Development and validation of UV-spectrophotometric methods for quantitative estimation of Drotaverine HCl injection

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ABSTRACT

Introduction: The objective of this study is to develop and validate spectrophotometric method for Drotaverine HCl injection analysis. A very simple, unique, novel, protective, secured, reliable and quick method of spectrophotometric estimation in UV-region has been developed for the assay of Drotaverine HCl injection formulation. **Method:** Methanol and water were used as diluents for the assay of Drotaverine HCl. **Results:** However no interference was observed in spectrophotometric determinations. ICH guidelines were followed during selection of parameters and these were then validated statistically using neat chromate grams by RSD and %RSD. The Beer's law was followed when 8-40 mcg/ml concentration was used. Accuracy, linearity, precision and ruggedness were used for the validation of this method. Recovery studies were used to statistically validate the proposed method. **Conclusion:** UV spectrophotometer was used to scan the standard solution at wavelength between 200 nm to 400 nm on spectrum mode. Maximum absorbance was found at 242 nm.

Keyword: Beer's law, Drotaverine hydrochloride, Parameters, Accuracy.

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INTRODUCTION

Method validation is a procedure that demonstrates and manifests that analytical method is adequate and satisfactory as it shows intentional and deliberate objectives.[1] The guidelines for the validation in pharmaceutical procedures were obtained from the United States Pharmacopeia (USP), Food and Drug Administration (FDA) and International Conference on Harmonization (ICH).[2] The methods for regulatory submission must involve studies on linearity, accuracy, precision, specificity, detection Copyright by the American Chemical Society.[3] Chemically Drotaverine Hydrochloride is (1-[(3, 4-diethoxyphenyl)- methylene]-6, 7-diethoxy-1, 2, 3, 4- Tetrahydroisoquinoline1 and its molecular formula is C24H31NO4. Structure of drotaverine is closely related to papaverine.[4] It causes relaxation of smooth muscles by inhibiting phospodiesterase -4 (PDE-4).[5] Therapeutically it is used to treat kidneycholic and also in order to accelerate labor.[6] Drotaverine also block calcium channels and thus progress muscle anti spasmodic properties.[7] However it is not considered as an official drug. Literature demonstrates various methods (like HPLC3, 4, RP-HPLC , HPTLC) that determine Drotaverine HCl.[4] The objective of this study is to develop a simple, reasonable, accurate and precise spectrophotometric procedure for Drotaverine HCl determination in injection dosage form and also to validate it according to ICH guidelines.[8] Beer's law usually explain the relationship between absorbance and concentration which means that intensity of parallel beam(monochromatic radiation) has the tendency to decrease exponentially with the number of absorption molecules.[9] Method validation results can be used to check the quality, consistency and reliability of analytical method.[10]

Drotaverineis a drug which is structually related to papaverine and relives the muscle spasm.[11] It is phosphodiesterase 4 inhibitor, thus inhibits the conversion of cAMP into AMP and shows cholinergic properties.[12] When it is administered in animal models then it shows analgesic effects in dose-dependantmanners.[13]

Drotaverine is beneficial to treat kidney colic pain in 80% of the individuals as shown in few small studies.[14] It also increases the cervical dilation thus accelerates labor, however results of this study are conflicting.[15] Drotaverine is also administered in combination with mefenamic acid to prevent the pain attacks during endometrial biopsy andhysteroscopy in paracervical block.[16] Butylscopolamine (Bascopan) is used to treat diarrhea in patients of Irritable bowel syndrome.[17] Drotaverine is also used as an antiviral drug with concomitant use of rimantadine against influenza of type A and B.[18]

Drotaverine is eliminated from the body through non-renal mechanism as revealed by a small study. Variations in therapeutic responses were also observed when administered through oral route.[19] Side effects of drotaverine are very rare as only 0.9% adverse effects are reported.[18]

Experimental

Drotaverine

Chemically Drotaverine is1-(3,4-Diethoxybenzylidene)-6,7-diethoxy-1,2,3,4 tetrahydroisoquinoline. (C24H31NO4)

MATERIALS AND METHODS

Drotaverineraw material (Sigma Aldrich USA) HCl (Merck KGA, Germany), Drotaverine injection (Sanofi Aventis). Double beam UV/Visible Spectrophotometer of Shimadzu (1800), analytical balance of Shimadzu (1600) and double distilled water were used in the experiment. Analytical grade materials were used throughout the experiment without further purification.

Diluent preparation

0.1 molar HCl and water in ratio of 15:85(v/v) was used as a diluent.

