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Research Article

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UV Spectrophotometric Estimation of Alprazolam by second and third order derivative Methods in Bulk and Pharmaceutical Dosage Form

Rajan V. Rele* and Amey V. Deshpande

Central Research Laboratory, D. G. Ruparel College, Matunga, Mumbai 400016.

ABSTRACT

Simple and economical UV spectrophotometric methods by second order and third 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 at 234 nm for the second order derivative and at 220 nm for third order derivative methods. Beer's law was obeyed in the concentration range of 1 to 12 µg / ml with coefficient of correlation value 0.9994 for second order and 0.9996 for third order derivative method respectively. A validation of above methods was carried out as per ICH guidelines. The precision expressed as relative standard deviation were of 1.063978 % and 0.889569 % for the above two methods respectively. The proposed methods were easily applied for the estimation of alprazolam in pharmaceutical dosages forms. Results of the analysis were found to be satisfactory statistically. The proposed methods are simple, low-cost and require relatively inexpensive instruments.

Keywords: Alprazolam, UV spectroscopy, Derivative spectroscopy, 0.1 N hydrochloric acid

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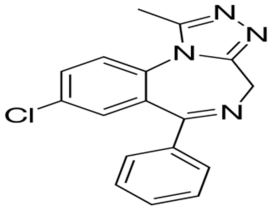
INTRODUCTION

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



EXPERIMENTAL SECTION

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

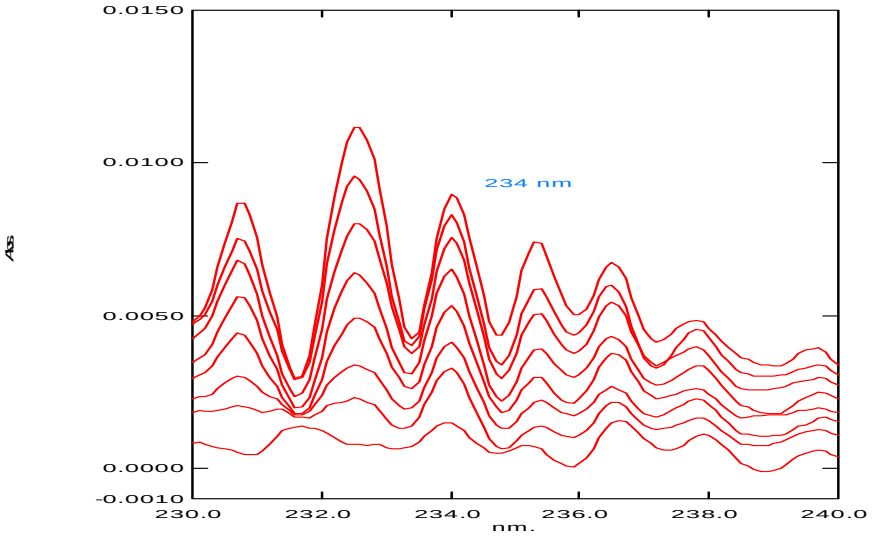
Twenty tablets were weighed accurately and average weight of each tablet was determined. Powder equivalent to 10 mg of alprazolam was weighed and transferred in 100 ml of volumetric flask. A 30 ml of 0.1N hydrochloric acid was added and sonicated for 15 minutes and filtered. The filtrate and washing 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 : Second order derivative method

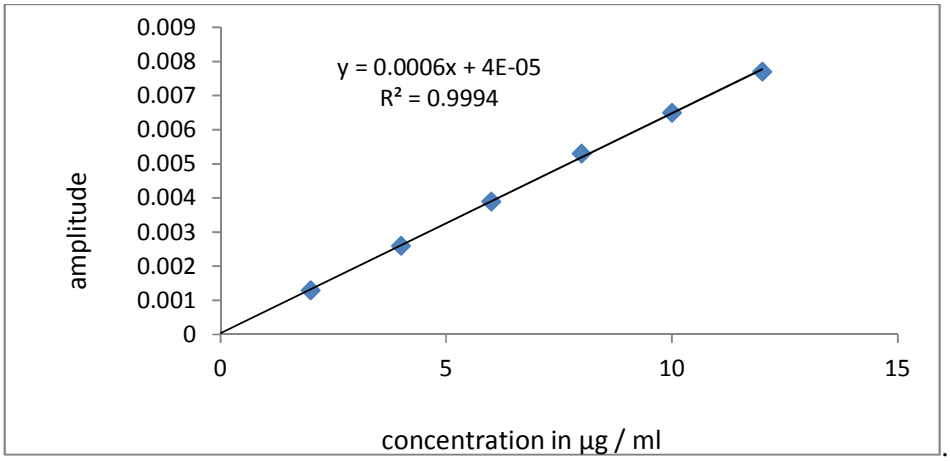
For the selection of analytical wavelength, 10 µg /ml solution of alprazolam was scanned in the spectrum mode from 300 nm to 200 nm by using 0.1N hydrochloric acid as blank. The second order derivative spectrum was obtained by using derivative mode by UV probe 2.42 software. From the spectrum, the amplitude of the derivative spectrum was measured 234 nm (Fig. 3).

