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Research Article

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Method development, validation and determination of Alprazolam in its pharmaceutical dosage by 2,3-dichloro 5,6-dicyano-1,4-benzoquinone

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ABSTRACT

Alprazolam is a known sedative and hypnotic drug. A simple visible spectrophotometric method is developed and validated for Alprazolam in its pharmaceutical dosage, using 2, 3 dichloro 5, 6 dicyano 1, 4 benzoquinone (DDQ) at $38^0 C$ *. The maximum absorbance of Alprazolam is found to be at* λ_{max} *=266 nm. On reaction with DDQ solution Alprazolam forms yellow colored salt derivative with maximum absorbance at* λ_{max} *=426nm. The solution obeys Beer's law in the concentration range of 1-20* $\mu g / mL$ *.The coefficient correlation for the linear curve equation Y=0.037x+0.004, is 1.00. From the average absorbance value of 0.2902, analytical, statistical and optical parameters were calculated and found to be within limits. An average recovery of the solution was made to 94.6%. The proton NMR spectrum of Alprazolam and its derivative were recorded to verify the possible mechanism which is also to supported by HPLC.*

Key words: Alprazolam, DDQ, validation, spectrophotometry, NMR, HPLC.

INTRODUCTION

Alprazolam (Fig-I) 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 [1]. It also possesses sedative, hypnotic, anticonvulsant, amnesic and skeletal muscle relaxant property [2-4].It has fast onset action and symptomatic relief [5].It has been determined by voltametry [6], GC-MS [7-11], HPLC [12-16]. The reagent DDQ (Figure-II) is chemically 2, 3-Dichloro-5, 6-dicyano-1,4-benzoquinone.It is a known oxidizing reagent and is stable in mineral acids. It abstracts hydride ion from allyllic and benzyllic positions. It is used in Organic synthesis of dehydrogenation of alcohols [17], phenols [18] and steroids [19].It has been used to determine drugs as Paroxetine, Famotidine and Itraconazole [20] spectrophotometrically.

A method for the validation of Alprazolam in its pharmaceutical dosage in UV region is reported recently [21]. However in the present method Alprazolam has been determined in its pharmaceutical dosage using DDQ by visible spectrophotometry. At room temperature Alprazolam is a colorless powder, whereas DDQ is an orange colored powder. On heating DDQ with Alprazolam in aqueous methanol at $38^0 C$ a bright yellow colored solution. This solution is subjected to visible spectrophometry and been validated as per ICH guidelines.

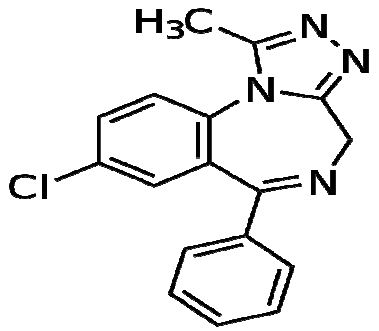


Figure-I Alprazolam.

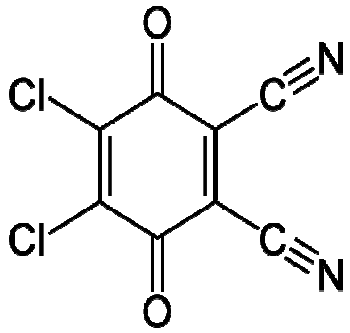


Figure-II DDQ

EXPERIMENTAL SECTION

Chemicals and reagents

DDQ (Sd fine), Alprazolam (Zepro-M, Pharmacia India Ltd) Chloroform, Benzene, Potassium dihydrogen phosphate, Methanol, NaOH and HCl used were of analytical grade.

Instrumentation

The instruments used in this method are
NMR spectrophotometer, 400 MHz (Bruker Biospin)
UV-Visible spectrophotometer-117(Systronics)
UV-Visible spectrophotometer-3000+ (Labindia)
Analytical balance (Mattler toledo B2048)

Preparation of reagent solution

0.01gm of DDQ was accurately weighed and transferred into 50 ml standard flask. Few drops of Concentrated HCl was added and made up with distilled water. The concentration of the resultant solution was 0.001M. The solution was labeled as reagent solution.

