ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ESTIMATION OF DENAVERINE HYDROCHLORIDE IN BULK AND INJECTABLE PHARMACEUTICAL DOSAGE FORM BY UV-SPECTROPHOTOMETRIC METHOD

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Abstract:

It has been accomplished the development of a simple and rapid UV-spectrophotometric method to estimate for the evaluation of Denaverine hydrochloride, which is used in the treatment of antispasmodic drug in Veterinary medicine. The present study describes the method developed a simple and reproducible. UV-Visible spectrophotometric method for the quantitative determination of Denaverine hydrochloride. A UV-Visible spectrophotometric method with 1cm quartz cells and distilled Methanol solvent was employed. The proposed method was successfully applied for the estimation of Denaverine hydrochloride in commercial pharmaceutical preparation with UV detection at 221 nm. It obey's Beer law in the concentration range of 3 to 15μg/mL having correlation coefficient of 0.9997, Percentage recovery (100.0%). In terms of linearity, precision, accuracy, recovery, limit of detection (LOD), and limit of quantitation (LOQ), the developed technique was within limit as per ICH guidelines.

Key words: Denaverine hydrochloride, Absorbance Maxima, UV Spectrophotometry.

INTRODUCTION

Denaverine hydrochloride[1-6] is a muscle relaxant. It was developed and patented in Germany in 1974. Under the brand name Sensiblex, Denaverine hydrochloride is used in Veterinary medicine as a muscle relaxant for the myometrium of cows and dogs during parturition. Now,the drug is in trial with human plasma to treat urogenital and gastrointestinal spasms under the brand Spasmalgan.

Denaverine hydrochloride, is a neurotropic–musculotropic spasmolytic with analgesic effect. It's used to treat gastrointestinal and urogenital smooth muscle spasms, as well as postoperative abdominal pain and obstetrics. Despite the fact that denaverine hydrochloride has been used successfully in therapy for over 30 years, there was little information on its biotransformation in humans.

Structure
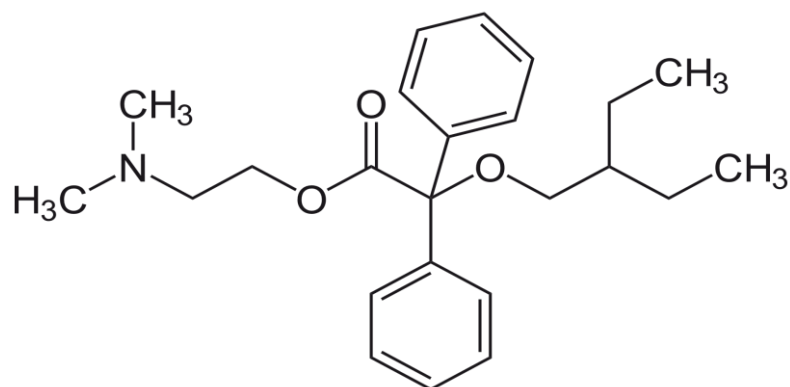
Fig. 1 Chemical Structure of Denaverine hydrochloride

Chemical name: 2-dimethylaminoethyl2-(2-ethylbutoxy)-2,2diphenylacetate hydrochloride

Mechanisms of Action

Denaverine inhibits the enzyme phosphodiesterase. A phosphodiesterase inhibitor is a drug that inhibits the inactivation of the intracellular second messengers cyclic Adenosine MonoPhosphate (cAMP) and cyclic Guanosine MonoPhosphate (cGMP) by one or more of the five subtypes of the enzyme PhosphoDiEsterase (PDE). It has anticholinergic properties. Anticholinergics (anticholinergic agents) are a class of drugs that prevent the neurotransmitter acetylcholine (ACh) from acting at synapses in the central and peripheral nervous systems.

MATERIALS AND METHODS:

Instrument: A Shimadzu UV-1600 UV/VIS Spectrophotometer was used with 1 cm matched quartz cell.

Materials and Reagents:

All the chemicals used were of analytical grade. An analytically pure sample of Denaverine hydrochloride was procured as gift sample from HITECH LABORATORY,Chennai.

