Analytical Method Development and Validation of Denaverine Hydrochloride in Bulk and Injectable Pharmaceutical Dosage Form by HPLC Method

Ravindar Bairam.[1], Vinay Raj Vangala[2], P. Balakrishanaiah[3], Manjunath S.Y[4]

Department of Pharmaceutical Chemistry & Analysis
Corresponding Author: Ravindar Bairam

Abstract

A new, simple, accurate, precise and rapid Reverse-Phase High Performance Liquid Chromatographic (RP-HPLC) method has been developed and subsequently validated for the estimation of Denaverine HCl in bulk and injection dosage form, which is used in the treatment of antispasmodic drug in Veterinary medicine. The proposed method is based on the separation of the drugs in reversed-phase mode using Symmetry C_{18} Column (4.6 x 150mm, 5 □m, Make: XTerra) The optimized mobile phase was disodium hydrogen phosphate buffer (pH 3.5): Acetonitrile (30:70 %v/v). The flow rate was at 0.6 mL/min and UV detection at 306 nm. The retention time was 3.2 min for Denaverine HCl. The method was validated according to ICH guidelines. It was found to be accurate and reproducible. Linearity was obtained in the concentration range of 10-50µg/mL for Denaverine HCl. Mean percent recovery of samples at each level for both drugs were found to be 101.3 %v/v for Denaverine HCl. 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. The proposed method can be successfully applied in the quality control of bulk and injectable pharmaceutical dosage forms.

Keywords: Denaverine hydrochloride, validation, estimation, HPLC.

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:

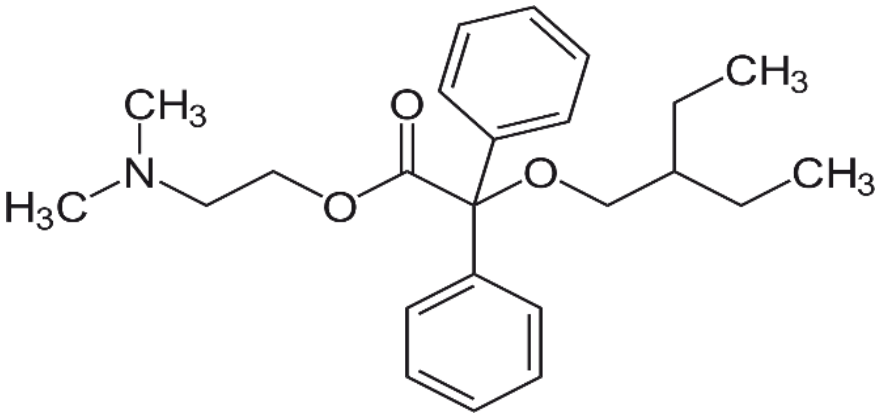


Fig. 1 Chemical Structure of Denaverine hydrochloride

2-dimethylaminoethyl 2-(2-ethylbutoxy)-2,2 diphenylacetate 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.

EXPERIMENTAL MATERIALS AND METHODS

Instrumentation: A Shimadzu Prominence HPLC iLc2030

Materials and Reagents:

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

RP-HPLC method

Preparation of Phosphate buffer

7.0 grams of Potassium di hydrogen Phosphate was weighed and transferred into a 1000 mL beaker, dissolve and diluted to 1000 mL with HPLC water. The pH to 3.5 was adjusted with ortho phosphoric acid.

Preparation of mobile phase

Phosphate buffer 300 mL (30%) and 700 mLof Acetonitrile HPLC (70%) was mixed well and degassing in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Chromatographic Conditions

Mode of operation	:	Isocratic Instrument : HPLC Waters
Detector	:	UV detector
Column	:	Symmetry C18 (4.6 x 150mm, 5 □m, Make: XTerra)
Temperature	:	Ambient
Flow rate	:	0.6 ml/min
Wave length	:	306 nm
Runtime	:	5 min
Sample size	:	20 □L
Mobile Phase	:	Phosphate buffer: Acetonitrile (30:70 %v/v)

Standard Solution Preparation

Accurately weigh and transfer 10 mg of Denaverine working standard into a 10 mL volumetric flask add about 7 mL of mobile phase and sonicate to dissolve it completely and make volume up to the mark with the same solvent (1000 µg/mL). Further 0.3 mL was pipette out (1 mg/ml) of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with mobile phase (30 µg/mL). Then the solution was mixed well and filter through 0.45µm filter.

