EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 9
Issue: 6
360-363
Year: 2022

DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR ESTIMATION OF ALPRAZOLAM IN TABLET DOSAGE FORM

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Article Received on 13/04/2022 **Article Revised on 03/05/2022** **Article Accepted on 24/05/2022**

ABSTRACT

Objective: To develop and validate simple, rapid, linear, accurate, precise and economical UV Spectroscopic method for estimation of Alprazolam in tablet dosage form. **Methods:** The drug is freely soluble in analytical grade methanol. The drug was identified in terms of solubility studies and on the basis of melting point done on melting point apparatus of Equiptronics. It showed absorption maxima were determined in analytical grade methanol. The drug obeyed the Beer's law and showed good correlation of concentration with absorption which reflect in linearity. The UV spectroscopic method was developed for estimation of Alprazolam in tablet dosage form and also validated as per ICH guidelines. **Results:** The drug is freely soluble in analytical grade methanol, slightly soluble in acetone and insoluble in water. So, the analytical grade methanol is used as a diluent in method. The melting point of Alprazolam was found to be 228-229˚C (uncorrected). It showed absorption maxima 270 nm in analytical grade methanol. On the basis of absorption spectrum the working concentration was set on 10µg/ml (PPM). The linearity was observed between 6-14 µg/ml (PPM). The results of analysis were validated by recovery studies. The recovery was found to be 98.75, 101 and 98.33% for three levels respectively. The % RSD for precision was found to be 0.49% and for Ruggedness is 0.54%. **Conclusion:** A simple, rapid, linear, accurate, precise and economical UV Spectroscopic method has been developed for estimation of Alprazolam in tablet dosage form. The method could be considered for the determination of Alprazolam in quality control laboratories.

KEYWORDS: Alprazolam, UV Spectrophotometer, Melting Point, Assay Method, Validation, Accuracy, Linearity, Ruggedness, Precision.

INTRODUCTION

Alprazolam (8-chloro-1-methyl-6-phenyl-4H-[1,2,4] triazole [4,3,-α]-[1,4] benzodiazepine) is a benzodiazepine derived from 1.4-benzodiazepines of new generation.[1,2] It is a benzodiazepine mainly used to treat anxiety disorders. On a short time basis it is used to palliate symptoms of anxiety or anxiety associated to symptoms of depression. The exact mechanism of action of Alprazolam is unknown. Benzodiazepines bind nonspecifically to benzodiazepine receptors BNZ1, which mediates sleep, and BNZ2, which affects muscle relaxation, anticonvulsant activity, motor coordination, and memory.[3,4] As benzodiazepine receptors are thought to be coupled to gamma-amino butyric acid-A (GABAA) receptors, this enhances the effects of GABA by increasing GABA affinity for the GABA receptor. Binding of the inhibitory neurotransmitter GABA to the site opens the chloride channel, resulting in a hyperpolarized cell membrane that prevents further excitation of the cell.[5,6,7]

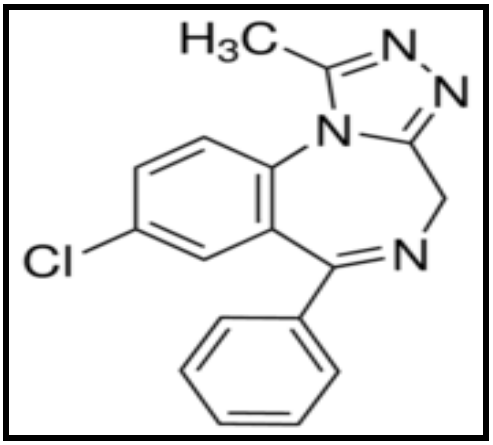


Fig. 1: Chemical Structure of Alprazolam.

Most of the analytical methods carried out by high performance liquid chromatography (HPLC) to determine alprazolam[8] found in the consulted literature,

are aimed at quantifying alprazolam in biological fluids, for postmortem analysis[9-13], to determine the raw material[12] and its related substances.[14,15] Few methods reported on UV by using ferric chloride and indigo carmine and UV method of alprazolam area under curve and first order derivative[13,14,15,16] but no method reported on alprazolam for routine analysis on UV. So, the work planned accordingly.