Preparation of Solution

Standard Solution Preparation

Accurately weighed and transfered 10 mg of Denaverine working standard into a 10 mL volumetric flask. About 7 mL of methanol was added and sonicate to dissolve it completely. Then the solution was made volume up to the mark with the same solvent (1000 μg/mL). Further 1.0 mL of the Denaverine stock solution was pipette out into a 10 mL volumetric flask and dilute up to the mark with solvent (100μg/mL). Further 0.9 mL of the Denaverine stock solution was pipette out into a 10 mL volumetric flask and dilute up to the mark with solvent (9μg/mL).

Sample Solution Preparation

0.25mL of injection (Sensiblex 40mg/mL) was measured accurately and transfered the sample (equivalent to 10 mg of Denaverine hydrochloride) into a 10 mL volumetric flask. 7 mL of methanol was added and sonicate to dissolve it completely. Then it was made volume up to the mark with same solvent (1000 μg/mL). The solution was mixed well and filter through 0.45 μm filter. Further pipette 1 mL of the Denaverine solution into a 10 mL volumetric flask and dilute up to the mark with solvent (100μg/mL). Further pipette 0.9 mL of the Denaverine stock solution into a 10 mL volumetric flask and dilute up to the mark with solvent (9 μg/mL).The sample solution (9 μg/mL) absorbance was measured against blank at 221nm. Percentage purity was calculated by using the following formula.

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.Wt}{Label\ Claim} \times 100$$

Method development and validation

Linearity:

From the stock solution, the aliquots of stock solution of Denaverine (1000 µg/mL) further dilution make up to (0.3 to 1.5 mL of 1000 µg/mL) and transferred in to 100 mL volumetric flask and made up to the mark with solvent. The absorbance of the solution of different concentration were measured at 221 nm against blank. The calibration curve was plotted using Concentration Vs Absorbance. The curve obtained was linear with concentration range 3-15 µg/mL.

Recovery:

0.25mL of injection (Sensiblex 40mg/mL) was measured accurately and transferred the sample (equivalent to 10 mg of Denaverine hydrochloride) into three separate 10 mL volumetric flask. Then 5mg, 10mg and 15mg (50%, 100%, 150%) of standard were accurately weighed and added. 7 mL of methanol was added and sonicate to dissolve it completely. Then the solution was made volume up to the mark with the same solvent. 0.9 mL was pipette out from each flask and transferred to separate 100 mL volumetric flask. Then the solution was made volume up to the mark with the same solvent. Absorbance was measured at 221 nm against blank. The amount of drug recovered was calculated by using slope and intercept values.

Precision:

Repeatability and intermediate precision studies were done to the precision of the method. Repeatability studies were done by consequently measuring the absorbance of standard solution (9µg/mL). These solutions were prepared in duplicate and absorbance were measured at 221 nm against blank and calculate the % RSD.

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

The LOD and LOQ were estimated from the set of s calibration curves used to determine method linearity.

LOD= 3.3 σ /S and LOQ=10* σ /S

Where, σ= the standard deviation of y-intercepts of regression lines.

S the slope of the calibration curve.

RESULTS AND DISCUSSION

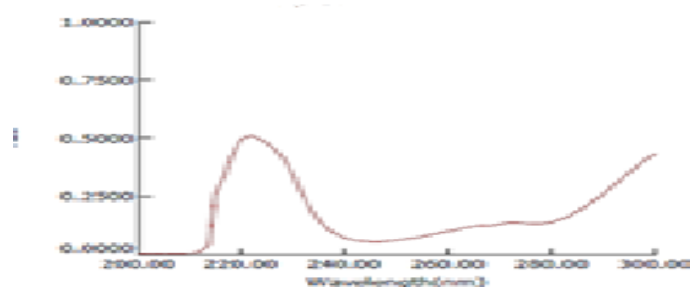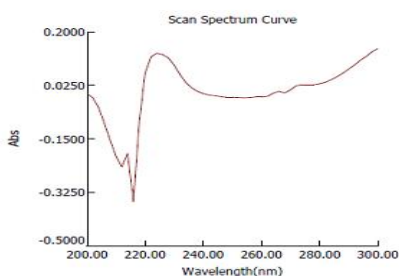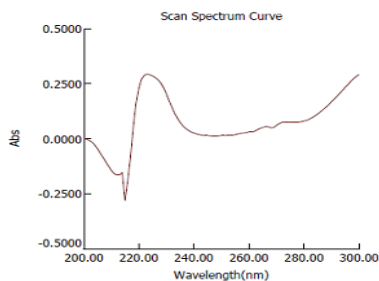* λ Max was observed at 221nm