Preparation of diluents solution

0.68 gm of Potassium di hydrogen phosphate was taken into 100ml of standard flask, and was made up with distilled water. Further, the solution p^H was adjusted to 6.4 with 0.1M KOH and 0.2M HCl solution and the solution were labeled as diluents solution.

Preparation of standard solution

6 mg of Alprazolam (API) was accurately weighed and transferred into 20ml standard flask and made up with the diluents solution. The solution is labeled as 0.001M of standard solution of Alprazolam.

Preparation of dosage solution

25 tablets of Alprazolam (Zepro-M), 10.25 mg each (0.25mg of Alprazolam and 10mg of Propanolol) each was finely powdered and thoroughly mixed, of this 6 mg was accurately weighed and transferred to 20ml of standard flask and made up with the diluents solution. The solution was sonified and filtered for the removal of placebo and labeled as 0.001M dosage solution.

Method development

5ml of dosage solution and 5ml of reagent solution was taken in 20 ml of standard flask and left to react for about 30 minutes at 38^0C . Pale yellow color develops, soluble in alcoholic KOH solution. The resultant solution is labeled as sample solution, which is chemically a salt derivative of Alprazolam and DDQ. The maximum absorbance of the standard solution was recorded at λ_{max} =266nm, and for the sample solution at λ_{max} =426nm. The sample solution is now subjected to ICH guidelines to validate this method.

Validation of the method developed

Linearity and range

Five different concentrations 1, 3,5,10 and 20 ppm were made from the sample solution. Each of these solution were subjected to absorbance and the values obtained were recorded (Table-1) and linearity plot was obtained at 426nm. The sample solution obeys Beer's law in the concentration range 1-20ppm (Figure-II).The regression for the equation, Y=0.037+0.004 was 1.00, established from the absorbance values.

Table-1 Linearity and range

Alprazolam Derivative (ppm)	Absorbance value
01	0.039
03	0.115
05	0.189
10	0.371
20	0.737

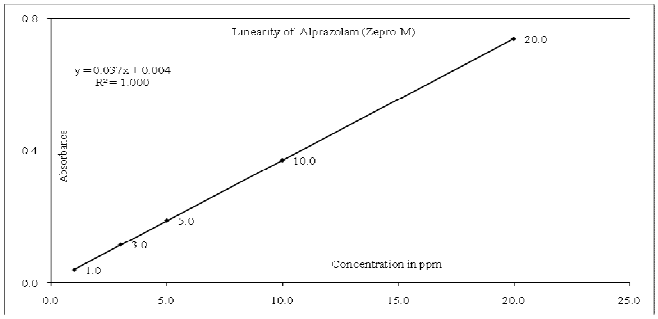


Figure-III Linearity & range of Alprazolam

Specificity

In the present method, Alprazolam was selectively made to react with DDQ, in the presence of Propanolol, placebo and impurities. According to standard ICH guideline.

Accuracy

Recovery studies in the present work confirms that the method is accurate.1ml of the standard solution was taken and concentration of 2,4 and 6 ppm were made and their absorbance measurements were made and listed in table-4.An average recovery of 94.6 was made in present studies.

Table- 2 Accuracy of the method at λ_{max} =426nm

Concentration of Derivative(ppm)	Label claim(mg)	Amount found	% of Recovery
02	0.25	0.23	92
04	0.25	0.24	96
06	0.25	0.24	96

Repeatability

From the freshly prepared sample solutions of 5, 10 and 15 ppm and 5, 10 and 15 ppm for standard solution, Intraday precision and Inter day precision were recorded at 266nm and 426nm. The absorbance values obtained were tabulated in table-3.

Table- 3 Repeatability for the proposed method

Formulation	Concentration Taken (ppm)	Intra-Day Precision %	Inter-Day Precision %
Alprazolam (Formulation)	5	4.810	4.950
	10	10.30	10.80
	15	13.90	14.60
Alprazolam salt Derivative	05	5.100	4.910
	10	10.20	9.780
	15	14.80	14.30

Ruggedness

For a freshly prepared sample solution of 25ppm, absorbance values were recorded on different days and were also recorded by two different analysts as given in table-4.

