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Validated RP-HPLC PDA Method for estimation of Trientine Hydrochloride in Pharmaceutical dosage form

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Abstract : The present work reports a reverse phase high performance liquid chromatography (RP-HPLC) method for the determination of Trientine hydrochloride in pharmaceutical dosage form. HPLC was performed using Waters reliant C_8 column (250 mm x 4.6 mm ID, 5µm particle size) using a mixture of Acetronitrile : Ammonium formate buffer pH 5.3 ± 0.05 as mobile phase. Ultraviolet detection was carried out at 220 nm. The retention time of Trientine hydrochloride was found to be 12.497minutes. The developed method was validated as per ICH guidelines. The proposed method was found to be suitable for the quantification of the selected drug in pharmaceutical dosage form.
Keywords : RP-HPLC, Validation, Capsule, Trientine hydrochloride.

Introduction

Trientine hydrochloride (TNT) is chemically known as N'-(2-(2-aminoethylamino)ethyl)ethane-1,2-diaminehydrochloride[1]. The chemical structure of trientine hydrochloride is shown in fig1.It is the metal chelating agent used to bind and remove excess copper in the body to treat Wilson's disease. Also a potent anti-angiogenic activity, in addition, trientine may inhibit copper-induced secretion of interleukin-8(IL-8). Literature review revealed spectroscopic method[2], fluorimetric methods[3,4], RP-ion pairing HPLC and conductivity detection[5] a for estimation of TNT and simultaneous estimation of trientine and its two major metabolites by HPLC[6] . In the present work, RP-HPLC method using acetronitrile : ammonium formate as mobile phase in a gradient technique has been reported.

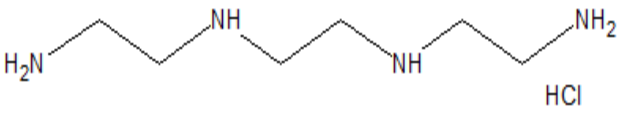

Figure 1. Structure of Trientine hydrochloride

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Experimental

Instrumentation

The water HPLC 2996 system equipped with photo diode array detector was used for the study. Software used for this system is Empower 3.HPLC was performed using Waters Reliant C_8 column (250 mm x 4.6 mm ID, 5µm particle size).

Reagents and chemicals

TNT ≥99.0% was obtained from SaimirraInnopharm Pvt Ltd. All the chemicals and reagents used were of HPLC grade.

Mobile phase

The mobile phase used in the present work was Acetronitrile: Ammonium formate buffer pH 5.3 in a gradient technique. The flow rate was set to 20µL/min.

Preparation of standard stock solution

About 200mg of TNT standard was accurately weighed and transferred into a 100ml volumetric flask, dissolved and made upto the volume with diluent.10ml of standard stock solution was pipetted out into a 100ml volumetric flask, 40ml of diluent, 1ml of 1N sodium hydroxide and 1.5ml of ammonium hydroxide solution were added and shaken well. The flask was kept in an ice-bath to allow the solution to reach the temperature 2 to 8ºC and the same temperature was maintained for about 15 min. 2ml of benzoyl chloride was added to the flask and kept in ice-bath for 2 min.The flask was kept at room temperature for about 30 min and made upto the volume with diluent.

Standard stock solution of TNT containing 200µg/ml was prepared. From the above stock solution, concentrations in the range of 40-120 µL/mL of TNT were obtained. The peak area for the different concentrations of TNT were recorded. The chromatogram in Figure 2 shows the retention time of TNT as 12.497mins.The calibration curve was constructed between concentrations and respective peak area. Figure 3 represents the linearity curve of TNT.