Analytical procedure

Analysis procedure involves the method or steps in order to perfrom the analytical test. Quality of the product can be checked and maintained through method validation.The most suitable ptotocol and validation method must be selected to demonstrate the procedure.

Determination of Drotaverine HCl injection is base on UV-Visible Spectrophotmetry technique, detailed testing method is mentioned. Drotaverine HCl injectionis formulated as 40 mg per Ampoule. The method is developed and subjected to validation by applying above mentioned parameters according to planned protocols. All laboratory resources and UV-Visible Spectrophotmetry Shimadzu-1800 series and reagent are used. Before validation of method it is very difficult to say that the method will comply all the parameters of validation. The every step of method has its own rationale.

Procedure
Assay (Contents of DrotaverineHCl)
Standard Preparation

Transfer accurately weighed 40 mg of Drotaverine HCl working standard (raw material) into a 100 ml V/Flask. Add 50 ml 0.1M HCl. Sonicate for 15 min. Make upto the mark with same solvent and mix well. Filter through whatman No 42 filter paper discard the 1st 15 ml for rinsing. Dilute 1ml of the above solution to 50 ml with 0.1 M HCl.

Sample Preparation

Transfer, accurately 2 mL Of Sample equivalent to 40 mg of Drotaverine HCl into a 100 ml V/Flask. Add 50 ml 0.1 M HCl. Sonicate for 15 minutes. Make upto the mark with same solvent and mix well. Filter through whatman No 42 filter paper discard the 1st 15 ml for rinsing. Dilute 1ml of the above filtrate to 50 ml with 0.1 M HCl.

Procedure

Take absorption spectrum of sample and standard in the range 200-400 nm using water a reference solvent. The absorption maxima should occur at wavelength about 242 nm. Note absorption values of standard and sample solution at this maximum and calculate the results as bellow.

Calculation

Abs. of Sample Solution   = A

Abs. of Standard Solution = B

$$\text{Assay (\% age)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of s tan dard}} \times 100$$

RESULT AND DISCUSSION
Idnetification of raw material

FTIR technique was used to identify the purity of drotaverin raw material [Figure 2].

Selection of wavelength

UV spectrophotometer was used to scan the standard solution at wavelength between 200 nm to 400 nm on spectrum mode, Diluent was used as a blank. Maximum absorbance was found at 242 nm. Simple, accurate, precise and reproducible method was proposed in this study as shown in Figure 3.

System Precision or System suitability

The performance parameters i.e. the system suitability must be evaluated in order to start the validation of analytical procedure. It involves precision; which is determined from various samples of a homogenous sample and closeness of agreement between their results was determined. The precision study must be performed under following prescribed condition using the sample and standard solutions:

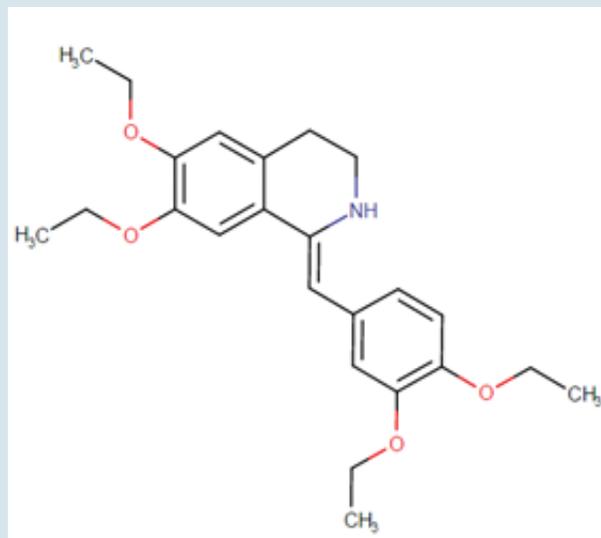- System precision by applying three replicates of standard.

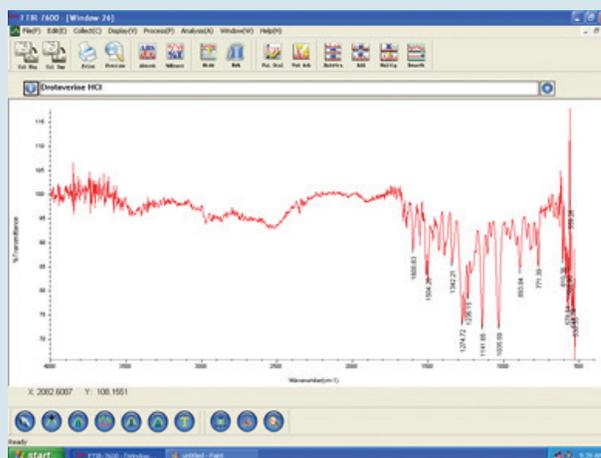**Figure 1:** Chemical structure of drotaverine (Drug Bank).

