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# Development of Analytical Method by RP-HPLC Technique for Determination of Alprazolam in Pharmaceutical Dosage Form

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**Abstract :** A novel reverse phase liquid chromatographic method was developed and validated for estimation of short acting anxiolytic drug, alprazolam in its dosage form i.e. tablets. The reverse phase HPLC analysis was carried out on isocratic system. The column used was Peerless basic C18 (50mm x 4.6mm, 3µm) with ambient temperature. The mobile phase consisted of Buffer: acetonitrile in proportion 60: 40 % (v/v). The flow rate was maintained at 1 ml / min. The detection was carried out at wavelength 225 nm. The method was validated as per ICH guidelines for system suitability, linearity, accuracy and precision. The linear ranges were 5-15 µg/ml for alprazolam. The accuracy and precision were found to be well within the acceptable limit. The method was successfully applied for determination alprazolam of in dosage form with good recoveries.
**Keywords :** Alprazolam Potassium dihydrogen phosphate Acetonitrile HPLC.

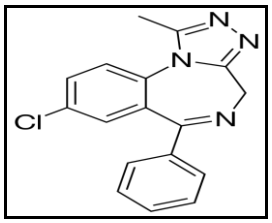
## Introduction

This research article provides important insight into development, validation and application of reverse phase high pressure liquid chromatographic method for the assay of alprazolam in bulk drug and pharmaceutical dosage form.

Alprazolam is chemically 8-chloro-1-methyl-6-phenyl-4H-(1,2,4) triazolo (4,3a)1,4-benzodiazepine, which is a short acting anxiolytic drug. It also possesses sedative, hypnotic, anticonvulsant, amnesic and skeletal muscle relaxant property. Alprazolam is a short-acting drug of the benzodiazepine. It is used to treat moderate to severe anxiety disorders and panic attacks and is used as an adjunctive treatment for anxiety associated with moderate depression.

Alprazolam may be habit-forming, and long-term use and abuse may cause a physical dependence to develop along with withdrawal reactions during abrupt or rapid discontinuation. Although the side-effect profile of alprazolam may occur in some patients. Some side-effects may disappear with continued treatment. If signs of an allergic reaction occur - such as hives; difficulty breathing; swelling of face, lips, tongue, or throat**.**

Literature survey reveals the HPLC[1] and spectrophotometric [2-8] methods for the estimation of alprazolam. Methods [9-13] for assay of alprazolam with other drugs combination and miscellaneous[14-23] for assay of other anxiolytic drugs . Simple, rapid and reliable UV spectrophotometric methods are developed for the determination of alprazolam. These methods can be used for the routine analysis. In the proposed methods optimization and validation of this method are reported.




**Structure of alprazolam**

## Experimental

### Instrumentation

The HPLC system Merck-Hitachi equipped with separation module and UV detector (L-7400) was used. The chromatogram was recorded and peaks are quantified by means EZChrom Elite software. A Shimadzu analytical balance with 0.01 mg was used.

## Materials and reagents

Reference standard of alprazolam were obtained from reputed firms with certificate of analysis. Acetonitrile and potassium dihydrogen phosphate used were of analytical grade from Merck and the HPLC grade water was obtained by using Millipore water system.

### Procedures

### Standard stock solution

About 10 mg of standard alprazolam was weighed accurately and transferred in 10 ml volumetric flask. About 5 ml of diluent (buffer: acetonitrile (60:40 % v/v)) was added and sonicated for 5 minutes. The volume was adjusted to the mark with diluents to give concentration as 1000 µg/ ml. The working standard solution was prepared by diluting 1 ml of 1000 µg/ ml solution to 10 ml with diluent to get concentration 100 µg/ ml.

### Sample preparation:

Twenty tablets were weighed accurately and average weight of each tablet was determined. The powder equivalent to 10 mg of alprazolam was weighed accurately and transferred in 10 ml volumetric flask. About 5 ml of diluent (buffer: acetonitrile (60:40 % v/v)) was added and sonicated for 5 minutes. The volume was adjusted up to mark with diluent to give concentration as 1000 µg /ml. The working sample solution was prepared by diluting 1 ml of 1000 µg/ ml solution to 10 ml with diluent to give 100 µg/ ml. A 15 µl was injected for analysis.

