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# Dissolution study and method validation of alprazolam by high performance liquid chromatography method in pharmaceutical dosage form

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## Abstract

*Dissolution study was carried for alprazolam tablets containing 0.25 mg of active pharmaceutical ingredient. From the solubility study, conditions selected for paddle was 100 rpm, using 500 ml potassium dihydrogen phosphate of pH 6.8 at temperature as 37°C. These conditions are useful for % release of alprazolam coated 0.25 mg. The validation of dissolution of alprazolam was achieved on symmetyshield RP8 (150 x 3.9 mm i.d, 5 µ.) column was most suitable for dissolution study with mixture of (60:40 % v/v) of buffer and acetonitrile as mobile phase. A 0.1 % orthophosphoric acid was used as a buffer and to tri-ethylamine was used to adjust the pH 3.0 of buffer. The potassium dihydrogen phosphate of pH 6.8 was used as a diluent. The detector wavelength was set at 225 nm. Flow rate was adjusted at 0.8 ml/min. The validation of method was performed as per ICH guidelines. The linearity was found between 50- 150% with coefficient of co-relation was 0.996. The % RSD of precision was 0.66%.*

**Keywords:** Alprazolam, High performance liquid chromatography, Dissolution apparatus, Phosphate buffer pH 6.8.

## Introduction

The 8-chloro-1-methyl-6-phenyl-4H-(1,2,4) triazolo (4,3a)1,4-benzodiazepine is a chemical name of alprazolam. The alprazolam is used treat short acting anxiolytic disorders. The drug also used for sedative, hypnotic, anticonvulsant, amnesic and skeletal muscle relaxant property. It is a long term use drug and abuse causes a physical dependence. Some side effects of alprazolam were occurs in patients. If signs of an allergic reaction occur - such as hives; difficulty breathing; swelling of face, lips, tongue, or throat**.** In the literature the liquid chromatography[1,2] and spectrophotometric[3-7] and HPTLC[8] methods were suggested for the assay of alprazolam. The aim of present work was to develop, simple, accurate and precise method for the determination of alprazolam. For the analysis of alprazolam, a new developed method was more useful. In the suggested method validated of was carried out as per ICH guideline.

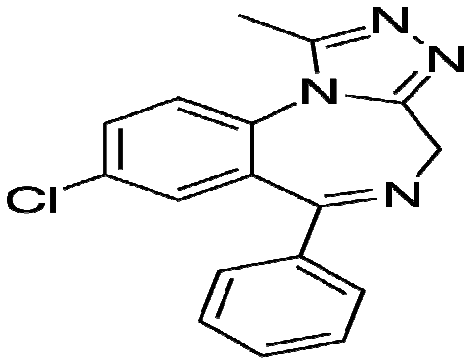


**Figure-1:** Structure of alprazolam.

## Materials and methods

**Instrument and reagents:** i. HPLC system was used equipped with separation module and UV detector [Merck-Hitachi], ii. The dissolution study Lab-India Disso 2000 dissolution tester model was used at a temperature of 37°C, iii. Analytical balance of made Shimadzu was used.

**Reagents and materials:** i. Alprazolam reference standard was obtained from reputed firm with certificate analysis. ii. Millipore water was used for preparing dissolution media. The chemical used was analytical reagent grade and HPLC grade. Alprazolam tablets containing Alprazolam (0.25 mg) where procured from reputed firm.

**Dissolution study: Method development:** Individual six tablets where weighed containing 0.25 mg alprazolam and transferred into separate dissolution apparatus bowls containing 500 ml of potassium dihydrogen phosphate buffer pH 6.8.

The determination solubility and amount of drug release was determined in 500 ml of potassium dihydrogen phosphate buffer pH 6.8. The USP paddle method (USP apparatus II) at 100rpm was used for determination of percentage drug release tests. The bowl temperature was stabilized at 37°C. After 2, 4, 8, 10 and 12 hrs, the aliquots were taken from bowl. Filtered the aliquots with 0.45µ nylon filter and injected. The formulated results are presented in Table-1.

The amount and percentage of drug release was calculated using HPLC method.

