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Research Article

METHOD DEVELOPMENT AND VALIDATION OF LORAZEPAM BY USING RP-HPLC IN PHARMACEUTICAL FORMULATION

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ABSTRACT

High pressure liquid chromatography (RP-HPLC) is a column chromatographic technique employing high pressure pump to pass both mobile phase and sample mixture through stationary phase column and perform efficient separation. A C-18 column with mobile phase containing Acetonitrile: methanol (80: 20) was used. The flow rate was 1.0ml/min. and were monitored at 210 nm. The retention time for Lorazepam was 1.3 min. The method was validated for linearity, accuracy, precision, limit of detection. The Mean percent recovery of Lorazepam from tablet formulation was found to be 94.80 %.

INTRODUCTION

Lorazepam is commonly used for the treatment of anxiety, insomnia. The molecular formula is $C_{15}H_{10}Cl_2N_2O_2$. It is white and crystalline powder and soluble in water and insoluble in ethanol and slightly soluble in methylene chloride.

The Lorazepam half-life is 14 hours and its absorption half -life is calculated as 55 minutes. Lorazepam is does not redistributed.

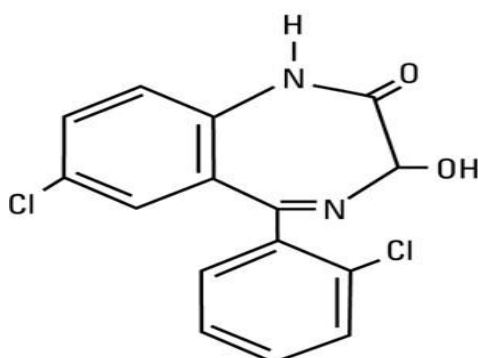


Fig. 01 - Lorazepam

MATERIALS AND METHODS

Instrument

The liquid chromatographic system consisted of Shimadzu HPLC model (VP series) containing LC-10AT (VP series) pump, variable wave length programmable UV/visible detector SPD-10AVP and rheodyne injector (7725i) with 20 μ l fixed loop. Chromatographic analysis was performed using Intersil ODS Ultra sphere 5 μ m or equivalent ODS C-18 column with 4.6mm x 15cm internal diameter and 5 μ m particle size. Shimadzu electronic balance (AX-200) was used for weighing purpose.

The Mobile Phase

A mixture of Methanol:Water in the ratio of 80:20 v/v was prepared and used as mobile phase.

Preparation of mobile phase

Accurately measured 80ml of Acetonitrile and 20ml of methanol were mixed by using sonication for 5 min.


Diluent Preparation

The mobile phase was used as the diluent.

Blank Preparation

Place unused swab in 10ml of solvent. Sonicate for 5 minutes. Squeeze swab out well. Filter through a 0.45 μ m filter.

Preparation of Standard Solution: A standard solution of lorazepam was prepared by accurately weighing 0.05gms and add 5ml of methanol and

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sonicate for 10 mins. Take 1ml from it and then, add 9ml of methanol. Take 10µg/ml from it and observe the absorbance of the solution in UV-Visible Spectrometry. The UV scan and gives the absorbance is 210nm.

Preparation of Sample Solution

Weigh the tablet weight and crush them by using motor and pestle. Ativan total weight – 2mg, one tablet weight – 0.20gm. Whereas Clonofit total weight – 0.25mg, one tablet weight – 0.09gms. Both Ativan and Clonofit crushes separately and makeup them into 100ml.

DISCUSSION

Linearity

Linearity: From the prepared stock solution, a series of calibration standards were prepared at concentrations of 100, 200, 300, 400, 500, 600µg/ml using mobile phase as solvent. Each of these drug solutions was injected into the column, the peak area and retention times were recorded. Results are in Table -01.

Precision: Six replicate analysis of stock solution, the retention time and area which are not changed while it doing even three or five times, the values must be same. we considered that concentration is more precised. Here, 300µg/ml is precised. Results are in Table -02

Table 1: Results of linear response

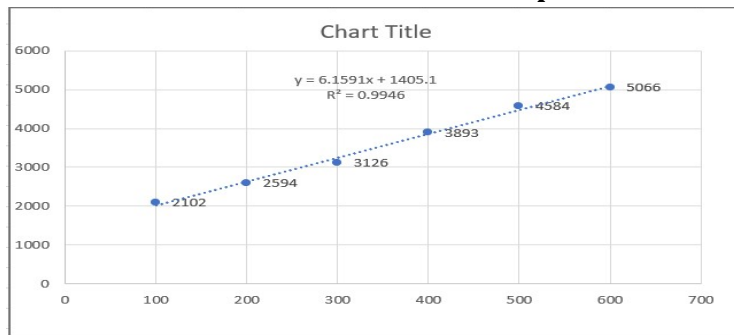


Fig.02 – Calibration curve

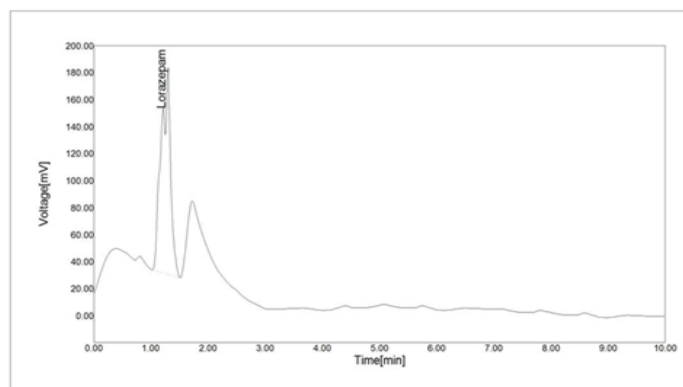
Slope	Y=6.1591x+1405.1
Intercept	0.9946
Correlation coefficient	0.999

Precision

Table 2: Results of Precision Response

S.no	Injection	Area value
1	Injection 1	954
2	Injection 2	712
3	Injection 3	717
4	Injection 4	84

Formulation: The sample solution prepared at a concentration of 100µg/ml was analysed in the developed method conditions. The method can successfully separate. The method was found to be suitable for routine analysis of Lorazepam and formulations. The first formulation Atvian gives 76.78%. The second formulation Clonofit gives 94.08%. We considered first formulation because it is near to accurate limit percentage (97%). The resultant graph is shown in below.



CONCLUSION

The estimation of Lorazepam was done by RP-HPLC. The assay of Lorazepam was performed with tablets and the % assay was found to be 94.08% which shows that the method is useful for routine analysis. The linearity of Lorazepam was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 94.08% for lorazepam. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The acceptance criterion for LOD and LOQ is 3 and 10. The LOD and LOQ for lorazepam was found to be 3.02 and 9.98. The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

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