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Method Development and Validation of UV Spectrophotometric Method for Alprazolam in Pharmaceutical Dosage Form Using *Ferric chloride* and *Indigo carmine*

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Abstract: A simple, novel, sensitive and specific extractive Derivative Spectrophotometric method was developed and validated for the determination of Alprazolam (ALP) in bulk and its dosage form. These methods are based on the formation of Ferric Chloride and Indigo carmine ion-association complexes maximum at 521nm. Reaction conditions were optimized to obtain the maximum colour intensity. The absorbance was found to increase linearly with increase in concentration of Alprazolam (ALP), which was corroborated by the calculated correlation coefficient values (0.9994 and 0.9998). The systems obeyed Beer's law in the range of 5-45 µg/mL for Alprazolam (ALP) respectively. No interference was observed from common excipients present in tablets. Various analytical parameters have been evaluated and the results have been validated by statistical data.

Key words: Alprazolam · Derivative spectroscopy · Ion-association complexes

INTRODUCTION

Alprazolam (ALP) is chemically 8-chloro-1-methyl-6-phenyl-4H-[1, 2, 4] triazolo [4,3,-α]-[1,4] benzodiazepine derived from 1,4-benzodiazepines of new generation [Fig.1]. It is a benzodiazepine mainly used as anxiolytic in humans and may be effective in the treatment of depression and panic disorder. It should be borne in mind that neither alprazolam nor any other benzodiazepine is effective when it comes to treating anxiety and strain caused by daily stress. Besides this, ALP is also used to treat panic disturbances with or without agarophobia [1,2]. Most of the analytical methods in the literature to Alprazolam pharmaceutical formulation [3-5] or to determine its related substances [6-10] and include analysis using high-performance liquid chromatography (LC), LC/tandem mass spectrometry (LC/MS), gas chromatography/MS (GC/MS), Spectroflourimetry, high-performance thin layer chromatography (HPTLC) and capillary electro chromatography (CEC). Author of the article and his research team has developed a UV Method development different pharmaceutical dosage form [11-25] using Ferric Chloride [26-27].To the best of our knowledge, there is no reported Spectrophotometric or pharmacopoeial method for simultaneous determination of ALP using Ferric Chloride in pharmaceutical formulations.

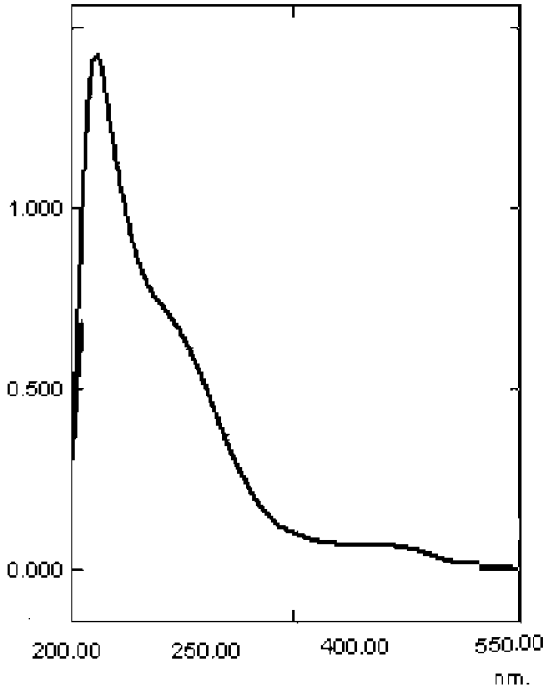

Fig. 1: Overlain derivative spectroscopy spectra of Alprazolam

Thus, efforts were made to develop fast, selective and sensitive analytical method for the estimation of ALP.