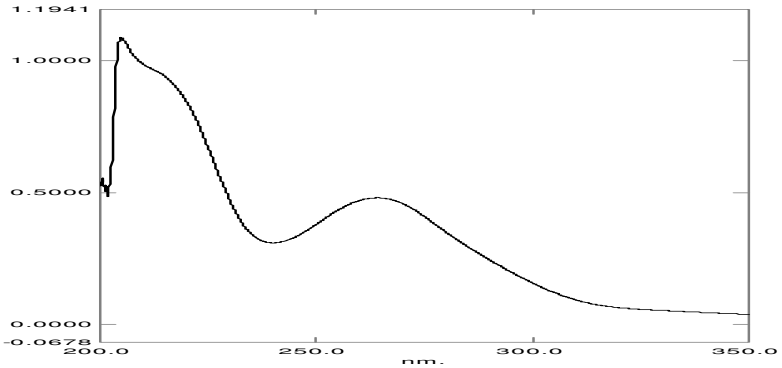


**Figure-2:** UV spectrum of Alprazolam.

**Table-1:** Results of development of alprazolam for dissolution study.

| Time interval | | Area | % drug released |
|---|---|---|---|
| After 2 hrs. | bowl-1 | 170582 | 27.12 |
| | bowl-2 | 167009 | 26.56 |
| | bowl-3 | 173966 | 27.66 |
| | bowl-4 | 164606 | 26.17 |
| | bowl-5 | 163541 | 26.00 |
| | bowl-6 | 166210 | 26.43 |
| After 4 hrs. | bowl-1 | 273824 | 43.54 |
| | bowl-2 | 232094 | 36.90 |
| | bowl-3 | 247134 | 39.30 |
| | bowl-4 | 249936 | 39.74 |
| | bowl-5 | 241172 | 38.35 |
| | bowl-6 | 267363 | 42.51 |
| After 8 hrs. | bowl-1 | 426529 | 67.82 |
| | bowl-2 | 438172 | 69.67 |
| | bowl-3 | 415383 | 66.05 |
| | bowl-4 | 427003 | 67.90 |
| | bowl-5 | 440691 | 70.07 |
| | bowl-6 | 435222 | 69.20 |
| After 10 hrs. | bowl-1 | 542914 | 86.33 |
| | bowl-2 | 537006 | 85.39 |
| | bowl-3 | 530644 | 84.38 |
| | bowl-4 | 543736 | 86.46 |
| | bowl-5 | 570904 | 90.78 |
| | bowl-6 | 564503 | 89.76 |
| After 12 hrs. | bowl-1 | 618025 | 98.27 |
| | bowl-2 | 602127 | 95.74 |
| | bowl-3 | 598196 | 95.12 |
| | bowl-4 | 585854 | 93.15 |
| | bowl-5 | 602127 | 95.74 |
| | bowl-6 | 637596 | 101.38 |

**Method validation: Linearity:** To assess the linearity, from initial concentration, 50 % - 150 % level solution was injected. The results are presented in Table-2.

**Table-2:** Results of Linearity for dissolution study.

| Factors | Values |
|---|---|
| Value of Correlation Coefficient | 0.996 |
| Intercept | 1000000 |
| Slope | 13910 |

**Accuracy:** By adding known amount of alprazolam standard (50%, 100%, 150% level) to the placebo and calculate accuracy of the percentage drug recovered. The results are presented in Table-3.

**Precision:** The repeatability of drug release was determined using six tablets analysis for 12 hrs. The percentage RSD is not more than 5 (Table-4).

**Robustness:** The variations of the dissolution method was demonstrated by - Change in RPM ±10 rpm., Change in temperature ± 2°C, Change in value of wavelength ± 0.5.

The percentage RSD values were within specified limit indicating the robustness of dissolution method.

**HPLC Method: Standard solution:** Weighed and transferred 5 mg of alprazolam standard into 100ml volumetric flask, added about 50 ml of diluent. This solution was sonicate 1 minute and adjusted the volume up to the mark with diluents to obtained concentration as 50 µg / ml. Dilute 1 ml of 50 µg/ ml solution to 10 ml volumetric flask with diluents to get concentration 5 µg/ ml. Further dilute 1 ml of above solution to 10 ml and diluted with diluent. Final concentration of solution was obtained 0.5 µg / ml.