Table –4 Intermediate precision (Ruggedness)

Concentration of salt Derivative	Factor Considered	Absorbance
25 ppm	Day-1	0.938
25 ppm	Day-2	0.919
25 ppm	Analyst-1	0.909
25 ppm	Analyst-2	0.920

Robustness

It involves absorbance scanning of the sample solution at different wavelength, temperature and P^H .The values of absorbance are given in table-5.

Table -5 Robustness

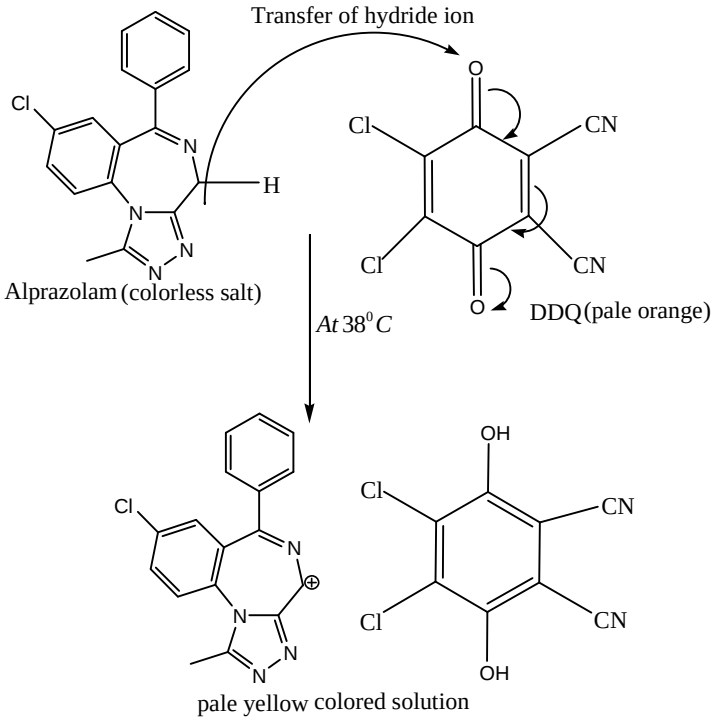
Concentration of salt Derivative(ppm)	Factor Considered	Absorbance
20	λ_{max} =430nm	0.721
	λ_{max} =422nm	0.701
20	P^H =6.0	0.720
	P^H =7.2	0.712
20	$At\ 25^0C$	0.700
	$At\ 35^0C$	0.733

RESULTS AND DISCUSSION

DDQ is well known oxidizing reagent, for its selectivity for Allylic and Benzylic position. In the present studies 0.001M of DDQ was made to react with Zepro-M, which is a combination drug of 0.25mg of Alprazolam and 10mg of Propanolol. DDQ selectively reacts with Alprazolam at its 4H position to form salt derivative. Alprazolam absorbance wavelength λ_{max} =266nm, whereas its salt derivative absorbance wavelength, λ_{max} = 426nm.The amount of Alprazolam present in Zepro-M is directly proportional to the amount of its salt derivative. Since placebo and other combined drugs (Propanolol) [22] are inert to DDQ, the method can be validated accurately. In the present studies the salt derivative of Alprazolam was subjected to standard ICH guidelines [23] to validate Alprazolam in its pharmaceutical dosage. The method was validated in two steps.

According to ICH guidelines [23-25] the sample solution linearity and range was found to obey Beer's law in the concentration range of 1-20ppm.The regression for the linear equation, Y = 0.037x + 0.004 was 1.00. Average recoveries of the sample solution were made to 94.6.The intraday and inter day precision, absorbance values were calculated for the average concentration 25 ppm. A sample solution of 25ppm concentration was tested for its ruggedness towards time, analyst and found to obey ICH guidelines. Robustness for the present method was testified by slight variation in p^H , λ_{max} and temperature for the sample solution. The results show that the method is robust. The stability [26] of the sample solution was found to be 12 hours, which is sufficient to validate Alprazolam in its dosage. The optical and statistical data obtained for this method is given in table-6 and table-7, which are under the prescribed limits [27].