### Method Development

### Chromatographic condition

Different columns containing octyl and octadecyl silane stationary phase were tried for separation and resolutions. It was found that Chromatopak Peerless Basic C18 (50mm x 4.6mm x 3µm) column offered more advantage over other columns. The mobile phase was a mixture of buffer and acetonitrile (60:40 % v/v). The buffer was 8.6 g of potassium dihydrogen phosphate dissolved in 1000 ml of HPLC grade water. Drug solution was injected into column. The flow rate of the mobile phase was adjusted to 1 ml /min. The detection was carried out at wavelength 225 nm. (Fig.1) The injection volume of the standard and sample solution was set at 15.0 µl. Elution and resolution parameters of drug were recorded at the wavelength range 200 nm to 380 nm and its response optimization was compared. The choice of wavelength 225 nm was considered satisfactory, permitting the detection of the drugs with adequate sensitivity. It produced well shaped peaks for the drug assay. A UV spectrum of the drug is given in fig 1 and chromatogram of the drug assayed is depicted in fig. 2.

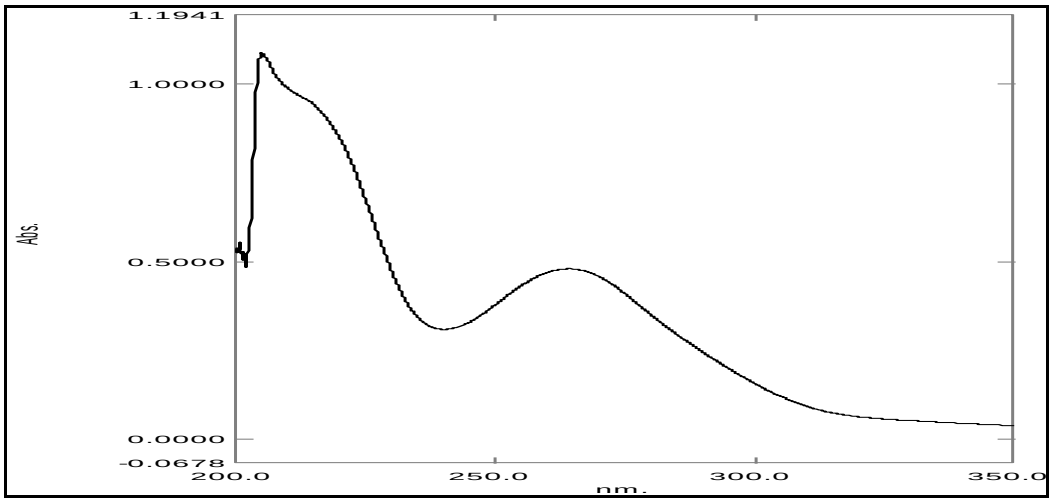



**Fig no. 1. UV spectrum of alprazolam**

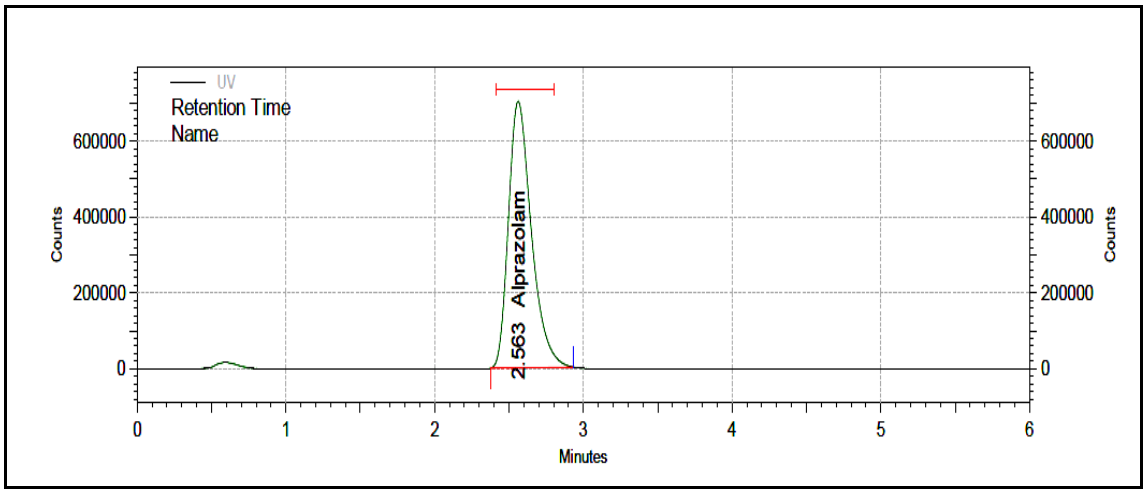



**Figure.2: chromatogram of standard alprazolam**

**Method validation**

**System suitability**

System performance parameters of developed HPLC method were determined by injecting standard solutions. Parameters such retention time, area, % area and asymmetry were shown in Table-1.It indicated good performance of the system.