**Sample solution:** By Accurate weighing average weight of twenty tablets was determined. From the tablet of alprozolam

powder equivalent to 0.25 mg was weighed, to this added 50 ml of diluents and sonicated for 1 minute and adjusted volume up to 100 ml with diluents to give concentration as 50 µg/ ml. Filter this solution with 0.45µ nylon filter. A 0.1 ml of 50 µg/ ml of filtered solution were further diluted to 10 ml with diluent to get concentration with diluents to get concentration 0.5 µg/ ml.

**Chromatographic conditions:** The separation of alprazolam was confirmed at room temperature on symmetyshield RP8 (150 x 3.9 mm i.d.5µ) column. A mixture of (60:40 % v/v) of buffer and acetonitrile was used as mobile phase. 0.1 % orthophosphoric acid adjusted the pH 3.0 with tri-ethylamine was used as buffer. The 225 nm was set as detector wavelength and the 30 µl was an injection volume.

**Method validation: Suitability:** System suitability parameter was confirmed by injecting standard solution. Suitability was shown in Table-5. It shows that the system is suitable. The chromatogram is given in Figure-3.

**Table-3:** Results of accuracy of alprazolam for dissolution study.

| Stage | No. of replicate | Weight in mg | Pek-area | Quantity recovered (%) | % recovery | Mean recovery |
|---|---|---|---|---|---|---|
| 50% | 1 | 0.125 | 335817 | 50.74 | 101.48 | 100.30 |
| | 2 | 0.125 | 331946 | 50.16 | 100.31 | |
| | 3 | 0.125 | 327919 | 49.55 | 99.09 | |
| 100% | 1 | 0.251 | 661343 | 99.93 | 99.93 | 99.27 |
| | 2 | 0.252 | 654064 | 98.83 | 98.83 | |
| | 3 | 0.251 | 655689 | 99.07 | 99.07 | |
| 150% | 1 | 0.374 | 989320 | 149.48 | 99.65 | 99.05 |
| | 2 | 0.375 | 975586 | 147.41 | 98.27 | |
| | 3 | 0.375 | 985059 | 148.84 | 99.23 | |
| | | | | Mean recover of all level | | 99.54 |

**Table-4:** Method Precision: Repeatability.

| Test | Amount of alprazolam in tablet (in mg) | Area | % drug released |
|---|---|---|---|
| Solution-1 | 0.25 | 665272 | 100.32 |
| Solution-2 | 0.25 | 632161 | 95.33 |
| Solution-3 | 0.25 | 644474 | 97.18 |
| Solution-4 | 0.25 | 654498 | 98.69 |
| Solution-5 | 0.25 | 655046 | 98.78 |
| Solution-6 | 0.25 | 639740 | 96.47 |
| | Mean Assay | | 97.79 |
| | Standard Deviation | | 1.810 |
| | Relative Standard Deviation | | 1.85 |

**Table-5:** System suitability for alprazolam.

| | Retention time | Tailing factor | No. of plates |
|---|---|---|---|
| Alprazolam | 4.440 | 1.20 | 4214 |

**Linearity:** The five concentration levels from 50 to 150µg/ml were prepared for determination of linearity of alprazolam. The graph was plotted response factor against concentration of the drugs. The regression equation was given as y = 2000000 x + 24010. The correlation coefficient ($r^2$) was 0.999 and concentration range indicated above. The results are presented in the Table-6.

**Table-6:** Linearity.

| | **Resulted values** |
|---|---|
| Co-relation Coefficient (r) | 0.9990 |
| Intercept (y) | 2000000 |
| Slope (m) | 24010 |

**Accuracy:** The recovery for the alprazolam was done by injecting known amount of alprazolam standard and placebo in triplicate samples. The data is presented in Table-7.