Sample Solution Preparation

0.25mLof injection (Sensiblex 40mg/mL) was measured accurately and transfer the sample (equivalent to 10 mg of Denaverine hydrochloride) into a 10 mL volumetric flask.7 mL of mobile phase was added and sonicate to dissolve it completely. Then it was made volume up to the mark with mobile phase (1000 µg/mL). The solution was mixed well and filter through
0.45 µm filter. Further 0.3 mL was pipette out of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with mobile phase (30 µg/mL). Mix well and filter through 0.45µm filter.

Method Validation

The method was validated in accordance with ICH guidelines[7-9]. The parameters such as Linearity, Accuracy, Precision, Specificity, Assay, Limit of Detection (LOD), Limit of Quantification (LOQ), Robustness, Ruggedness and Stability of the solution were assessed asper ICH guidelines.

RESULTS AND DISCUSSION

Chromatographic Conditions

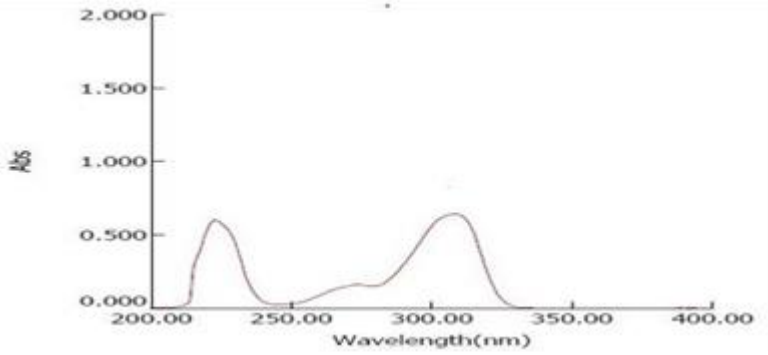
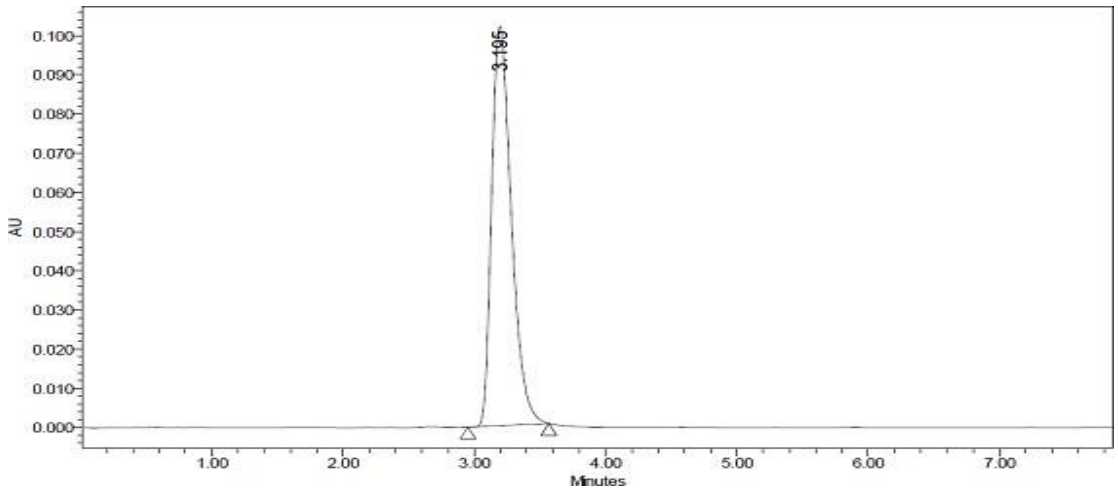


Fig 1: UV Spectrum of Denaverine In Phosphate Buffer pH 3.5:



	RT	Area	Height	USP Plate Count	USP Tailing
1	3.195	1058299	102124	2178.3	1.4

Fig 2: Optimized Chromatogram Phosphate Buffer pH3.5: Acetonitrile (30: 70%V/V)

System suitability

The system suitability studies carried out as specified in ICH guidelines and USP. Theparameters like tailing factor, number of theoretical plate were calculated.