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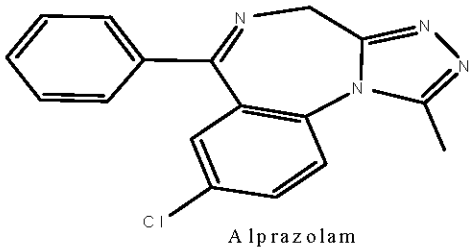

Alprazolam

Experimental: UV Visible spectrophotometer was employed with spectral bandwidth of 1 cm and wavelength accuracy of 0.3 nm (with automatic wavelength correction with a pair of 1 cm matched quartz cells). All chemicals and reagents used were of AR/HPLC grade, Chloroform and methanol (A.R., Ranbaxy Ltd., New Delhi) were used for preparation and as solvent. All chemicals used in this study were analytical grade and used without further purification. Commercial Tablets containing 10 mg of Alprazolam were procured from local market.$FeCl_3$ $6H_2O$ (0.8%) [9] and $1x10^{-3}$ M solution in ethanol standardized against standard $KMnO_4$ after reduction. Indigo carmine (200 μg mL^{-1}): A 1000 μg mL-1 stock standard solution was first prepared by dissolving accurately weighed 112 mg of dye (S.D. Fine Chem., Mumbai, India, 90 % dye content) in water and diluting to volume in a 100 mL calibrated flask. The solution was then diluted 5-fold to get the working concentration of 200 μg mL^{-1}.

Standard Stock Solutions: Accurately transfer volumes of standard drug solution in ethanol (1mg/ml) equivalent to 5 mg Alprazolam into a of 10 ml volumetric flask add 0.5 ml of 0.8 % $FeCl_3$ and 200 μg mL^{-1} Indigo carmine heated in a water bath at 65±5°C for 15 minutes, cool and complete volume with ethanol. Measure the absorbance of an orange chelate of Alprazolam with Fe (III) at 521 nm against reagent blank.

Preparation of Sample Solution: From the above stock solution 1 ml was transferred into 10 ml volumetric flask and volume was made up to the mark with methanol to make 10μg/ml. Spectrophotometry using indigo carmine (method). Varying aliquots (1.0-3.0 mL) of a standard 200 μg mL^{-1} ALP solution were transferred into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was brought to 3 mL by adding water. The content was mixed well and the flasks were kept aside for 10 min with intermittent shaking. Finally, 1 ml of 200 μg mL^{-1} indigo carmine solution was added to each flask, the volume was diluted to the mark with water, mixed well and absorbance measured against a reagent blank at 470 nm after 10 min. The concentration of the unknown was read from the calibration graph or computed from the regression equation derived using Beers' law data.

Table 1: Optical Characteristics Data for Method

Working λ max	Alprazolam
Beer's law limit (μg/ml)	5-45
Absorptive E (1%,1cm)*	521 nm
Molar absorptivity (l/mol.cm)*	27654
Correlation coefficient*	0.9999
Intercept*	0.741
Slope*	0.3997

Table 2: Summary of validation parameters for the proposed method

Parameter	Alprazolam
LOD[a]	0.045 μg/mL
LOQ[b]	0.151μg/mL
Accuracy, %	99.36-100.29
Repeatability (RSD % = 6)	0.228
Interday (n=3)	0.0325 - 0.765
Intraday (n=3)	0.465 - 0.665

Table 3: Regression analysis of calibration curves for the proposed Derivative Spectrophotometric method

Parameter	Alprazolam
Concentration range	5-45 μg/mL
Slope	0. 3665
Standard deviation of the slope	0.5323
Intercept	1.76442
Standard deviation of the intercept	2.875
Correlation coefficient	0.9999

Method: Derivative Spectrophotometry [28]: Stock solutions were prepared separately in 100 ml methanol to obtain 10 μg/ml of all drugs. The six working mixed standards were prepared by dilution of stock solution in same solvent system in concentration range 5-45 μg/ml of Alprazolam initially scanned for determining sampling wavelength. Sampling wavelengths were 521 nm for Alprazolam showed zero crossing point. Calibration graphs were constructed from the absorbances at respective wavelength. Calibration graphs were constructed from the absorbances at respective wavelength.