MATERIALS AND METHODS

- **Instruments**

Shimadzu double beam UV-visible spectrophotometer 1700 Ultra with matched pair Quartz cells corresponding to 1 cm path length and spectral bandwidth of 1 nm, Bath sonicator and citizen weighing balance.
Melting point apparatus of Equiptronics were used.

- **Materials**

Alprazolam was obtained as a gift sample. Alprazolam tablets were procured from local pharmacy. Methanol used was of analytical grade was used throughout the experiment. Freshly prepared solutions were employed.

Method development

A. Determination of ƛ max (10 PPM)[17, 18]

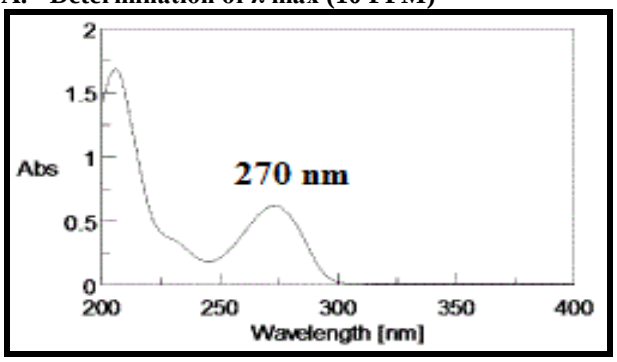


Fig. 2: Calibration Curve.

50 mg weighed amount of Alprazolam was dissolved into 100 ml of volumetric flask with analytical grade methanol. Pipette out 1 ml and added in 50 ml of volumetric flask dissolved and diluted up to the mark with analytical grade methanol. This solution was subjected to scanning between 200-400 nm and absorption maximum was determined.

B. Preparation of Working concentration

Preparation of Standard stock solution

Standard stock was prepared by dissolving 25 mg of Alprazolam in 25 ml of analytical grade methanol to get concentration of 1000 µg/ml (PPM).

Preparation of Standard solution

Pipette out 1 ml from standard stock solution and diluted up to 100 ml with analytical grade methanol to get concentration of 10 µg/ml (PPM).

C. Procedure for UV reading

Blank Solution: (For Auto zero)
Fill the cuvette with analytical grade methanol. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Standard Solution

Fill the cuvette with standard solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Sample Solution

Fill the cuvette with sample solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

D. Procedure for sample preparations[19,20,21]

For analysis of commercial formulations; twenty tablets are taken weighed it and powdered. The powder equivalent to 25 mg of Alprazolam was accurately weighed and transferred into the 25 ml of volumetric flask, added 15 ml analytical grade methanol, the solution was sonicated for 20 min. After sonication cool the flask and diluted upto 25 ml with analytical grade methanol. Filtered the solution through nylon syringe filter 0.45 µ. Pipette out 1 ml of the filtered solution and diluted up to 100 ml with analytical grade methanol. The absorbance was measured at 270 nm. The absorbance was recorded:

Table 1: Absorbance of Dosage Form.

Sunrise Remedies Pvt. Ltd. (100 mg)		
Sr. no.	**Sample**	**Absorbance**
1	Blank	0.0000
2	Standard	0.5515
3	Sample	0.5440

Table 2: Dosage Form Specifications.

Type	Brand / Company	M.D.	E.D.	Batch No.	Avg wt (g)	Assay (%)
1	ALPRAMAX - 5 Betamax Remedies Pvt LTD (5mg)	10/2021	10/2023	AQ5145	0.0504	98.64

E. Method of validation[18,20,21]

The proposed method was developed by using linearity, accuracy, precision and ruggedness as per ICH guidelines, 1996.

Linearity

The linearity of the proposed assay was studied in the concentration range 6 - 14 PPM at 270 nm. The calibration data showed a linear relationship between concentrations.

Table 3: Linearity Studies.

Sr. no.	Sample Concentration	Absorbance
1	6 PPM	0.3385
2	8 PPM	0.4353
3	10 PPM	0.5496
4	12 PPM	0.6473
5	14 PPM	0.7398
Correlation coefficient	0.9988 ~ 0.999	

Accuracy

To ensure the accuracy of the method, recovery study was performed by preparing 3 sample solutions of 80, 100 and 120% of working concentration and adding a known amount of active drug to each sample solution and dissolved in 100ml of volumetric flask with analytical grade methanol and measuring the absorbance at 270nm.