Table 1: System suitability

Parameter	**Denaverine**
Tailing factor	1.6
No of Theoretical plate	2951
Retention time	3.2

Linearity:

From the stock standard (1000 µg/ml), the aliquots (0.1 to 0.5 mL of 1000 µg/mL) solution taken 0.1, 0.2, 0.3, 0.4, 0.5 mL were taken in a separate 10 mL volumetric flasks and made up to 10 mL with mobile phase (10–50 µg/mL).. This solution can inject into the chromatographic system and record the Chromatogram. The calibration graph was plotted withpeak area in the Y axis and concentration of standard solution in the X axis.

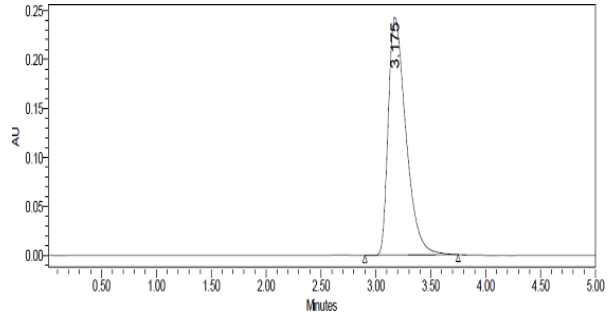


Fig 3: Linearity Chromatogram 10µg/mL

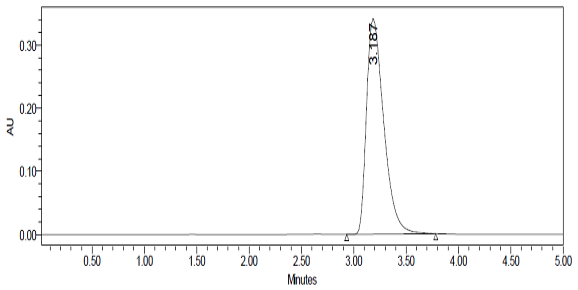


Fig 4: Linearity Chromatogram 20µg/mL

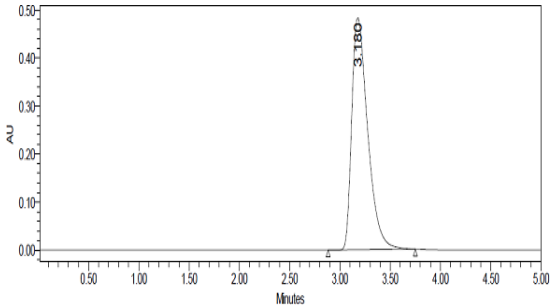


Fig 5: Linearity Chromatogram 30µg/mL

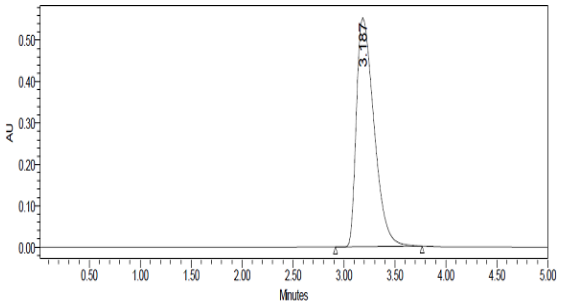


Fig 6: Linearity Chromatogram 40µg/mL

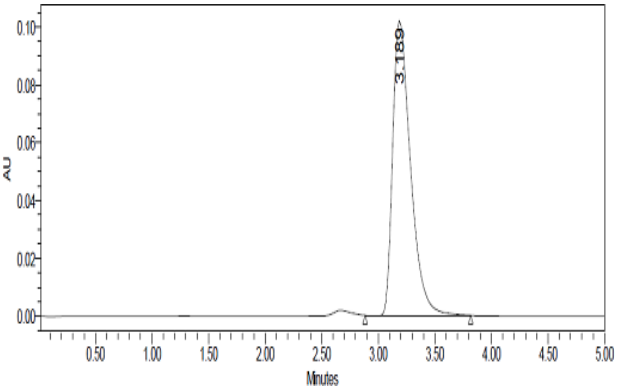


Fig 7: Linearity Chromatogram 50µg/mL

RT	Area	Height (µV)
3.189	1113634	102352
3.175	2712792	244484
3.187	3908404	342239
3.180	5328851	484112
3.187	6652686	555333

linearity -Data

Table. 2 Results of Linearity

S.No	Concentration (µg/ml)	Peak Area	LOD	LOQ
1	10	1323634	0.014	0.0465
2	20	2712792		
3	30	3998490		
4	40	5328851		
5	50	6652686		