Fig. 2 **Spectrum of Denaverine hydrochloride**
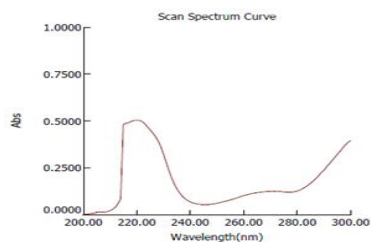
Linearity:

The Linearity for Denaverine hydrochloride was found to be concentration range 3-15 µg/mL with Correlation Coefficient 0.9997. LOD and LOQ were found to be 0.216 µg/mL and 0.655 µg/mL. Calibration data and %RSD is shown in table 1 and curve is shown in figure 8.
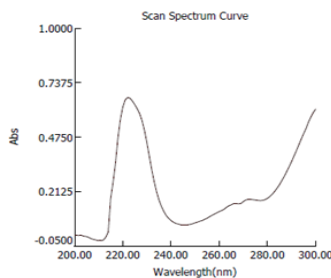

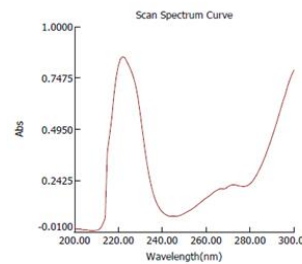**Fig. 3 Linearity Spectrum (3µg/mL)**   **Fig. 4 Linearity Spectrum (6µg/mL)**
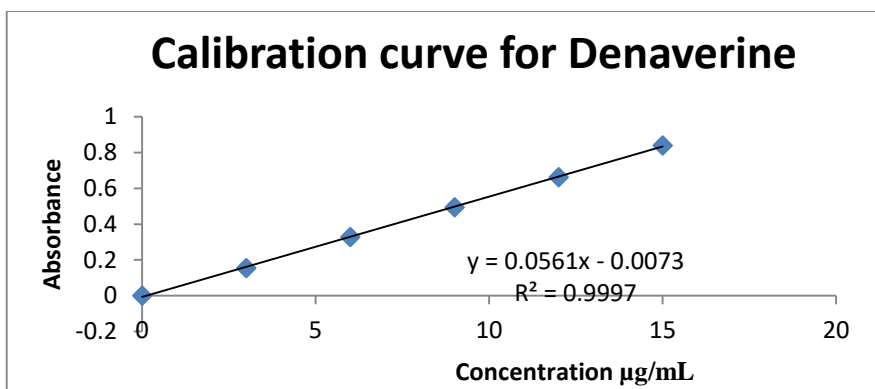

Fig. 5 Linearity (9µg/mL)   **Fig. 6   Linearity (12µg/mL)**   **Fig. 7 Linearity (15µg/mL)**

Table. 1 Results of Linearity

S. No	Concentration	Average Absorbance	Correlation Coefficient	LOD	LOQ	Slope	Intercept
1	3µg/mL	0.155					
2	6µg/mL	0.328					
3	9µg/mL	0.495	0.9997	0.216	0.655	0.056	-0.007
4	12µg/mL	0.663					
5	15µg/mL	0.840					

Calibration curve for Denaverine

$y = 0.0561x - 0.0073$
$R^2 = 0.9997$

Fig. 8 Calibration curve for Denaverine

Quantification of Formulation (Assay)

The percentage of Denaverine in marketed formulation (Injection) was calculated from the calibration curve of Denaverine. %Assay was found to be 99.95% as shown in Table 2

Table. 2 Results of Quantification of formulation (Assay)

S.No	Standard Absorbance	Sample Absorbance	Percentage purity (%)	Average Percentage (%)	SD	%RSD
1	0.495	0.490	98.98			
2	0.482	0.485	100.62			
3	0.484	0.488	100.82	99.95	0.7610	0.7613
4	0.480	0.481	99.79			
5	0.470	0.468	99.57			

*mean of five readings.