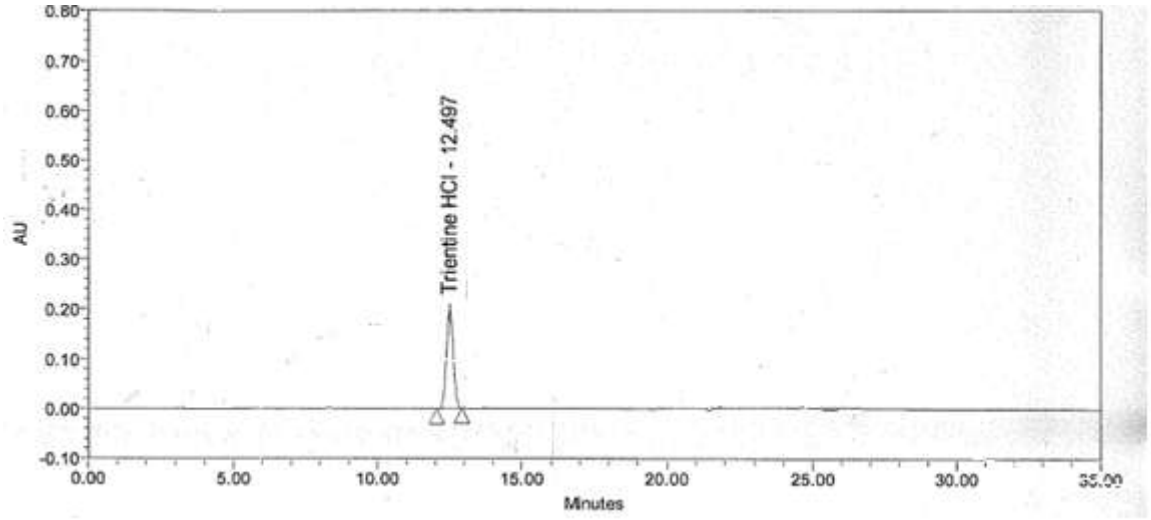

Figure 2.Chromatogram of Trientine hydrochloride.

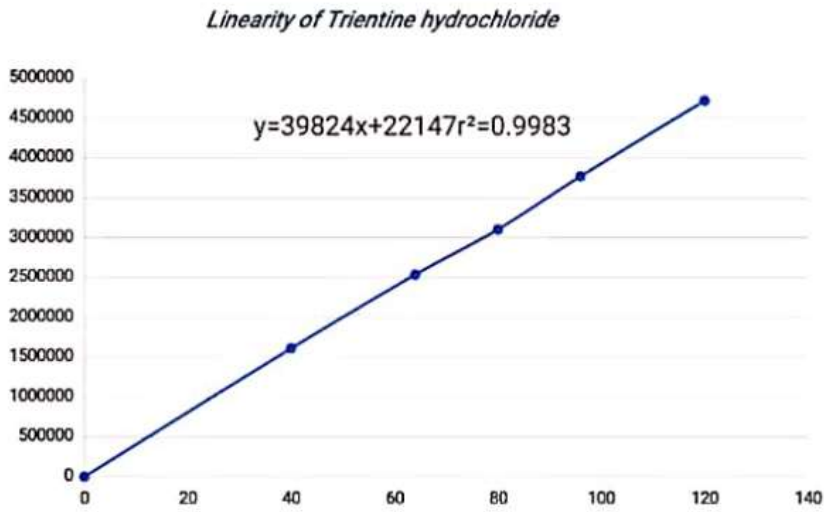

Figure 3.Linearity curve of TNT

Analysis of formulation

For analysis of the capsule dosage form, twenty capsules (Saimirra Pvt.Ltd) containing 250 mg of TNT was weighed and the average weight was determined.The powder equivalent to the weight of 200 mg of TNT was transferred to a 100ml volumetric flask, dissolved and made upto the volume with diluent. 10ml of standard stock solution was pipetted out into a 100ml volumetric flask, 40ml of diluent, 1ml of 1N sodium hydroxide and 1.5ml of ammonium hydroxide solution were added and shaken well. The flask was kept in anice-bath to allow the solution to reach the temperature 2 to 8ºC, the same temperature was maintained for about 15 min. 2ml of benzoyl chloride was added to the flask and kept in an ice-bath for 2 min. The flask was kept at room temperature for about 30 min and made upto the volume with diluent. 10ml of the above solution was pipetted out into a 25ml volumetric flask and made upto the volume with diluent to get the final concentration of 80µg/mL.20µL of solution was then injected for quantitative analysis.

Validation

The validation of an analytical method verifies that the characteristics of the method satisfy the requirements of the application domain[7-13]. The proposed method was validated in the light of ICH guidelines. The developed method was validated for linearity, accuracy, precision, robustness, selectivity and specificity study as per ICH guidelines. The validation study was carried out by replicate injection of the sample and standard solutions.

Results and Discussion

Column chemistry, solvent type, solvent strength, detection wavelength and flow rate were varied to determine the chromatographic conditions using the best separation. The mobile phase conditions were optimised so that the components were free from the interference of solvent and excipients. Mobile phase and the flow rate selection was based on the peak parameters like height, area, tailing, theoretical plates and the run time. The best result was obtained by use of gradient mobile phase A and mobile phase B (Programmed) under the optimum chromatographic conditions, the retention time obtained for Trientine hydrochloride was 12.497mins.