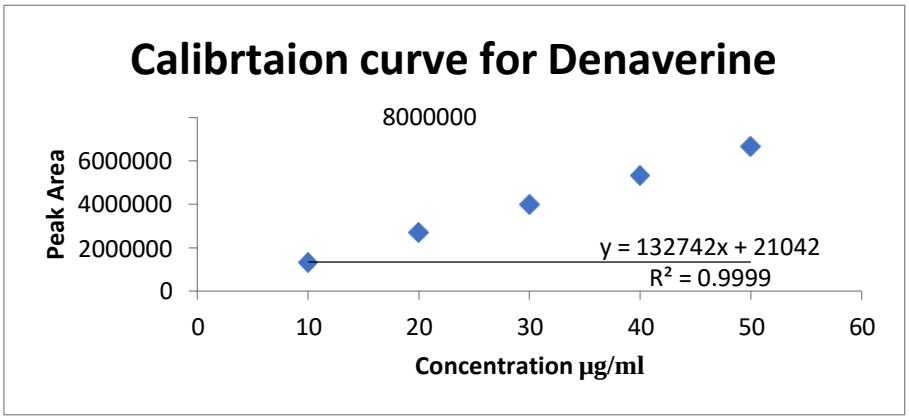
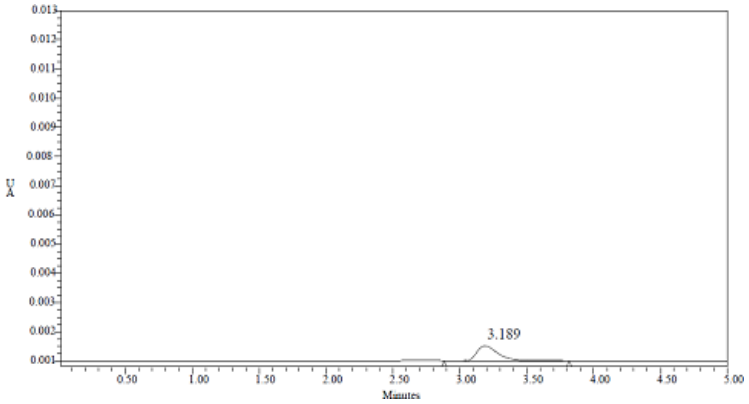