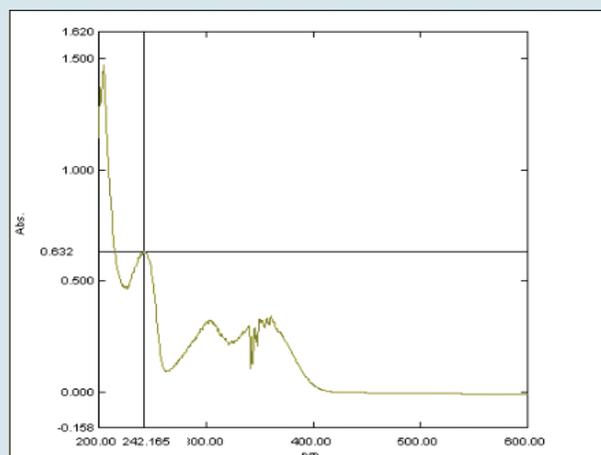**Figure 2 :** FTIR of pure Drotaverine HCl raw material..
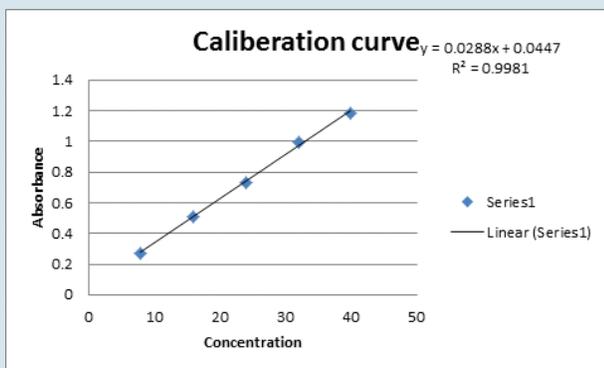

Figure 3: UV spectrum of Drotaverine injection.

Figure 4: caliberation curve of drotaverin.

Table1: Sample response without active

S. No.	Sample Identity	Results/ Absorbance/ Percentage etc.	Remarks
01	Sample 01 [Placebo (without Active)]	0.00	OK
02	Sample 02 [Placebo (without Active)]	0.01	Ok

Table 2: System Precision of Drotaverine HCl

No. of Replicate	Results Absorbance
1	0.267
2	0.268
3	0.261
4	0.258
5	0.268
Mean	0.259
% RSD	1.77599377

Table 3: Intermediate precision (Reproducibility/Repeatability)

Analyst 1	
Sample No.	Percent Assay
1	0.268
2	0.269
3	0.253
4	0.259
5	0.264
6	0.249
Mean	0.2603

Table 4: Intermediate precision (Reproducibility/Repeatability)

Analyst 2	
Sample No.	Percent Assay
1	0.262
2	0.259
3	0.259
4	0.269
5	0.269
6	0.259
Mean	0.26283

Table 5 : caliberation curve parameter

standard ID	concentration(mcg/ml)	Absorbance
1	8	0.267
2	16	0.512
3	24	0.731
4	32	0.991
5	40	1.18

Table 6: validation parameters

Absorption maxima	242
Linearity range mcg/ml	8 mcg-40 mcg
Standard regression equation	y=0.028×0.044
Correlation coefficient	0.998
Limit of quantification	0.63
Limit of detection	2.09
Beer's law limit	8-40 µg/ml

- Calculated %RSD of three replicates should be within 2%.

Specificity

When there are multiple sample components in the test solution; like excepients, degradation material, intermediate products etc; then methods ability to measure the analyte response is known as specificity. Analyte response in the test mixture was determined and compared with the analyte response in the sample containing analyte only. Samples for specificity are prepared according to method and blank / diluent and Placebo are also treated as sample, all the samples are analyzed on UV-Visible Spectrophotometer according to method and interference is in limit (Table 1) .

LINEARITY

The linearity is the test procedure to determine the results; directly or indirectly through mathematical calculations; which depend upon the analyze concentration in the sample. At least six standards of concentration range 80–120% of the expected concentration must be used to obtain results of linearity. Intercept will be obtained when a linear regression equation is applied on the results. Significant zero and significant non-zero intercept may be obtained. No effect on method's accuracy was demonstrated by significant non-zero intercept.

