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UV Spectrophotometric Estimation of Alprazolam by Area Under Curve And First Order Derivative Methods in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

Simple and economical UV spectrophotometric methods by area under curve [AUC] and first order derivative have been developed for the estimation of alprazolam in bulk and its tablet formulation. The standard and sample solutions of alprazolam were prepared in 0.1N hydrochloric acid. Alprazolam was estimated between 255-270 nm for area under curve (AUC) method the zero order spectrum and 251 nm for the first order derivative method. Beer's law was obeyed in the concentration range of 1 to 12 µg / ml with coefficient of correlation value 0.9991 for area under curve method while 1 to 14 µg / ml for first order derivative method with coefficient of correlation value as 0.9997 respectively. A validation of above methods was carried out as per ICH guidelines. The precision expressed as relative standard deviation were of 0.0233 % and 0.724381 % for the above two methods respectively. The proposed methods were easily applied for the estimation of alprazolam in pharmaceutical dosages. Results of the analysis were were found to be satisfactory statistically. The proposed methods are simple, low-cost and require relatively inexpensive instruments.

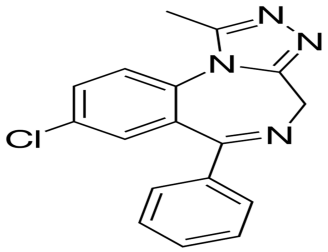
Keywords: Alprazolam, UV spectroscopy, Derivative spectroscopy, Area under curve method.

INTRODUCTION

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 [2-4] methods for the estimation of alprazolam. 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



MATERIALS AND METHODS

Shimadzu UV-1800 was used with 10 mm matched quartz cell to measure absorbance of solution.

A Shimadzu analytical balance with 0.01 mg was used.

CHEMICAL AND REAGENTS

Reference standard of alprazolam was obtained from reputed firm with certificate analysis. All spectral absorbance measurements were made on Shimadzu UV-1800 with 10 mm matched cell.

PREPARATION OF STANDARD SOLUTION

About 10 mg of standard alprazolam was weighed accurately and transferred in 100 ml of volumetric flask. About 30 ml of 0.1N hydrochloric acid was added and sonicated for 15 minutes. The volume was adjusted up to the mark with 0.1N hydrochloric acid to give concentration as 100 µg /ml.

Estimation from tablets

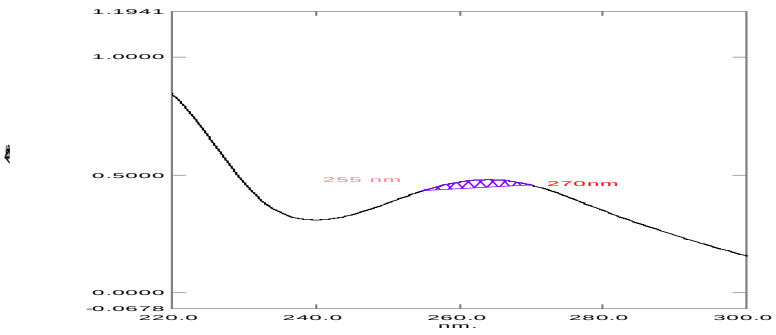
Twenty tablets were weighed accurately and average weight of each tablet was determined. Powder equivalent to 10 mg of alprazolam was weighed and transferred in 100 ml of volumetric flask. A 30 ml of 0.1N hydrochloric acid was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with 0.1N hydrochloric acid to give concentration as 100 µg /ml. Such solution was used for analysis.

Experimental

Method : Area under curve (AUC) method

Area under curve method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths such as λ_1 and λ_2. The area under curve between λ_1 and λ_2 were calculated by UV probe 2.42 software. In this method, 10 µg/ml solution of alprazolam was scanned in the spectrum mode from 300 nm to 200 nm. From zero order spectrum the AUC calculation was done. The AUC spectrum was measured between 255 nm to 270nm (Fig. 1).