Fig 8: Calibration curve for Denaverine

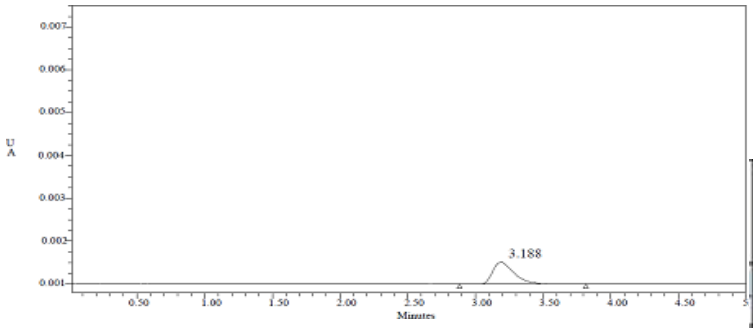
LOD



Retention Time (min)	Area (µV*sec)	Height (µV)
3.189	1545	142

Fig 9: LOD Chromatogram

LOQ



Retention Time (min)	Area (µV*sec)	Height (µV)
3.188	5277	485

Fig 10: LOQ Chromatogram

Quantification of Formulation

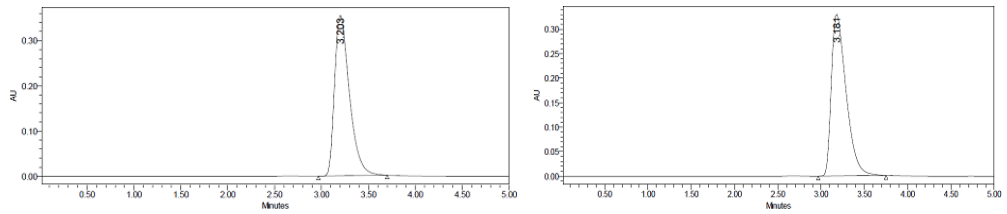
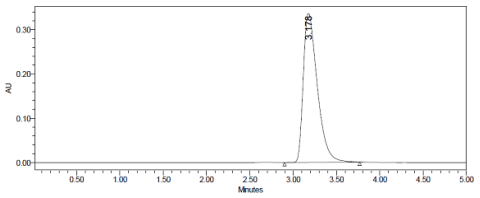
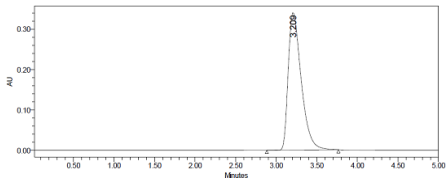


Fig 11: Sample Chromatogram





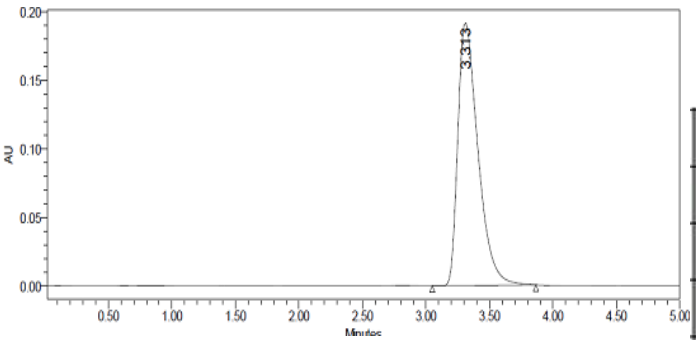
RT	Injection	Area
3.178	1	3775295
3.209	2	3778402

Fig 12: Standard ChromatogramTable. 3 Results of Assay

S.No	Standard Peak area	Sample Peak Area	Percentage purity (%)	Average Percentage(%)	SD	%RSD
1	3775295	3912105	101.65	101.52	0.1767	0.1740
2	3778402	3907203	101.40			

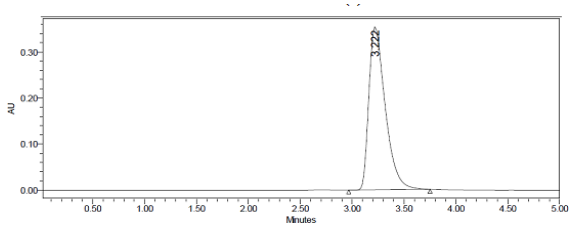
Recovery:

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 accuratelyweighed and added. 7 mL of mobile phase was added and sonicate to dissolve it completely. Then the solution was made volume up to the mark with the same. 0.3 mL was pipette out fromeach flask and transferred to separate 10 mL volumetric flask. Then the solution was made volume up to the mark with the same. 20µl solution was injected in to chromatographic systemand the chromatogram was recorded.



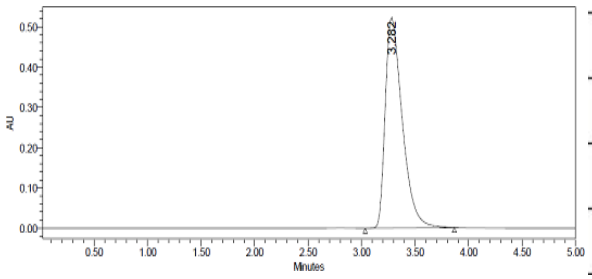
RT	Injection	Area
3.313	1	2108215
3.184	2	2109521
3.235	3	2114103

Fig 13: Recovery-50%



RT	Injection	Area
3.222	1	3875174
3.191	2	3888449
3.217	3	3893469

Fig 14: Recovery -100%



RT	Injection	Area
3.282	1	5781825
3.189	2	5779001
3.238	3	5773680

Fig 15: Recovery-150% Table. 4 Results of Recovery

%Concentration pecificationLevel)	Area	Amount Added(mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	2110613	5.5	5.57	101.3%	101.3%
100%	3885698	10.1	10.2	101.5%	
150%	5778169	15.1	15.2	101.0%	

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. These solutions were prepared in duplicate and absorbances were measured at 306 nm against blank and calculate the % RSD.