Proton-NMR of Alprazolam (Figure-IV) and its sample solution (Figure-V) gives a strong evidence for this method, as the salt derivative. DDQ gains aromaticity and does not show any color in the developed solution, the only contributor for the color developed is the salt form of Alprazolam. UV-Vis spectrum of Alprazolam (Figure VI) and its salt derivative (Figure-VII) also supports validation of the method developed. Another evidence for the formation of salt derivative was confirmed from HPLC. Alprazolam in its dosage and its salt derivative were separately scanned on HPLC, the chromatograms (Figure-VIII and IX) shows no significant structural change. The formation of salt derivative is as shown in scheme-1.



Scheme-1 Formation of salt derivative of Alprazolam

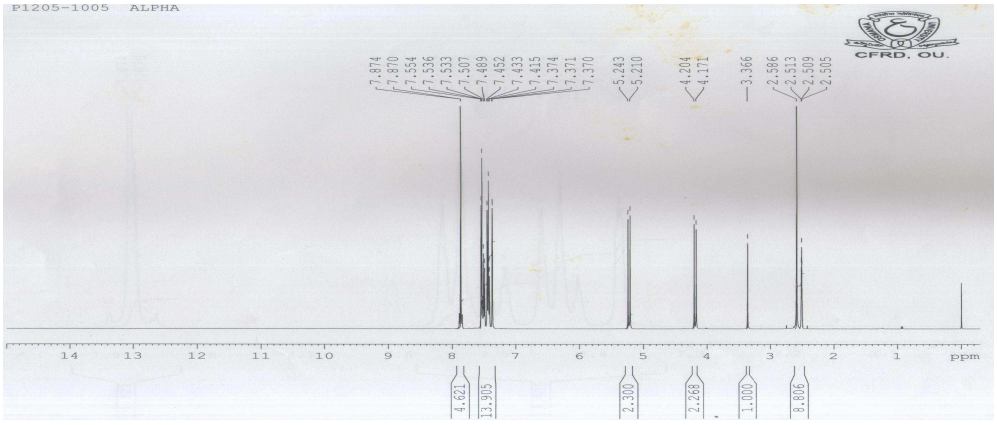


Figure- IV Proton NMR spectrum of Standard Alprazolam in its API form

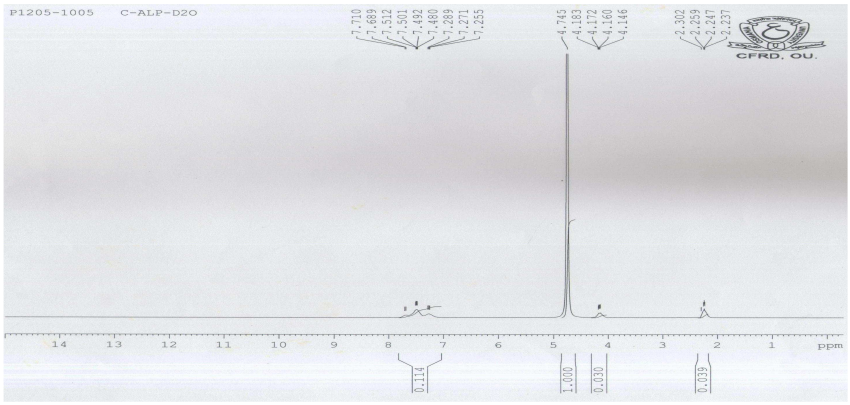


Figure-V Proton NMR spectrum of salt derivative of Alprazolam in D_2O

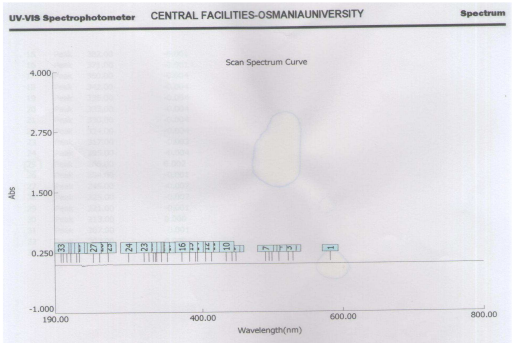


Fig-VI UV-Vis spectra of Alprazolam showing 266nm

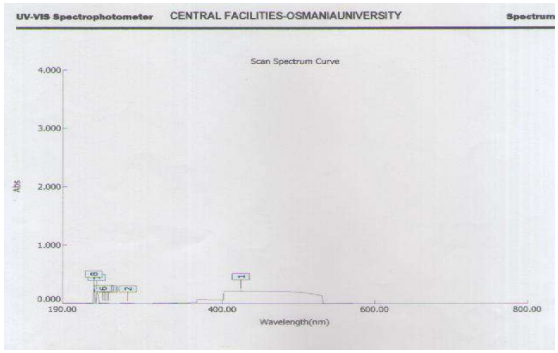


Fig-VII UV-Vis spectra of Alprazolam salt derivative at 426nm

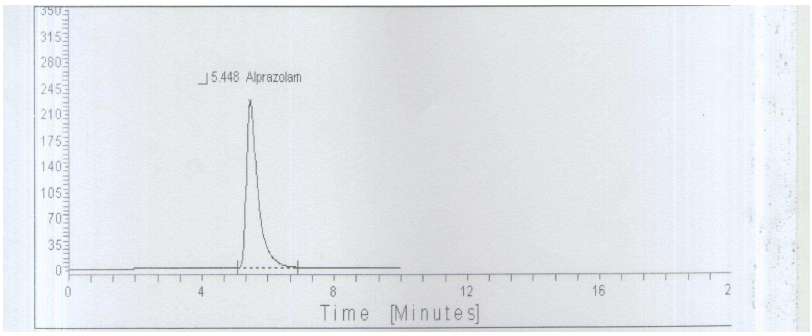


Fig-VIII HPLC chromatogram of Alprazolam in its dosage

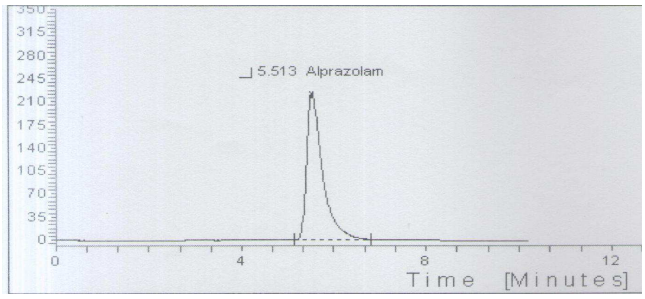


Fig-IX HPLC Chromatogram of Alprazolam salt derivative

Table-6 Optical parameter s involved in the method

Optical parameter	Coresponding value
Color of Alprazolam in Zepro-M	White powder