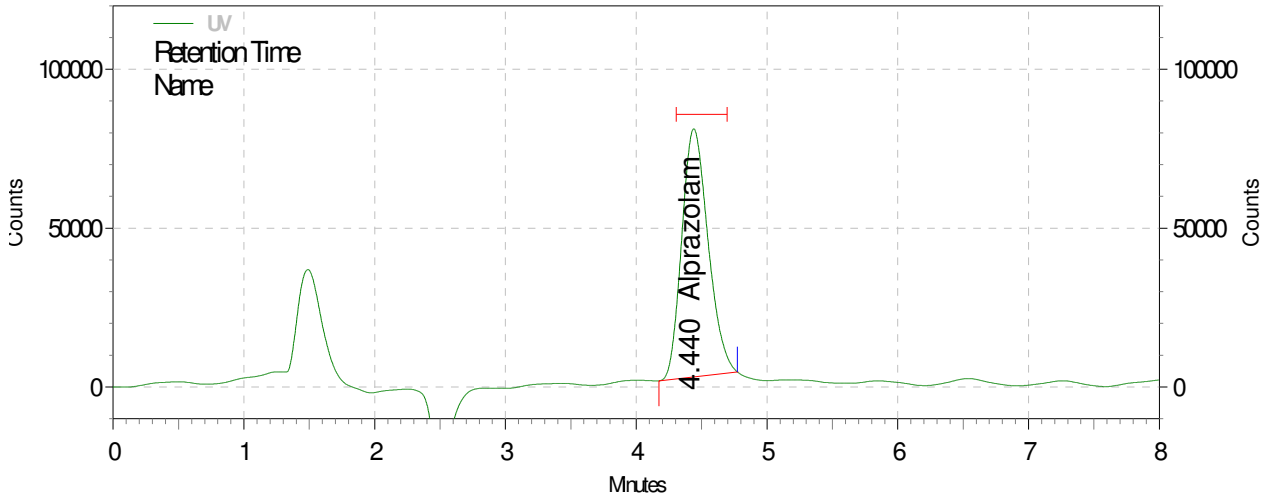


**Figure-3:** Chromatogram of alprazolam (standard).

**Table-7:** Accuracy - % Recovery.

| Stage | Test | Weight (mg) | Area of peak | Added Alprazolam µg /ml | Alprazolam obtained in µg /ml | % recovery | Total recovery stage |
|---|---|---|---|---|---|---|---|
| 50% | 1 | 5.38 | 529622 | 0.266 | 0.262 | 98.37 | 98.87 |
| | 2 | 5.41 | 532795 | 0.266 | 0.265 | 99.51 | |
| | 3 | 5.32 | 537583 | 0.266 | 0.263 | 98.74 | |
| 100% | 1 | 5.49 | 1062271 | 0.532 | 0.536 | 100.67 | 99.78 |
| | 2 | 5.42 | 1050472 | 0.532 | 0.523 | 98.28 | |
| | 3 | 5.28 | 1101440 | 0.532 | 0.534 | 100.39 | |
| 150% | 1 | 5.39 | 1608728 | 0.798 | 0.796 | 99.79 | 99.87 |
| | 2 | 5.53 | 1575177 | 0.798 | 0.800 | 100.24 | |
| | 3 | 5.35 | 1617566 | 0.798 | 0.795 | 99.59 | |
| | | | | | Mean recovery of all level | | 99.51 |

** Average of triplicate analysis.*

**Precision:** The precision parameters were confirmed by analyzing six replicate analysis of alprazolam sample. The value of percentage RSD is shows that the method is precise and shown in Table-8.

**Table-8:** Method precision.

| Test no. | Alprazolam in mg | Area of Alprazolam | % assay |
|---|---|---|---|
| 1 | 5.23 | 1069978 | 99.95 |
| 2 | 5.39 | 1097554 | 99.48 |
| 3 | 5.31 | 1085665 | 99.89 |
| 4 | 5.22 | 1064138 | 99.59 |
| 5 | 5.35 | 1088729 | 99.42 |
| 6 | 5.34 | 1084043 | 99.18 |
| | Mean Assay | | 99.59 |
| | SD | | 0.293 |
| | RSD | | 0.29 |

**Stability of solution:** The solution stability study of alprazolam was determined after 24 hr at room temperature. It confirmed the drug showing the stability of solution.

**Robustness:** Variation in parameters of the HPLC method was demonstrated by - Change in flow ± 0.2 ml/min., Change in mobile phase ± 0.2 units, Change in wavelength ± 5 nm.

**Method application:** The method validation of HPLC method was applied for quantification of alprazolam its dosages. By Accurate weighing average weight of twenty tablets was determined. From the tablet of alprozolam powder equivalent to 0.25 mg was weighed, to this added 50 ml of diluents and sonicated for 1 minute and adjusted volume up to 100 ml with diluents to give concentration as 50 µg/ ml. Filter this solution with 0.45µ nylon filter. A 0.1 ml of 50 µg/ ml of filtered solution were further diluted to 10 ml with diluent to get concentration with diluents to get concentration 0.5 µg/ ml. A 30µl of this solution was injected and confirmed the analyte peaks with respective standard (Figure-4). The assay results expressed as mg / tablets are shown in Table-8. It shows that the developed method is applicable for alprazolam. The chromatogram of sample is given in Figure-4.