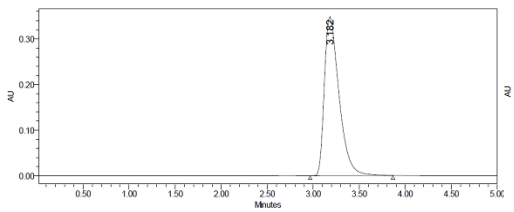


Fig 16: Precision study -1

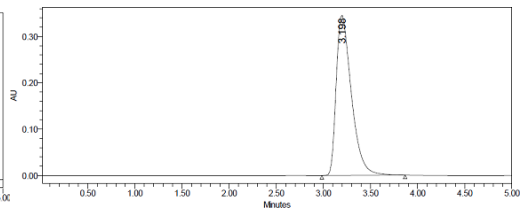


Fig 17: Precision study -2

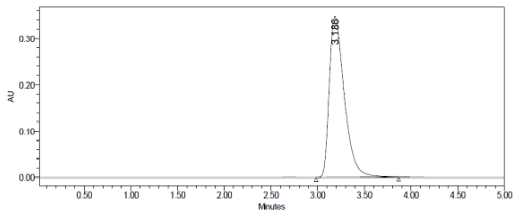


Fig 18: Precision study -3

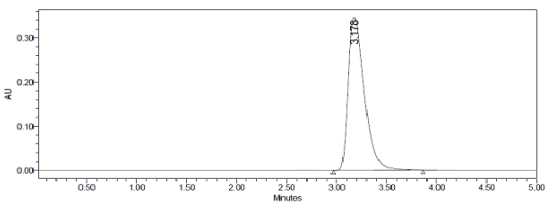
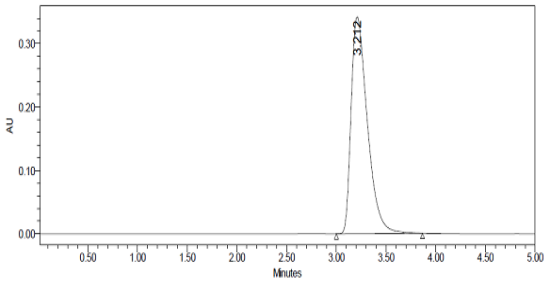


Fig 19: Precision study -4



RT	Injection	Area
3.182	1	3855508
3.198	2	3865126
3.186	3	3871273
3.178	4	3878408
3.212	5	3882797

Fig 20: Precision study -5

Table. 5 Results of Precision

S.No	ıcentration(µg/ml)	Peak Area	Average	SD	%RSD
1	30	3855508	3870622	10815.8	0.28
2		3865126			
3		3871273			
4		3878408			
5		3882797			