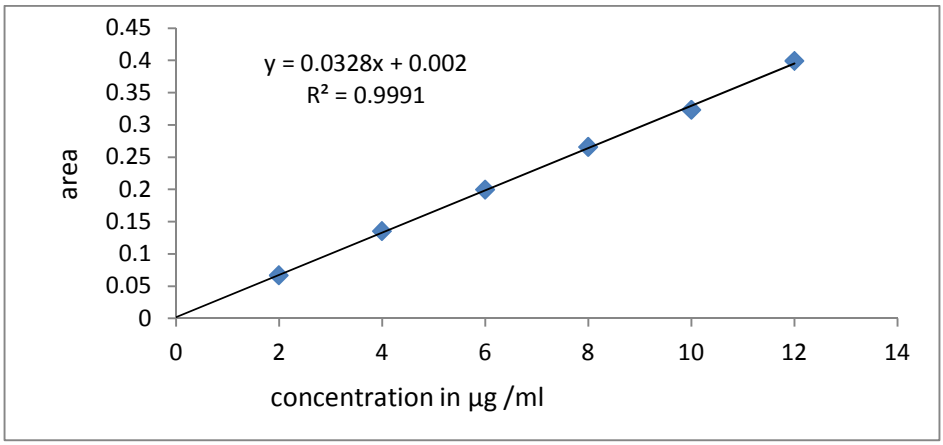
Fig. 1. Area under curve spectrum of alprazolam (10 µg/ml) showing area from 255 nm to 270 nm



Into series of 10 ml graduated flask, varying amount of standard solutions of alprazolam was pipetted out and volume was adjusted with 0.1N hydrochloric acid. Solutions were scanned between 300 nm to 200 nm in spectrum

mode. The AUC calculations were done and the calibration curve for alprazolam was plotted in the concentration range of 2 to 12 µg/ml (Fig. 2).

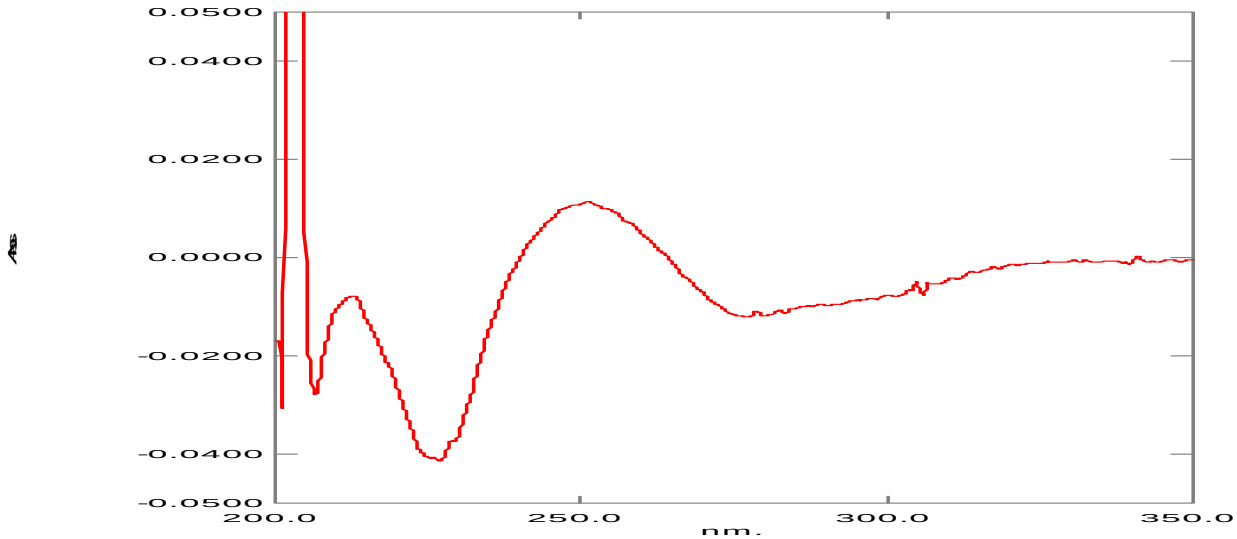
Fig. 2. Calibration curve for alprazolam by area under curve spectroscopy