Analysis of Commercial Formulation: Content of powder equivalent to 10 mg of Alprazolam taken and added in 100 ml of solvent sonicated for 10 min after sonication volume was made up to 100 ml. 1ml of this stock solution was diluted to 10 ml to get concentration equal to 10 μg/ml of Analyze the clear alcoholic filtrate claimed to contain

10 µg /ml Alprazolam by chelation with Fe (III) as mentioned above. This solution is scanned in range 200-400 nm taking solvent system as blank. The spectra obtained were converted Simultaneous equation method and first order derivative spectra absorbances were noted and concentrations were determined from regression equations generated from calibration graph.

Validation: Selectivity/specificity method is said to be specific when it produces a response only for a single analyte. Selectivity is the ability of the method to produce a response for the analyte in the presence of other interferences, in order to prove that the method chosen was specific and selective. Sensitivity Limit of detection (LOD) and Limit of quantification (LOQ) were calculated according to the 3:1 (S/N) and 10:1 (S/N) criterions respectively, where S is the signal of the sample and N is the noise of the corresponding curve. Linearity and range Linearity of the concentrations was taken in the range of 10-50µg/ml for ferric chloride and 20-60µg/ml for indigo carmine respectively. Accuracy of proposed method from excipients was determined by recovery experiments. Recovery experiments were carried out in three levels of concentration. The amounts of standard recovered were calculated in the terms of mean recovery with the upper and lower limits of % relative standard deviation. Intraday precision it was determined by calculating the %coefficient of variation (%CV) of the results obtained in the same day. Inter day precision it was determined by calculating the percentage coefficient of variation (% CV) of the results obtained over at least three days.

RESULTS AND DISCUSSIONS

The principle advantages of derivative spectroscopy are the improvement of resolution of overlapping absorption bands and the accuracy and precision compared to UV absorption methods; therefore, derivative spectroscopy has been used in quantitative analysis when the analytes to be determined present in admixture with other components. Calibration curves were constructed by plotting absorbance vs. concentrations of ALP and the regression equations were calculated. The calibration curves were plotted over the six different concentrations in the range 5-45 $\mu g.mL^{-1}$ for drugs. Accurately measured mixed standard solution of ALP (1, 3, 5 and 7 mL) were transferred to a series of 10 mL volumetric flasks and diluted to the mark with 0.1 M $FeCl_3$. Accurately measured mixed standard solution of ALP (1.0, 1.5, 2.0, 2.5 and 6.5 mL) were transferred to a series of 10 mL volumetric flasks and diluted to the mark with 0.1 mol L^{-1} $FeCl_3$. Absorbance was measured for each solutions at analytical wavelength of ALP (n = 6).The accuracy of the methods was determined by calculating recoveries of ALP by the standard addition method. Known amounts of mixed standard solution of ALP (4.0, 8.0 and 12.0 $\mu g.mL^{-1}$) were added to pre-quantified sample solutions of tablet dosage forms. The amounts of ALP were estimated by applying values of absorbance to the regression equations of the calibration curve. The precision of the instruments was checked by repeatedly scanning (n = 6) mixed standard solutions of ALP (10 µg.mL-1).The intermediate precision for the proposed method was determined by estimating mixed standard solution of ALP for three different concentrations for three times on the same day and on three different days. The results are reported in terms of relative standard deviation (RSD).The limit of detection (LOD) with signal to noise ratio of 3:1 and the limit of quantification (LOQ) with signal to noise ratio of 10:1 were calculated for both drugs using the following equations as per International Conference on Harmonization [29] guidelines. LOD = 3.3 × ó/S LOQ = 10 × ó/S Where ó = the standard deviation of the response and S = the standard deviation of y-intercept of regression line. To check the degree of repeatability of the methods, suitable statistical evaluation was carried out. Five samples of the formulations were analyzed for the repeatability study. The standard deviation, coefficient of variance and standard error was calculated. Sampling wavelengths were determined from scanning individual drug samples in 200-400 nm range. Sampling wavelengths were 521 nm for ALP respectively in derivative Spectroscopy mode. For this method equations generated were Y=-0.0854+0.0043x (r^2=0.9994) and Y=-0.0437+0.0043x (0.9998) for ALP. Linearity of proposed method was found to be 5-45 µg/ml for ALP. Limits of detection were found to be 0.0488 and limits of quantitation were found to be 0.041 ALP. This method utilizes the active analogue principle that lies at the spectroscopic method [26-27].