Recovery:

The percentage recovery study for Denaverine hydrochloride was found to be 98.8-100.53.
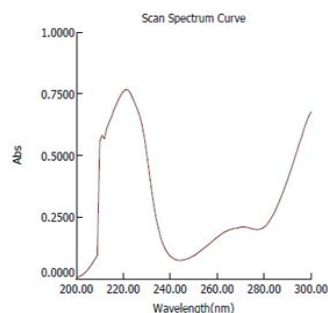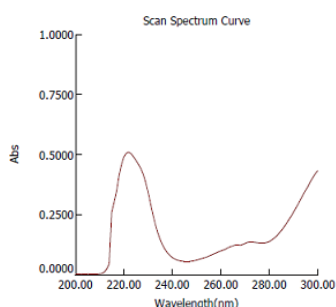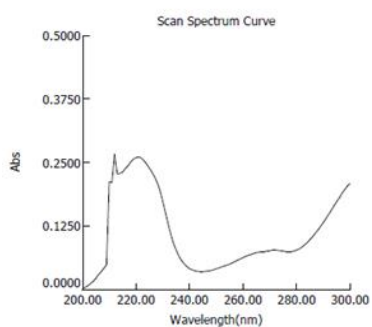
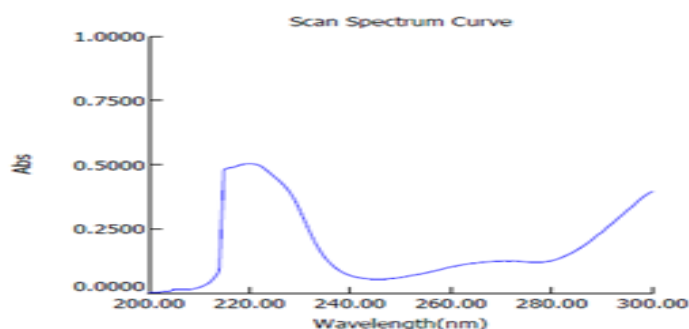Fig. 8 Recovery Spectrum (50 %) Fig. 9 Recovery Spectrum (100 %) Fig. 10 Recovery Spectrum (150 %)

Table. 3 Results of Recovery Data

% Concentration	Absorbance	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	0.261	5.1	5.12	100.53%	
100%	0.508	10.1	9.98	98.8%	100.0%
150%	0.769	15.0	15.10	100.7%	

*mean of three readings.

Precision:
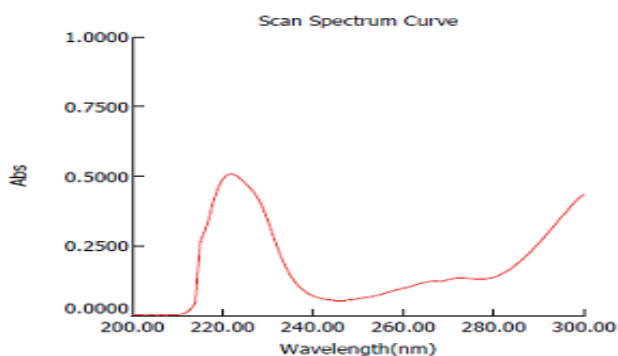
% RSD was found to be 0.16

Fig. 11 Precision

Table. 4 Results of Precision Data

S. No	Absorbance	Average Absorbance	SD	%RSD
1	0.511			
2	0.511			
3	0.512	0.5118	0.0008	0.16
4	0.512			
5	0.513			

*mean of five readings.

Intermediate precision:

% RSD was found to be 0.222

Fig. 12 Intermediate Precision Spectrum

Table. 5 Results of Intermediate Precision Data

S. No	Absorbance	Average Absorbance	SD	%RSD
1	0.510			
2	0.511			
3	0.513	0.511	0.0011	0.222
4	0.511			
5	0.512			

*mean of five readings.

Conclusion

The developed method was found to be simple, sensitive, accurate, precise, reproducible, and can be used for routine quality control analysis of Denaverine Hcl in bulk and pharmaceutical formulation.

ACKNOWLEDGEMENTS

REFERENCES

[1] H. Huller,W. Scheler, E. Schulz, Acta Biol. Med. German. 12 (1964) 682.

[2] H. Huller, W. Scheler, H. Oberender, R. Peters, Acta Biol. Med. German. 22 (1969) 751.

[3] H. H˙uller, Zbl. Pharmaz. 109 (1970) 115.

[4] V. Bredow, Zbl. Gynakol. 114 (1992) 551.

[5] E. Neumayer, Medicamentum 16 (1975) 264.

[6] B. Vesper, Medicamentum 12 (1971) 335.