Color of Alprazolam in Buffer	Colorless
Color of DDQ in mineral acid	Orange
Color of Alprazolam derivative	Pale yellow (In methanol)
Color after 12 Hours	Colorless
Absorption wave length of Alprazolam	λ_{max} =266nm
Absorption wavelength of Alprazolam salt Derivative.	λ_{max} =426nm
Molar absorptivity	$0.02902 M^{-1} Cm^{-1}$
Optical density	0.02902
Transmittance	51.3%
Sandells sensitivity	$3.5\times10^{5}\ \mu g / ml / Cm^{2}$

Table-7 Statistical parameter of the proposed method

Statistical parameter	Corresponding value
Concentration of Alprazolam	0.001M
Concentration of 2,4-DNPH	0.001M
Recovery (Average)	94.6%
Limit of Quantization (LOQ=3SD/m)	0.002 μ g/mL
Limit of Detection (10=SD/m)	0.37 μ g / mL
Length of the cell	1Cm
Signal-Noise ratio	1.165(Acceptable range)
Lower critical limit(LCL)	0.00159 mg / L
Upper critical limit(UCL)	0.00547 mg / L
Method detection limit(MDL)	0.00249 mg / L
Standard deviation (SD)	0.2490
Mean value	0.2902
Standard error value of mean	0.124
t-value	0.01
p-value	0.496
Average of absorbance	0.2902
Concentration range	1-20 $\mu g / ml$
Linear equation	Y = 0.037x + 0.004
Intercept	0.004
Slope	0.037
Regression Co-efficient	1.00
Stability time	12 hours

CONCLUSION

The method is simple, accurate and economical and can be used for validation of Alprazolam in its pharmaceutical dosage using DDQ, which imparts color to the solution that is directly proportional to the concentration of the Alprazolam, which can be estimated in visible spectrophotometry.

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