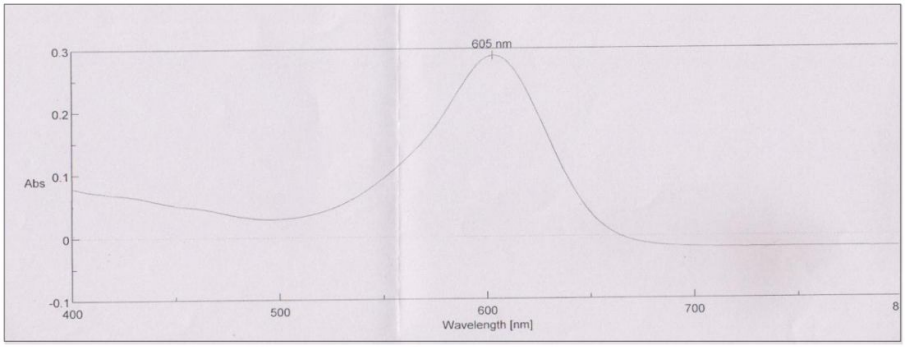


Fig. 1: λ_{max} for Drotaverine Hydrochloride

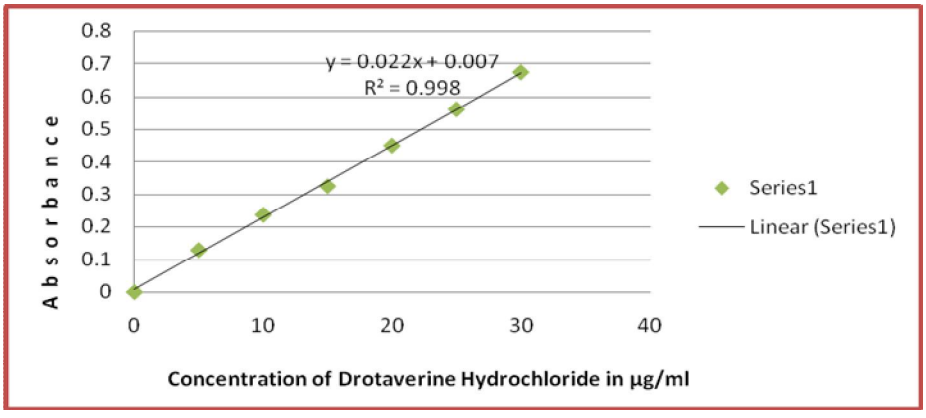


Fig. 2: Calibrated Curve for Drotaverine Hydrochloride at 605 nm

CONCLUSION

For routine analytical purpose, it is always necessary to establish methods capable of analyzing huge number of samples in a short time period with due accuracy and precision. A very few analytical method appeared in the literature for the determination of Drotaverine Hydrochloride. In view of the above fact, some simple analytical method was planned to develop with sensitivity, accuracy, precision and economical. In the present investigation, colorimetric method for the quantitative estimation of Drotaverine Hydrochloride in bulk drug and pharmaceutical formulations has been developed.

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