DEVELOPMENT AND VALIDATION OF SIMPLE UV-SPECTROPHOTOMETRIC METHOD FOR QUANTITATION OF BROMAZEPAM IN API AND SOLID DOSAGE FORMULATION.

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ABSTRACT
Bromazepam are a well-known class of psychoactive drugs; they are known primarily for their hypnotic and sedative effects. In this recent research a very simple, precise, least time consuming, versatile, inexpensive and efficient UV Spectrophotometric method developed for the quantitation of Bromazepam in Active Pharmaceutical Ingredient and its pharmaceutical solid dosage formulation. The assay is based on the Ultraviolet Spectroscopy (UV), measuring $\lambda_{max}$ at 285 nm for bromazepam. A sample of bulk was dissolved in 0.1N methanolic sulphuric acid to produce a desired concentration of solution containing bromazepam. Similarly, a sample of pharmaceutical formulation was grind and dissolved in 0.1N methanolic sulphuric acid and various dilutions were made. The absorbance of bulk, dosage formulation preparation was measured at 285nm against 0.1 N methanolic sulphuric acid (blank). Calibration curves were linear over the range of 5-40 $\mu$g/mL with a correlation coefficient is ≥ 0.9996 at $\lambda_{max}$ 285 nm. The analysis shows a linear relationship between the absorbance and concentration. The proposed method was statistically validated and successfully applied for routine analysis of bromazepam in bulk and tablet dosage forms.

KEYWORDS: Bromazepam, UV-Spectrophotometric Method, Active Pharmaceutical Ingredient, Solid dosage formulation, Absorbance ratio method.

INTRODUCTION
Bromazepam (Figure 1) is chemically 7-Bromo-5-(2-pyridyl)-3H-1, 4-benzodiazepin-2(1H)-one. It is a drug belonging to class 1, 4-benzodiazepine.[1] White or yellowish crystalline powder. Practically insoluble in water, slightly soluble or sparingly soluble in ethanol and in methylene chloride. The molecular formula is C$_{14}$H$_{10}$BrN$_{2}$O & molecular weight is 316.15. It is a well established and proven anxiolytic and hypnotic drug. It acts on the central nervous system as an inhibitor of the neurotransmitter gamma aminobutyric acid (GABA).[2] It metabolize into 3-hydroxybromazepam through oxidative biotransformation in liver.[3] It is frequently prescribed for hospitalized patients in whom anxiety is frequently observed.[4] It is formerly known as a tranquilizer commonly used to reduce tension, pathological anxiety, agitation and depression.[5] It misused may also cause or contribute sudden death.[6] Literature revealed that several methods available for the determination of Bromazepam in pharmaceutical dosage forms and in biological fluids such as spectrophotometry,[7,8] flow injection analysis (FIA) methods,[9] HPLC-MS.[10] One spectroscopy method based on second derivative absorption method for the determination of bromazepam in formulation.[11] Other spectrophotometric method used for the assay of bromazepam by making a complex of the drug with iron (II).[12] Various HPLC methods were also reported for determination of bromazepam.[14-16] I and our research group have developed different assay methods which are very simple and useful for different API and dosage formulation.[17-20] Here a rapid, simple, sensitive and precise UV spectroscopy method was developed for the quantitative assay of bromazepam which has been employed for our studies. This spectrophotometric method is simple, precise, rapid, and accurate as compared to other reported methods.

**Figure 1: Chemical Structure of Bromazepam.**
MATERIALS AND METHODS

Material and reagents
Pharmaceutical grade bromazepam was a kind gift from Martin Dow Limited and it dosage formulation, Nikuv® 3 mg Tablets were purchased from local market, the expiry of which was not less than 3 year at the time of study. All other chemical were analytical grade and obtained from Merck laboratories (Merck KGaA, Darmstadt Germany).

Statistical study
Standard regression curve analysis was performed by use of STATISTICA version 7.0 (USA), without forcing through zero. Linearity graphs were obtained by use of Micro- soft Excel 2007 software. SPSS software version 10.0 (Caryy, NC, USA) was used for the calculation of means, standard deviations.

Apparatus
Electrical balance (Mettler Toledo # AG245), UV visible spectrophotometer (Model 1601, Shimadzu, Japan) with 10-mm path length connected to a P-IV computer loaded with Shimadzu UVPC version 3.9 software was used in these studies. Deionizer (Stedec CSW-300), distillation unit (GFL Type 2001/2) used for deionization and distillation of water.

METHODS

Preparation of standard stock solution
Standard stock solution of Bromazepam (100 μg mL\(^{-1}\)) was prepared by weighing 10 mg of bromazepam and transferred to a 100 mL volumetric flask, add 3.3 ml distilled water and volume was made up to 100 mL with 0.1N methanolic sulphuric acid to get a concentration of 100μg mL\(^{-1}\), the prepared solution is sonicated for 10 minutes and filtered through thewhatman 41 filter paper. Appropriate volumes of this solution were further diluted to obtained final concentrations in range of 5 to 40 μg mL\(^{-1}\) (table 1). The spectrum of this solution was recorded using Shimadzu UV-VIS Spectrophotometer, in the range of 200-400 nm.