**Table – 1 : System performance parameters for alprazolam. (n = 6).**

| Retention time | symmetry factor | Area | % Area |
|---|---|---|---|
| 2.58 | 1.36 | 7514463 | 100.00 |

**Linearity**

The linearity of the method was determined for alprazolam at six concentrations level ranging from 5 to 15 µg/ml. The calibration curve was constructed by plotting response factor against concentration of the drugs. The regression equation was given as y = 678592 x + 35410. The correlation coefficient ($r^2$) was 0.999 and concentration range indicated above. The results of the same are tabulated in the table 2.

**Table 2 : Linearity – regression analysis data**

| Parameters | Values |
|---|---|
| Correlation Coefficient (r) | 0.9991 |
| Intercept (y) | 35410 |
| Slope (m) | 678592 |

Y =mx + c

**Accuracy**

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and percentage recovery was calculated and presented in Table 3.

**Table 3 : Accuracy - %Recovery**

| **level** | **Sr. No. of Test sample** | **Weight in mg** | **Area** | **Quantity of std. added in µg/ ml** | **Quantity recovered in µg/ ml** | **% recovery** | **Mean recovery** |
|---|---|---|---|---|---|---|---|
| **80%** | 1 | 10.31 | 6218054 | 8.96 | 9.26 | 103.39 | **103.29** |
| | 2 | 10.36 | 6222914 | 8.96 | 9.27 | 103.47 | |
| | 3 | 10.32 | 6194535 | 8.96 | 9.23 | 103.00 | |
| **100%** | 1 | 10.25 | 7536292 | 11.2 | 11.23 | 100.25 | **100.03** |
| | 2 | 10.21 | 7509967 | 11.2 | 11.19 | 99.90 | |
| | 3 | 10.13 | 7513034 | 11.2 | 11.19 | 99.94 | |
| **150%** | 1 | 10.22 | 9103868 | 13.44 | 13.56 | 100.92 | **101.09** |
| | 2 | 10.29 | 9136127 | 13.44 | 13.61 | 101.28 | |
| | 3 | 10.36 | 9118879 | 13.44 | 13.59 | 101.09 | |

* Average of triplicate analysis.

**Precession**

The method precision was established by carrying out the analysis of alprazolam. The assay was carried out of the drug using analytical method in six replicates. The value of relative standard deviation lies well with the limits (0.10 %). The results of the same are tabulated in the table 4.

**Table 4 : Precision – method precision.**

| Test | **Weight of test** | **Area** | **% assay** |
|---|---|---|---|
| Solution-1 | 1.12 | 7514463 | 99.96 |
| Solution-2 | 1.1 | 7522667 | 98.28 |
| Solution-3 | 1.11 | 7514463 | 99.07 |
| Solution-4 | 1.09 | 7528723 | 99.24 |
| Solution-5 | 1.05 | 7518809 | 98.15 |
| Solution-6 | 1.07 | 7507923 | 98.95 |
| | **Mean Assay** | | **98.94** |
| | **SD** | | **0.664** |
| | **RSD** | | **0.671** |

**Stability of solution**

The stability studies of the solutions under study were established by keeping the solutions at room temperature for 24 hours. The results indicated no significant change in the assay results of the same solutions. It confirmed the stability of the drug in the solvents used for the analysis.

**Robustness**

Robustness study of the method was performed by making slight changes in the chromatographic conditions.

In flow rate, variation ± 0.2˚ C.
In mobile phase composition, variation ± 0.2 units
In wavelength, variation ± 2 units

The chromatograms demonstrated have no marked changes that developed HPLC method was robust.

**Method Application**

The validated high performance liquid chromatographic method was applied for determination of alprazolam its formulation. Twenty tablets of alprazolam were used. A portion equivalent to 10 mg of alprazolam was weighed accurately. It was dissolved in 10 ml of diluent to obtain final concentration 1000 µg/ml. The working sample solution was prepared by diluting 10 ml of 1000 µg/ ml solution to 100 ml with diluent to give 100 µg/ ml.