Fig. 3. Overlain spectra of Second order derivative spectrum of alprazolam (2-14 µg/ml) showing absorbance at 234 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 second order derivative spectra were obtained by using derivative mode. Amplitudes of the resulting solutions were measured at 234 nm by using 0.1N hydrochloric acid as blank. The calibration curve was prepared in the concentration range of 1 to 12 µg/ml. (Fig. 4)

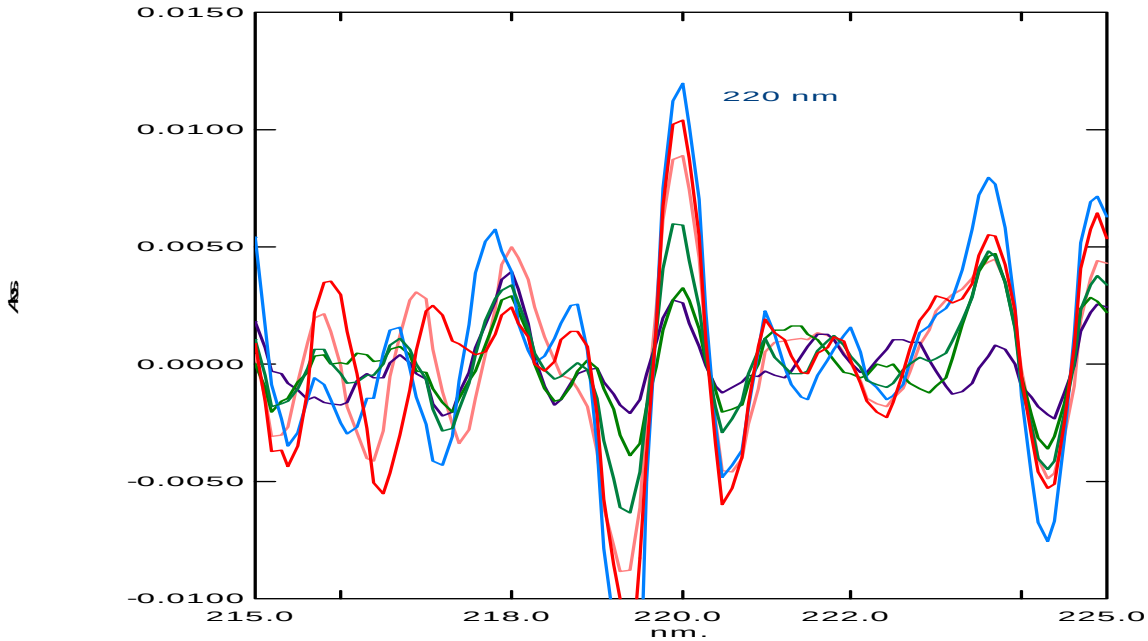
Fig. 4. Calibration curve for alprazolam at 234 nm by second order derivative Spectroscopy



Method : Third 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 third 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 234 nm (Fig. 3).