CONCLUSION

The method is precise and high percentage of recovery of as shown in table shows that the method is accurate. The method is simple, rapid, accurate and can be adopted in routine analysis of drug formulation. The accuracy and reproducibility of the proposed method was statistically validated by recovery studies.

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REFERENCES

1. Dawson, G.W., S.G. Jue and R.N. Brogden, 1984. Alprazolam: a review of its pharmacodynamic properties and efficacy in the treatment of anxiety and depression. Drugs., 27: 132.
2. Fawcett, J.A. and H.M. Kravitz, 1982. Alprazolam: Pharmacokinetic, clinical efficacy and mechanism of action. Pharmacotherapy, 2: 243.
3. Venkateswarlu, K., R.K. Venisetty, N.R. Yellu, S. Keshetty and M.G. Pai, 2007. Development of HPTLC-UV absorption densitometry method for the analysis of alprazolam and sertraline in combination and its application in the evaluation of marketed preparations. J. Chromatogr. Sci., 45: 537.
4. Zhang, J.L. and T.H. Zhou, 2004. A New Method of Separation of Four Benzodiazepines by RP-CEC. Chinese. Chem. Lett., 15: 935.
5. Patil, D. and B. Raman, 2001. Simultaneous estimation of sertraline and alprazolam in capsules by reverse phase liquid chromatography. Ind. Drugs., 38: 638.
6. Pujadas, M., S. Pichini, E. Civil, E. Santamarina, K. Perez and R. Torre, 2007. A simple and reliable procedure for the determination of psychoactive drugs in oral fluid by gas chromatography–mass spectrometry. J. Pharm. Biomed. Anal., 44: 594.
7. Hanysova, L., T. Grafnetterova, M. Dubovska and T. Klimes, 2005. Development of analytical method for LC-MS detection of unknown degradation product of Alprazolam. Chem. Pap., 59: 99.
8. Nudelman, N.S. and C.G. Cabrera, 2002. Spectrofluorimetric assay for the photo degradation products of alprazolam. J. Pharm. Biomed. Anal., 30: 887.
9. Basset, J., R.C. Denney and G.H. Jeffery, 1978. Vogel's textbook of Quantitative Inorganic Analysis fourth ed., 348-351: 370-372.
10. Gomes, P. and M. Steepe, 2006. First-Derivative Spectrophotometry in the Analysis of Rosiglitazone in Coated Tablets. J. AOAC Int., 89: 1296.
11. Sharma, M.C. and S. Sharma, 2010. Validated Simultaneous Spectrophotometric Estimation of Paroxetine HCl Bulk and Tablet Dosage Form using Ferric Chloride. J. Optoel. and Biomed. Mater., 2(4): 185-189.
12. Sharma, M.C. and S. Sharma, 2010. UV-Densitometric Determination of Sparfloxacin and its application to the Assay in Pharmaceutical Dosage Forms. J. Optoel. and Biomed. Mater., 2(4): 191-195.
13. Sharma, S., M.C. Sharma, R. Sharma and A.D. Sharma, 2010. Spectrophotometric Analysis of Nebivolol Hydrochloride in Tablet Dosage form using 5.0M Niacinamide solution as hydrotropic solubilizing agent. J. Pharm. Resea., 3(5): 1074-1076.
14. Sharma, S., M.C. Sharma, R. Sharma and A.D. Sharma, 2010. Simultaneous Estimation and Validation of Ezetimibe and Simvastatin in Combined Tablet Dosage Forms by Hydrotropic Solubilization Technique Using 3.0 M Urea. J. Pharm. Resea., 3(5): 1063-1067.
15. Sharma, M.C. and S. Sharma, 2010. Simultaneous Estimation and Validation of Pseudoephidrine Sulphate and Desloratidine from Bulk and Tablets as hydrotropic solubilizing agent. J. Curre. Pharma. Res., 1: 26-30.
16. Sharma, S., M.C. Sharma and A.D. Sharma, 2010. Hydrotropic solubilization phenomenon Spectrophotometric estimation of Tenfovir disoproxil fumerate tablet. J. Chemic. Pharmac. Res., 2(2): 411-415.
17. Sharma, S., M.C. Sharma and A.D. Sharma, 2010. Novel application and spectrophotometric estimation of Melitracen HCl tablet dosage form using Niacinamide as hydrotropic solubilizing agent. J. Chemic. Pharmac. Res., 2(2): 416-420.
18. Sharma, M.C. and S. Sharma, 2010. A Quantitative Estimation and Validation of Atorvastatin calcium and Pioglitazone in Pharmaceutical Tablet Dosage Form by Hydrotropic Solubilization Phenomenon. Intern. J. Chem. Tech. Resear., 2(4): 2487-2491.
19. Sharma, M.C. and S. Sharma, 2010. Novel method for Spectrophotometric analysis of Simultaneous Estimation of Bisoprolol Fumarate Tablet Formulations using hydrotropy solubilization Agents. J. Optoelect. Biomed. Mat., 2(4): 223225.