Intermediate precision

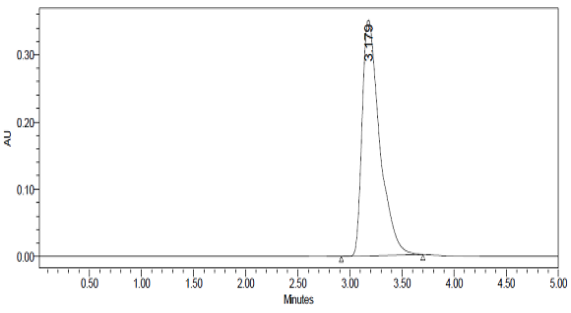


Fig 21: Intermediate precision-1

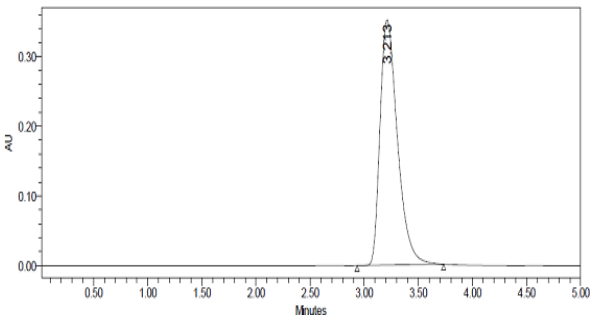


Fig 22: Intermediate precision-2

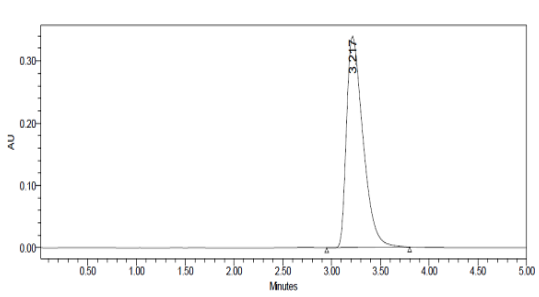


Fig23: Intermediate precision-3

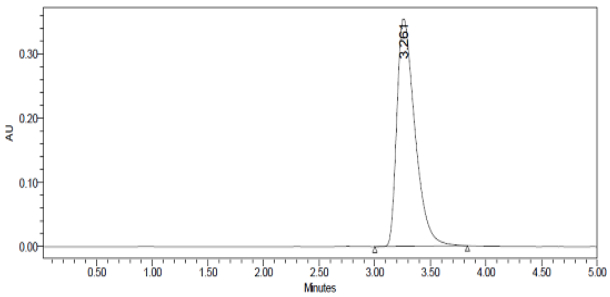
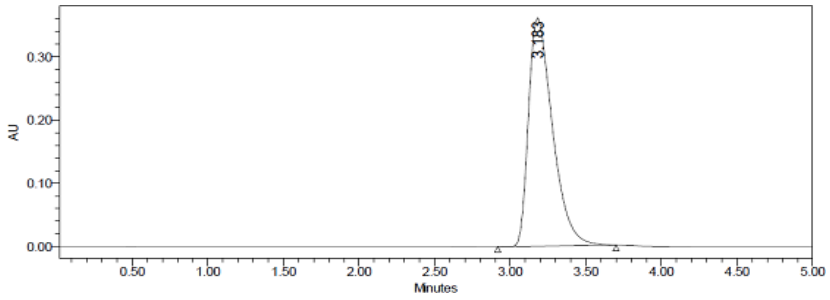


Fig 24: Intermediate precision-4



RT	Injection	Area
3.179	1	4095410
3.213	2	3935121
3.217	3	3963812
3.261	4	3990300
3.183	5	3976949

Fig 25: Intermediate precision-5

Table. 6 Results of Intermediate Precision

S.No	Concentration (µg/ml)	Peak Area	Average	SD	%RSD
1	30	4095410	3992318	61140.1	1.53
2		3935121			
3		3963812			
4		3990300			
5		3976949			

Robustness

For demonstrating the robustness of the developed method, experimental conditions were purposely altered and evaluated. The method must be robust enough to withstand such slight changes and allow routine analysis of the sample. For this present study mobile phase and flow rate has slightly changed and the assay was checked.

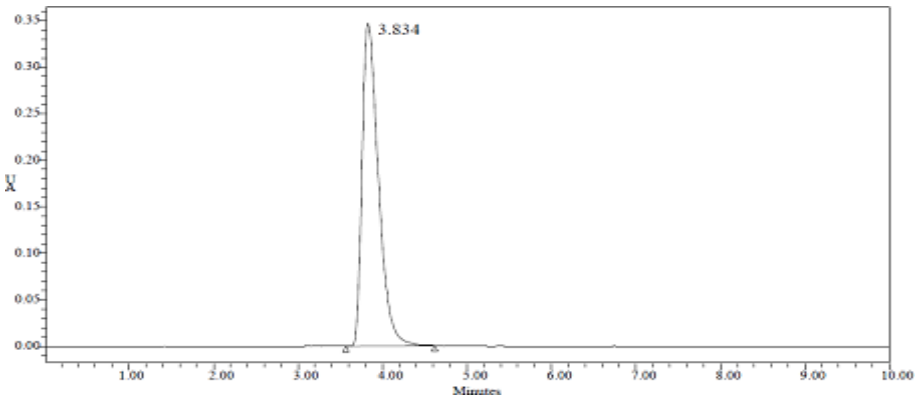


Fig 26: Robustness-Less Flow

Retention Time (min)	Area (µV*sec)	USP Plate Count	USP Tailing
3.834	4694313	2889.4	1.6