10 µl of this solution was injected under specified conditions. The analyte peaks were identified by comparison with respective standard and chromatogram was recorded. (Fig. no.3).

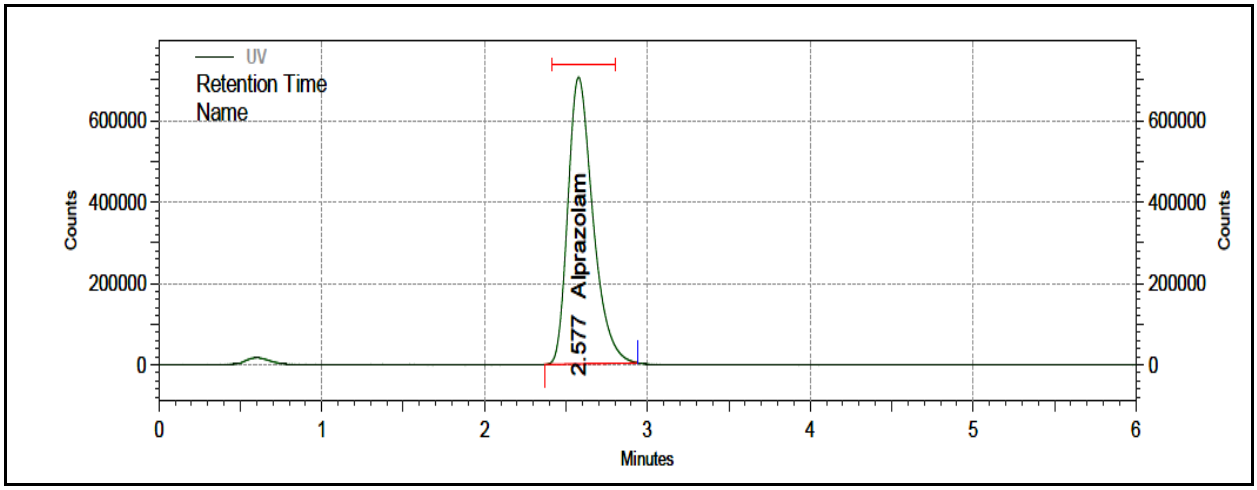



**Fig 3: chromatogram of alprazolam ( sample)**

The assay results expressed as mg / tablets are shown in Table-3. It indicated the amount of each drug in the product meet the requirement.

## Results

In the proposed method, the retention time of alprazolam was 2.57 min. The linearity was in the range of 5-15 µg/ml. The regression equation of the linearity was given as Y= 678592X+35410 where X is concentration of azelnidipine in µg /ml. and Y is corresponding peak area. The coefficient of co-relation was 0.9991. The result shows that an excellent correlation between peak area and concentration of alprazolam in the range indicated. The relative standard deviation for method precision was 0.671 ( limit %RSD < 2.0%). The mean recovery of the alprazolam was 101.47. The high percentage recovery indicates that the proposed method is highly accurate.

The use of potassium di-hydrogen phosphate in water and acetonitrile (60:40% (v/v) gave peak with good resolution. Method does not require any adjustment of pH value. The robustness studies indicated that there was no effect on the drug study. No interfering peaks were found in the chromatogram of the formulation within the run time indicted that excipents used in the formulation did not interfere the estimation of drug.

## Discussion

The reproducibility, repeatability and accuracy of the proposed method were found to be satisfactory which is evidenced by low values of standard deviation 0.664 and percent relative standard deviation 0.671.( Table no.4) The accuracy and reproducibility of the proposed method was confirmed by recovery experiments, performed by adding known amount of the drug to the pre-analyzed active pharmaceutical ingredient and reanalyzing the mixture by proposed method. (Table no.3) The percent recovery obtained indicates non-interference from the excipients used in the formulations. The methods reported in literature [1] method indicate large retention time. Hence more time will be required for validation of drug and its formulation as well it requires more amount of organic solvent.

This can be successfully used for validation of drug as well as for determining stability of drug in various conditions as per ICH guidelines.

## Conclusion

Thus the proposed RP-HPLC method is used for validation of alprazolam from active pharmaceutical ingredient and marketed formulation due to its simplicity and non interference of other peaks and relatively short retention time as 2.57 . It is more precise, accurate, linear, robust, simple and rapid method. Hence the proposed RP-HPLC method is strongly recommended for the quality control of the raw material, active pharmaceutical ingredient and pharmaceutical formulation per ICH guidelines.

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