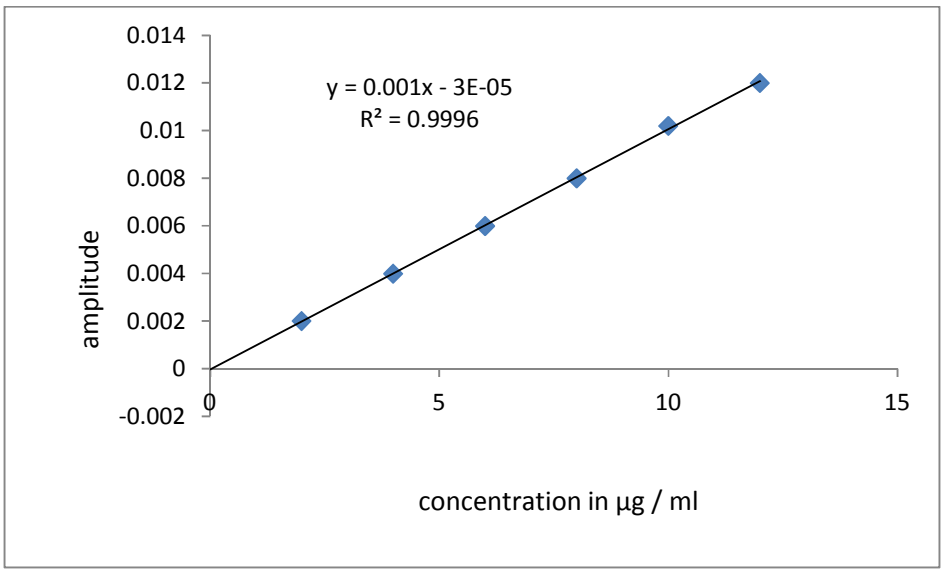
Fig. 3. Overlain spectra of third order derivative spectrum of alprazolam (2-14 µg/ml) showing absorbance at 220 nm



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 third order derivative spectra were obtained by using derivative mode. Amplitudes of the resulting solutions were measured at 220 nm by using 0.1N hydrochloric acid as blank. The calibration curve was prepared in the concentration range of 1 to 12 µg/ml. (Fig. 4)

Fig. 4. Calibration curve for alprazolam at 220 nm by third order derivative Spectroscopy



Results of analysis are given in table 1.

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

Parameter	Second order derivative method	Third order derivative method
Wavelength (nm)	234	220
Beer Law Limits (µg/ml)	1-14	1-14
Correlation coefficient(r^2)	0.9994	0.9996
Regression equation (y=b+ac)		
Slope (a)	0.0006	0.001
Intercept (b)	0.00004	-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 second 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	1.934066	96.7033	0.082234	4.251883
4	2	4.065934	101.6484	0.082234	2.022518
6	4	6.043956	100.7326	0.116297	1.924183
8	6	8.175824	101.9231	0.125615	1.54056
				Mean = 0.101595	Mean = 2.434786

Table 3: Results of recovery of alprazolam for third 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	2.042857	102.1429	0.09759	4.777133
2	2	4.028571	100.7143	0.075593	1.876419
2	4	6.028571	100.4762	0.075593	1.253911
2	6	8.057143	100.7143	0.09759	1.211223
				Mean = 0.086591	Mean = 2.279672

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.	
		Second order derivative method	Third order derivative method
1	10	10.000	10.200
2	10	9.846154	10.000
3	10	10.000	10.100
4	10	10.000	10.200
5	10	10.15385	10.200
6	10	9.846154	10.100
	Standard deviation	**0.106164**	**0.089974**
	%RSD	**1.063978**	**0.889569**

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 second order derivative method

A standard Solution of concentration 10 µg/ml was scanned between 300 nm to 200 nm in spectrum mode. The second order derivative spectrum was obtained by using derivative mode. Amplitude of the resulting solution was measured at 234 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 234 nm for second order derivative, table 5.

For third order derivative method

A standard Solution of concentration 10 µg/ml was scanned between 300 nm to 200 nm in spectrum mode. The third order derivative spectrum was obtained by using derivative mode. Amplitude of the resulting solution was measured at 220 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 220 nm for third order derivative, table 5.

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

Sr. no.	**Parameters**	**Second order derivative method**	**Third order derivative method**
(A)	Intra-day precision (n=3) Amount found ±% RSD	100.17% 0.1262	100.174% 0.1056
(B)	Inter-day precision (n=3) Amount found ±% RSD	99.456% 0.1157	99.456% 0.1023
(c)	Ruggedness Analyst to analyst(n= 3) %RSD	0.1047	0.1269

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	Second order derivative method	Third order derivative method
Limit of Detection (μg/ml)	0.293987	0.322047
Limit of Quantification (μg/ml)	0.890871	0.9759

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 second and third 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 methods were validated according to International Conference on Harmonization guidelines for validation of analytical procedures. Alprazolam has the absorbance maxima at 234 nm in second order derivative and 220 nm for third order derivative method respectively. 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 non interference 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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