Method : First order derivative method

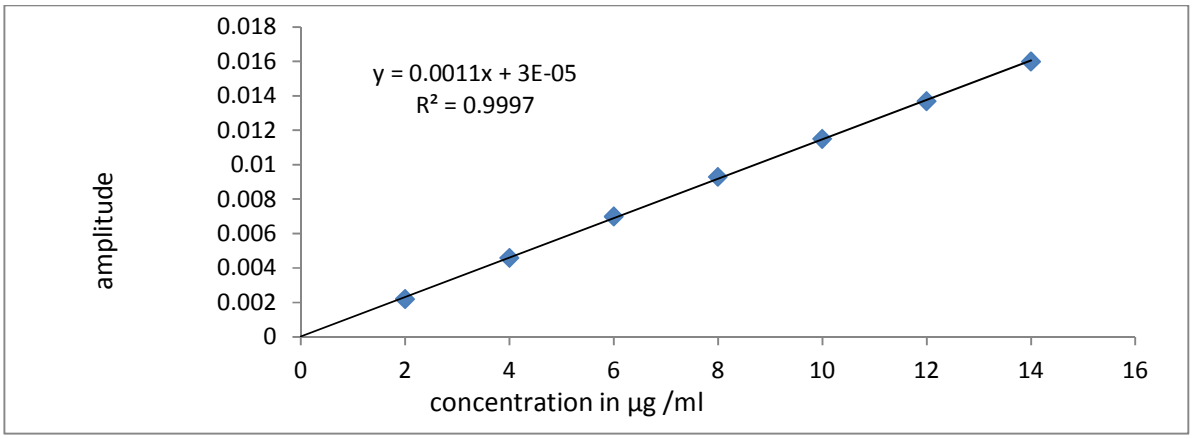
For the selection of analytical wavelength, 10 µg /ml solution of alprazolam was scanned in the spectrum mode from 300 nm to 200 nm by using 0.1N hydrochloric acid as blank. The first order derivative spectrum was obtained by using derivative mode by UV probe 2.42 software. From the spectrum, the amplitude of the derivative spectrum was measured 251 nm (Fig. 3).

Fig. 3. First order derivative spectrum of alprazolam(10 µg/ml) showing absorbance at 251 nm



Into series of 10 ml graduated flask, varying amount of standard solutions of alprazolam was pipette out and volume was adjusted with 0.1N hydrochloric acid as solvent. Solutions were scanned between 300 nm to 200 nm in spectrum mode. The first order derivative spectra were obtained by using derivative mode. Amplitudes of the resulting solutions were measured at 251 nm by using 0.1N hydrochloric acid as blank. The calibration curve was prepared in the concentration range of 1 to 14 µg/ml. (Fig. 4)

Fig. 4. Calibration curve for alprazolam at 251 nm by first order derivative Spectroscop



Results of analysis are given in table 1.

Table 1: Values of results of optical and regression of drug

Parameter	First order derivative method	Area under curve (AUC) method
Wavelength (nm)	251	255-270
Beer Law Limits (µg/ml)	1-14	1-12
Correlation coefficient(r^2)	0.9997	0.9991
Regression equation (y=b+ac)		
Slope (a)	0.0328	0.0011
Intercept (b)	0.002	0.00003

Validation

Accuracy

Accuracy of the proposed methods was carried as on the basis of recovery studies. It is performed by the standard addition method. Recovery studies were performed by adding standard drug at different levels to the pre-analyzed tablets powder solution and the proposed method was followed. From the amount of the drug estimated, the percentage recovery was calculated. The results of the analysis are shown in table (2, 3).

Table 2: Results of recovery of alprazolam for first order derivative method

Amount of Sample Added in (µg/ml)	Amount of Standard Added in (µg/ml)	Total amount recovered	Percentage recovery(%)	Standard deviation	Percentage of relative standard deviation (C.O.V.)
2	0	2.0124	104.34	0.0600	2.981
4	2	4.0248	102.173	0.06573	1.633
6	4	98.55	0.06841	1.147	1.147
8	6	8.0248	101.087	0.06573	0.8191
				Mean =0.0649	Mean =1.6453

Table 3: Results of recovery of alprazolam for area under curve (AUC) method

Amount of Sample Added in (µg/ml)	Amount of Standard Added in (µg/ml)	Total amount recovered	Percentage recovery (%)	Standard deviation	Percentage of relative standard deviation (C.O.V.)
2	0	1.998709	99.93546	0.003833	0.191753
2	2	4.071429	101.7857	0.003776	0.092739
2	4	6.002582	100.043	0.00474	0.078962
2	6	7.993976	99.9247	0.005217	0.092739
				Mean = 0.004391	Mean = 0.107179

Precision

The method precision was carried out by the analysis of homogenous powder blend of dosages i.e. tablets. The assay was performed out of drug in six replicates. The values of relative standard deviation lie well within the limits. It is indicated the sample repeatability of the method. The results are tabulated in table 4.