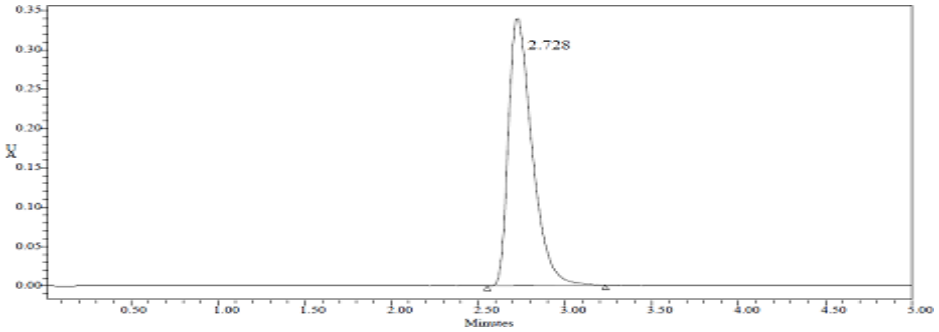


Fig 27: Robustness-More Flow

Retention Time (min)	Area (µV*sec)	USP Plate Count	USP Tailing
2.728	3231666	2961.0	1.5

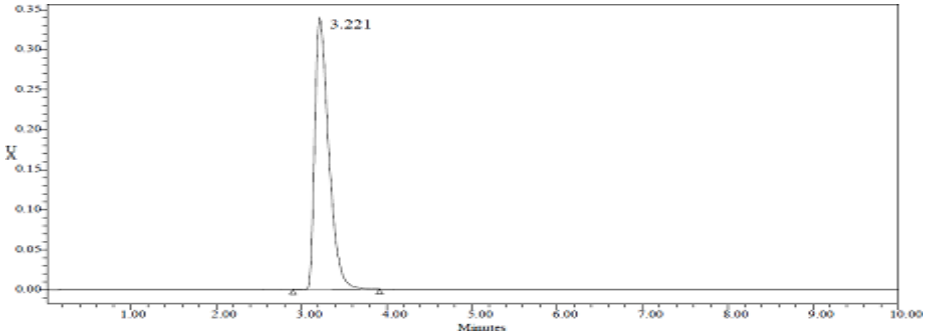


Fig 28: Robustness- Less Organic

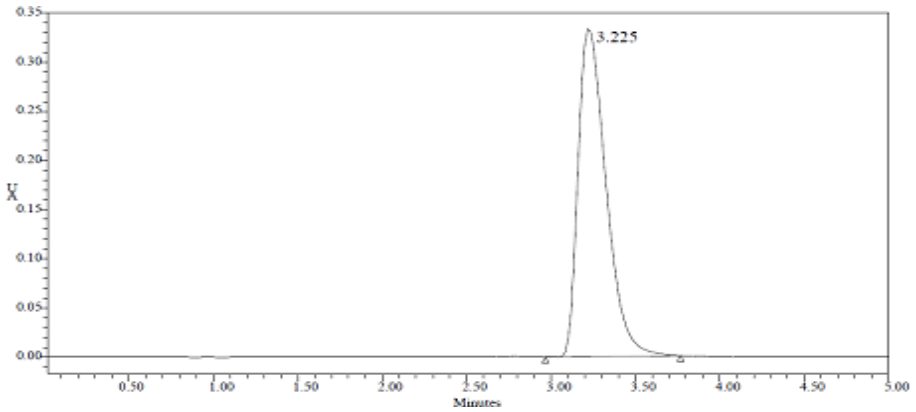


Fig 29: Robustness-More Organic

Retention Time (min)	Area (µV*sec)	USP Plate Count	USP Tailing
3.225	3828751	2856.9	1.5

Table. 7 Results of Robustness

Parameters	**Theoretical plate**	**Tailing factor**
Less flow (0.5 ml/min)	2889	1.6
More flow (0.7 ml/min)	2961	1.5
Less organic phase (60 %)	2874	1.6
More organic phase (80%)	2856	1.5

It shows that there is no change in the values even after making deliberate change in the analytical procedure.

Conclusions

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

ACKNOWLEDGEMENTS

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