Table 4: Accuracy Studies.

SPECTROPHOTOMETRIC METHOD			
Accuracy (%)	**Qty weighed (mg)**	**Qty found (mg)**	**Recovery (98-102%)**
80	0.8	0.78	98.75
100	1	1.01	101.00
120	1.2	1.18	98.33

Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies. Five sample solutions were made and the %RSD was calculated.

Table 5: Precision studies.

Sr. No.	Sample Solution	Absorbance
1	Sample Solution 1	0.5484
2	Sample Solution 2	0.5465
3	Sample Solution 3	0.5424
4	Sample Solution 4	0.5441
5	Sample Solution 5	0.5484
MEAN		0.5460
SD		0.0027
% RSD		0.4875

Ruggedness

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

Table 6: Results for Ruggedness Studies.

Sr. No.	Analyst	Results	Mean	% Assay	% RSD
1	Analyst 1	0.5419	0.5423	98.33	0.5444
		0.5426			
2	Analyst 2	0.5445	0.5465	99.09	
		0.5484			

RESULTS

1. Solubility of Alprazolam

Solubility test was passed as per criteria.

Table 7: Results for solubility studies.

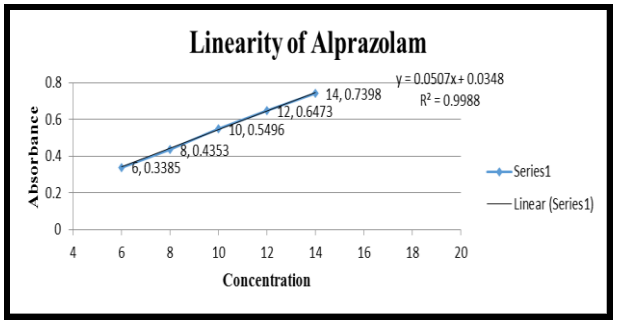
Sr. no.	Title	Result
1	Analytical grade Methanol	Freely Soluble
2	Acetone	Slightly soluble
3	Water	Insoluble

2. Melting point of Alprazolam

The melting point of Alprazolam was found to be 228-229˚C (uncorrected).

3. Results for linearity for assay method of Alprazolam

The linearity of method was determined at concentration level ranging from 6 to 14 µg/ml (PPM). The correlation coefficient value was found to be **(R^2) 0.9988 ~ 0.999**



`Fig. 3: Alprazolam Linearity Curve.

4. Results for accuracy for assay method of Alprazolam

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery were calculated and represented in Table - 4. The high percentage of recovery indicates that the proposed method is highly accurate. Accuracy results were found within acceptance criteria that are within 98-102%.

5. Results for precision for assay method of Alprazolam

The % RSD for different sample of precision was found to be 0.4875 ~ 0.49 and it is within acceptance criteria represented in Table - 5.

6. Results for ruggedness for assay method of Alprazolam

The %RSD for different sample of ruggedness was found to be 0.5444 ~ 0.54 and it is within acceptance criteria represented in Table - 6.

CONCLUSION

A method for the estimation of Alprazolam in tablet form has been developed. From the spectrum of Alprazolam, it was found that the maximum absorbance was 270 nm in analytical grade methanol. A good linear relationship

was observed in the concentration range of 6-14 µg/ml (PPM). The high percentage recovery indicates high accuracy of the method. This demonstrates that the developed spectroscopic method is simple, linear, accurate, rugged and precise for the estimation of Alprazolam in solid dosage forms. Hence, the method could be considered for the determination of Alprazolam in quality control laboratories.

ABBREVARTIONS

1. PPM - Parts per Million
2. nm - Nanometer
3. HPLC - High Performance Liquid Chromatography
4. UV - Ultra violet
5. LC - Liquid Chromatography
6. ICH - International Council for Harmonization
7. RSD - Relative Standard Deviation
8. SD - Standard Deviation
9. Qty - Quantity
10. °C - Degree Celsius
11. M.D. - Manufacturing Date
12. E.D. - Expiry Date
13. µg/ml - Microgram per milliliter
14. Avg - Average
15. Wt - Weight
16. g - gm
17. BNZ - Benzodiazepine
18. GABA - Gamma-Amino Butyric Acid

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