## Results and discussion

**Dissolution study:** The solubility of alprazolam, dose criteria was checked and the dissolution medium was selected. Potassium dihydrogen phosphate buffer was selected for the dissolution medium and study was done. The drug was feely soluble in phosphate buffer medium as it is insoluble in acidic medium. At 100 rpm, % drug release was better potassium phosphate pH 6.8 buffer. About 96% of drug was released at 12 hrs in potassium dihydrogen phosphate buffer pH 6.8 at 100 rpm. Hence phosphate buffer pH 6.8 was used as dissolution medium for further study, Table-1.

After 12 hrs at 100 rpm alprazolam drug was released completely. The cumulative percentages of drug released were shown in Tables-4.

Linearity was determined by injecting standard solution in the range of 50–150 µg/ml and the calibration curve was plotted by area against concentration levels. The representative linear equation was y = 1000000x - 13910 and correlation coefficient 0.996 for alprazolam. From this linearity data the method is linear.

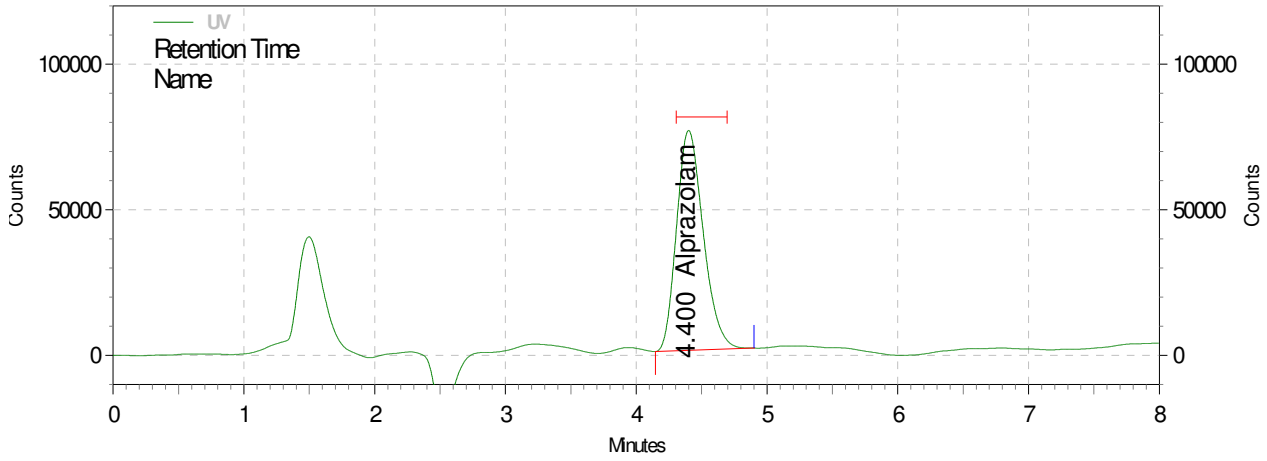


**Figure-4:** Chromatogram of alprazolam (sample).

The method was found to be accurate with % recovery of 99.05%–100.30% using standard addition method. For the precision of dissolution method, the % RSD for repeatability study was observed 1.85. The % RSD values indicating method if precise.

**HPLC method:** The revered phase HPLC method developed and validated as per ICH guidelines for the determination of alprazolam. The results which are shows limit value of standard deviation and percent relative standard deviation. The accuracy study reveals that mean recovery after spiking experiment were between 99.78 to 99.87, an indicative of accurate method.

Accordingly it can be conclude that the developed reverse phase HPLC method is meets validation criteria and it is strongly useful for study of alprazolam in regular analysis. Hence the proposed RP-HPLC method is strongly recommended for the quality control of the raw material, formulations and dissolution studies.

## Conclusion

The dissolution conditions were 500 ml of potassium dihydrogen phosphate buffer pH 6.8 at 37°C, paddle apparatus at stirring speed 100 rpm for 12 hrs. Thus proposed dissolution method and HPLC method can be applied successfully for quality control analysis of alprazolam in tablet formulation.

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