Table 7: Estimation of drotaverin in injection formulations

Label claim	Conc. prepared (µg/ml)	Conc. recovered (µg/ml)	Amount found (mg/2 ml)	%Labelclaim	Mean ± SD (n=3)
40 mg/2 ml	8	7.81	39.05	98.6	97.0 ± 1.00
		7.79	38.95	97.3	
		7.68	38.40	96.8	
	16	15.78	39.45	98.4	98.5 ± 0.8
		15.67	39.17	97.6	
		15.87	39.67	99.2	
	24	23.79	39.65	98.8	98.7 ± 0.4
		23.64	39.40	98.0	
		23.69	39.48	98.4	

Table 8: Recovery studies on marketed formulations

Drug	Drug amount mcg/ml	Level of addation %	Amount added mcg/ml	Drug found mcg/ml	% Recovery	Average recovery %
Drotaverine	10 mcg/ml	80	8 mcg/ml	7.94	99.3	100.1%
		100	10 mcg/ml	10.3	100.3	
		120	12 mcg/ml	12.08	100.7	

Different concentration of Drotaverine HCl is prepared as follows and single Results are taken for linearity purpose.

Accuracy (recovery test)

Recovery experiments were used to determine method accuracy. Different dilutions were prepared by adding calculated amount of drug. The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e., 80%, 100% and 120%. Result of recovery studies are reported in Table 8.

Robustness and stability of solutions

The robustness of the spectrophotometric method was determined with the change of reagents and equipment suppliers. The stability of the solutions was performed at room temperature (25°C) for 5 days and at different storage conditions. Standard solutions were prepared using volumetric flasks and transparent amber evaluating thus the photostability of Drotaverine.

Limit of detection (LOD) and limit of quantification (LOQ)

Detection limit is the minimum concentration of the substance that can reliably distinguished from the absence of that substance within 1% confidence limit. However the minimum concentration of the standard curve that can be measuredwith precision, variability and accuracy is known as limit of quantification(ICH guideline Q2B, 2005) (Table 6). The LOD and LOQ were calculated as:

$$LOD = 3.3 \times \frac{SD}{S}$$

$$LOQ = 10 \times \frac{SD}{S}$$

Where, S = slope of the linearity curve (0.028), SD = standard deviation of y-intercept

DISCUSSION

Analytical method precision is actually closeness of agreement between a series of measurement that was obtained from multiple sampling of the same homogenous sample using the said method and results of five replicates i.e1.77599377% RSD reveal the fact that the system is highly precise for the method (Table 2). Intermediate precision expresses with in the laboratory variation: different analyst and equipment etc. samples are analyzed by two different analysts and RSD of analytical results obtained by two different analysts found within two percent (2%) which is the acceptable (Table 3, 4). The linearity of analytical method is the ability to get the results of test which are directly proportional to the analyze concentration. The above-mentioned table reflects clearly that the concentration of standard i.e. Drotaverine HCl is directly proportional to results. Since linearity data does not show any trend in a single point calibration can be used within the specified to quantitative the Drotaverine HCl. The linear graph brings to close that the relationship between concentration and results is linear.

CONCLUSION

Specificity is the parameter by which one can proof that none of the ingredient interferes in the peak of interest. The said method is decidedly specific for quantitative and qualitative estimation of Drotaverine HCl.

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PICTORIAL ABSTRACT
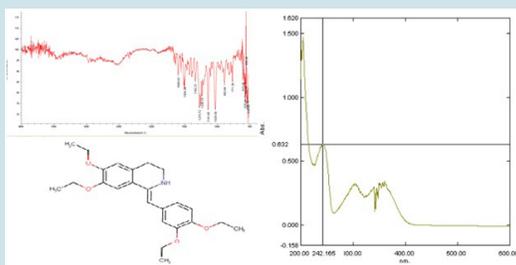
SUMMARY

• The objective of this study is to develop and validate spectrophotometric method for Drotaverine injection analysis. A very simple, unique, novel, protective, secured, reliable and quick method of spectrophotometric estimation in UV-region has been developed for the assay of Drotaverine HCl injection formulation. UV spectrophotometer was used to scan the standard solution at wavelength between 200 nm to 400 nm on spectrum mode. Maximum absorbance was found at 242 nm.

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Yasir Mehmood: Is working as Quality Control Manager in National pharmaceutical industry(A&A Pharmaceuticals). His current research interests are formulation development of different dosage form, clinical study design and different health related issues. He is also reviewer and editorial member of some journal.