Table 4: Precision- method precision

Experiment no.	Weight of alprazolam taken in mg	Content in mg.	
		First order derivative method	Area under curve method
1	10	10.08696	9.728916
2	10	9.913043	9.731928
3	10	10.08696	9.73494
4	10	10.000	9.728916
5	10	10.08696	9.731928
6	10	10.08696	9.731928
	Standard deviation	0.072753	0.002267
	%RSD	0.724381	0.0233

Precision in Inter-day and intra-day

A powder blend of tablets of equivalent to 10 mg of alprazolam was accurately weighed and transferred to 100 ml of volumetric flask. A 30 ml of 0.1N hydrochloric acid was added and sonicated for 10 minutes and filtered. The filtrate and washing were collected and diluted up to the mark with 0.1N hydrochloric acid. It gave solution of concentration 100 µg /ml. Such solution was used for analysis.

For first order derivative method

A standard Solution of concentration 10 µg/ml was scanned between 300 nm to 200 nm in spectrum mode. The first order derivative spectrum was obtained by using derivative mode. Amplitude of the resulting solution was measured at 251 nm by using 0.1N hydrochloric acid as blank. The amplitude of final solution was read at time interval of 0 hr., 3 hrs. and 6 hrs. Similarly the amplitude of the same solution was recorded on 1st, 2nd and 5th day. The amount of alprazolam was calculated by comparison with standard at 251 nm for first order derivative, table 5.

For area under curve method

A standard Solution of concentration 10 µg/ml was scanned between 300 nm to 200 nm in spectrum mode. The area under curve of resulting solutions was measured at between 255 nm to 270 nm by using 0.1N hydrochloric acid as blank. The area under curve of final solutions was read after 0 hr., 3 hrs. and 6 hrs. in 10 mm cell. Similarly area under curve of the same solution was read on 1st, 2nd and 5th day. The amount of alprazolam was estimated by comparison with standard at 255 nm to 270nm, table 5.

Table 5: Summary of validation parameter for intra-day and inter-day

Sr. no.	Parameters	First order derivative method	Area under curve (AUC) method
(A)	Intra-day precision (n=3) Amount found ±% RSD	100.04% 0.7244	99.93% 0.0235
(B)	Inter-day precision (n=3) Amount found ±% RSD	97.383% 0.5624	98.564% 0.0358
(c)	Ruggedness Analyst to analyst(n= 3) %RSD	0.5963	0.0487

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations-

$LOD = 3.3\ \sigma/S$ and $LOQ = 10\ \sigma/S$

Where σ is the standard deviation of the signal to noise ratio of the sample and S is the slope of the related calibrations graphs.

The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision and variability .The values of LOD and LOQ are given in table 6.

Table 6: Values of results of LOD and LOQ

parameters	First order derivative method	Area under curve (AUC) method
Limit of Detection (µg/ml)	0.2070	0.0128
Limit of Quantification (µg/ml)	0.6273	0.0387

Ruggedness

The ruggedness of the method is defined as degree of reproducibility of results obtained by analysis of alprazolam sample under variety of normal test conditions such as different laboratories, different analysts and different lots of reagents. Quantitative determination of alprazolam was conducted spectrophotometrically on one laboratory. It was again tested in another laboratory using different instrument by different analyst. The assays obtained in two different laboratories were well in agreement. It proved ruggedness of the proposed methods.

RESULTS AND DISCUSSION

The proposed area under curve and first order derivative methods are useful for routine analysis of alprazolam in bulk drug and dosage form. The derivative spectroscopy method applied has the advantage that it locates hidden peak in the normal spectrum. It eliminates the interference caused by the excipients and the degradation products present, if any, in the formulation. The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures. Alprazolam has the absorbance maxima at 251 nm in first order derivative and in the AUC spectrum method areas were measured between 255 nm to 270nm. The polynomial regression data for the calibration plots showed good linear relationship in the concentration range of 1 to 12 µg/ml and given in table1. Recovery studies were carried out by adding the pure drug to the previously analyzed tablet powder sample and shown in table 2, 3. The percentage recovery value indicates noninterference from excipients used in formulation. The reproducibility and accuracy of the method were found to be good, which was evidenced by low standard deviation.

CONCLUSION

The most striking features of two methods are its simplicity and rapidity, not requiring tedious sample solutions preparations which are needed for other instrumental methods. From the results obtained it can be concluded that the proposed methods are fully validated and found to be simple, sensitive, accurate, precise, reproducible, rugged and robust and relatively inexpensive. So, the developed methods can be easily applied for the routine quality control analysis of alprazolam in pharmaceutical formulation.

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