Linearity

Linearity was obeyed in the concentration range of 40-120 µg/ml for TNT. From the data obtained correlation coefficient, y- intercept and slope were calculated to provide mathematical estimates of the degree of linearity as shown in Table 1.

Table 1.Linear regression data for the calibration curve by HPLC method*

Parameter	Trientine hydrochloride
Linearity range	40-80 µg/mL
r^2	0.9983
Slope	39824
Intercept	22147
y Intercept	0.68%

*n=5

Accuracy

Accuracy of the developed method was carried out by adding known amount of drug corresponding to three concentration levels 50,100 and150 of the label claim. The accuracy was expressed as the percentage of analyte recovered by the assay method. The results of percentage recovery are shown in Table 2.

Table 2.Accuracy study of TNT by HPLC method*

Amount added (%)	Theoretical content(mg/caps)	Measured conc.±SD	Recovery (%)	%R.S.D
50	125.15	124.98	99.87	0.62
100	250.23	250.21	99.99	0.58
150	375.44	375.73	100.08	0.15

*n=9.

Table 3.Results of Precision study by HPLC method*

Drug conc. (µg/mL)	Repeatability Found conc.±S.D.	% R.S.D	Intermediate precision Found conc.±S.D.	% R.S.D
40	40.20±0.17	0.42%	39.90±0.04	0.09%
80	80.04±0.34	0.43%	80.39±0.43	0.53%
120	120.50±0.81	0.67%	120.15±0.56	0.47%

*n=9.

Precision

Precision was determined by repeatability and intermediate precision studies. The results are reported in terms of relative percentage standard deviation (% RSD) as in Table 3.

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

The lowest amount of the analyte in the sample which can be detected and the lowest amount of the analyte which can be quantitatively determined were studied and LOD and LOQ values are reported in Table 4.

Table 4.Limit of detection (LOD) and Limit of quantitation(LOQ) of TNT

Parameter	Trientine hydrochloride
LOD	1.785µg/Ml
LOQ	5.409µg/Ml

Table 5.Robustness evaluation of the method by HPLC (n=6)

Factor	Level	Retention time(t_r)	Asymmetry(T)
A. Columns from different manufactures			
Waters C18 column		12.521	1.02
Phenomenex C18 column		12.315	1.05
B. Column Temperature			
29°C		12.502	1.04
30°C	-1	12.499	1.02
31°C	0	12.501	1.01
C. Wavelength	+1		
218nm		12.511	1.11
220nm	-2	12.502	1.15
222nm	0	12.503	1.09
D. Buffer pH	+2		
5.2		12.498	1.03
5.3	1	12.497	1.05
5.4	0	12.502	1.07
	+1		

Robustness

The robustness of the method was determined by subjecting the method to slight changes in the chromatographic conditions. It was observed that there was no marked change in the chromatogram which demonstrated that the method developed is robust (Table 5).

Selectivity

The selectivity was checked by injecting the solution of the drug into the HPLC system. A sharp peak of TNT was obtainedat the retention time of 12.497 minutes. It was observed that the excipients did not interfere with the retention time of the drug, so the method developed is said to be selective.

Specificity

Specificity of the method was assessed by comparing the chromatogram obtained for the standard drug with the chromatogram obtained for capsule solution. The retention time of standard drug and the drug in the sample solution were same, so the method is specific. The results of the system suitability parameters are shown in Table 6.

Table 6.System suitability parameters

Parameter	**Trientine hydrochloride**	**Reference values**
Theoretical plates(N)	12765	NLT 2000
Tailing Factor	1.04	NMT 2.0

Table 7. Analysis of the marketed formulation by HPLC method*

Drug	Label claim	Drug content(%)±S.D	% RSD
TNT	250mg	250.11mg ± 1.07	0.43

Stability of analytical solution

Stability of sample solution was established by storage of the sample solution at 6°C for 48 hrs. TNT was reanalysed after 24 and 48 hrs time intervals and assay value was determined and compared against fresh sample. Sample solution does not show any appreciable change in assay value when stored at 6°C upto 48 hrs. The percentage labelclaimof TNT at 0, 24 and 48 hrs were found to be 99.9,100.2 and 100.1 respectively.

The values obtained for the validation parameters show that, the chromatographic conditions are appropriate for separation and determination of the selected drug.

Conclusion

A new RP-HPLC PDA method has been developed for analysis of Trientine hydrochloride in capsules. The developed method was found to be accurate, reproducible, precise, selective and specific proving the reliability of the method. The recovery studies indicate that there was no interference of excipients. The proposed method may be successfully applied to routine analysis of samples containing Trientine hydrochloride.

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