20. Sharma, M.C. and S. Sharma, 2010. Development and Validation of Simultaneous Estimation of Etoposide Solid Dosage form using hydrotropic Agents. J. Optoelect. Biomed. Mat., 2(4): 227-229.
21. Sharma, R., G. Pathodiya, G.P. Mishra and M. Sharma, 2010. Simultaneous Estimation and Validation of Cefixime Trihydrate and Ornidazole in Bulk and Tablets using Hydrotropic Solubilizing Agents. J. Pharm. Resea., 3(12): 2953-2955.
22. Sharma, M.C. and S. Sharma, 2011. Spectrophotometric determination of Lamivudine in Bulk and Pharmaceutical Formulation using hydrotropic Solubilization. Intl. J. Chem. Tech. Resea., 3(2): 988-991.
23. Sharma, S., R. Sharma and M.C. Sharma, 2010. Simultaneous Estimation and Validation of Poorly Water Soluble Drugs Rabeprazole Sodium and Itropide Hydrochloride Combined Tablet Dosage Form by Hydrotropic Solubilization Agents. Intern. J. Pure and Appl. Chem., 5(4): 305-311.
24. Sharma, M.C., S. Sharma and S.C. Chatuervedi, 2011. Spectrophotometric Methods for the Determination of Repaglinide in tablets Using Indigo Carmine. Intl. J. Pure and Appl. Chem., 6(1): 75-78.
25. Sharma, M.C. and S. Sharma, 2010. UV Spectrophotometric Methods for Estimation of Anastrazole Bulk and Tablet Dosage Form By derivative spectroscopy. J. Optoel. and Biomed. Mater, 2(4): 217-221.
26. Madhuri, D., K.B. Chandrasekhar, M. Ramakotaiah, G. Somasekhar, K. Harinadhababa and K. Ravi Kumar, 2010. Validation of Spectrophotometric determination of Rabeprazole using Ferric Chloride (FeCl3). Int. J. Res. Pharm. Sci., 1: 209-211.
27. Basavaiah, K. and U.R. Anil Kumar, 2006. New Sensitive Spectrophotometric Methods for the Determination of Raloxifene Hydrochloride in Pharmaceuticals Using Bromate-Bromide, Methyl Orange and Indigo Carmine. E.J. Chem., 3(13): 242-249.
28. Beckett, A.H. and J.B. Stenlake, 2004. Practical Pharmaceutical Chemistry, Fourth Edition, CBS Publishers and Distributors, New Delhi, India.
29. ICH, 1996. Q2 (R1) validation of analytical procedures: text and methodology. International conference on harmonization.