Preparation of sample solution
20 tablets of Lexotanil® 3 mg were accurately weighed and finally powdered. Amount of powder equivalent to 10 mg of API was transferred into a 100 mL volumetric flask, add 3.3 ml distilled water and add 45 ml 0.1N methanolic sulphuric acid and dissolved by sonication for 20 minutes. The flask was filled to mark and the resulting solution was filtered. Method was followed as describe under analytical procedure.

Preparation of 0.1N Methanolic Sulfuric Acid
Carefully dilute 5.1 gm of 96 % (m/m) sulfuric acid to 1000 mL with methanol. Prepare at least 24 hours before use.

Determination of Absorption maxima
For the selection of analytical wavelength, 20 μg mL\(^{-1}\) solution of bromazepam was scanned in the spectrum mode from 200 nm to 400 nm separately. From the spectra of drug, λmax, 285nm was selected for the analysis.

Validation
Linearity
The linearity of the analytical method is determined by taking concentration in between 5 to 40μg mL\(^{-1}\). From std. stock solution of bromazepam (100 μg mL\(^{-1}\)), pipette out aliquots of standard stock solution transferred to series of volumetric flasks and final volume made up to mark with 0.1N methanolic sulfuric acid as diluent to form solutions of 5 to 40 μg mL\(^{-1}\)of bromazepam (table 1). These solutions were then taking absorbance at 285 nm against 0.1 N methanolic sulfuric acid as blank and then calibration curve was plotted as absorbance vs. concentration to check the linear relationship between absorbance and concentration of bromazepam.

Acceptance Criteria: Correlation Coefficient should not be less than 0.999.

Precision
The precision of the system is determined by assay of six determinations at 20 μg mL\(^{-1}\) test concentration and relative standard deviation (%RSD) is calculated. The results of precision study were reported in terms of % relative standard deviation. Acceptance Criteria: The Relative Standard Deviation should not be more than 2%.

Accuracy
The accuracy of an analytical method is determined by applying the method to analyzed samples, to which known amounts of analyte have been added. The accuracy is calculated from the test results as the percentage of analyte recovered by the assay. The accuracy of developed method was carried out by calculating the % recovery of bromazepam by standard addition method at three different levels i.e. 80 %, 100 % and 120 %.

Procedure for Preparation of Sample Solution
It was carried out at three levels i.e. 80%, 100% and 120% of the nominal concentration. 80% Accuracy solution (16 μg mL\(^{-1}\)): It was prepared by diluting 4 ml of the stock solution up to 25ml with 0.1 N methanolic sulphuric acid. 100% Accuracy solution (20 μg mL\(^{-1}\)): It was prepared by diluting 10 ml of the stock solution up to 50 ml with 0.1N methanolic sulphuric acid. 120% Accuracy solution (24 μg mL\(^{-1}\)): It was prepared by diluting 6 ml of the stock solution up to 25ml with 0.1N methanolic sulphuric acid. The sample was prepared in triplicate and analyzed by using UV-spectrophotometer at wavelength 285 nm. Acceptance Criteria: Mean recovery should be in the range of 98-102%.
Limit of Detection
It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. Limit of detection can be calculated using following equation as per ICH guidelines. LOD = 3.3 × σ /S Where, σ = Standard deviation of the response and S = Slope of the corresponding calibration curve.

Limit of Quantification
It is the lowest concentration of analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions. Limit of quantification can be calculated using following equation as per ICH guidelines. LOQ = 10 × σ /S Where, σ = Standard deviation of the response and S = Slope of the corresponding calibration curve.

RESULT AND DISCUSSION
Method Development and Optimization
The present paper describes the application of absorption ratio method to estimation of bromazepam in API and solid dosage form. The method was validated for the linearity, accuracy, precision, LOD and LOQ.

Validation

Linearity
The calibration curve was taken in the range of 5-40 µg mL⁻¹ for bromazepam at λmax 285nm. The bromazepam was found to be linear within concentration range of 5-40 µg mL⁻¹ with regression coefficient of 0.9996 by absorbance ratio method (Table 1). There was an excellent correlation between absorbance and concentration (Figure 2).

Precision
The precision of an analytical method is determined by assaying six determinations at test concentration (20 µg mL⁻¹). % Relative Standard Deviation (% RSD) calculates statistically (Table 2). It was found to be less than 2% (i.e.0.5249%) indicate the high precision of the propose method.

Accuracy
The accuracy was assessed by the standard addition method of three replicate determinations of three different solutions containing 16, 20 and 24 µg mL⁻¹ (i.e.80%, 100% and 120% of the nominal concentration) of bromazepam. The average % recoveries for three different concentrations were found to be 100.015% as shown in Table 3.

LOD and LOQ
The limit of detection was found to be 0.4 µg mL⁻¹ and the limit of quantification was found to be 1 µg mL⁻¹. The criteria being the concentration should lie outside the range 0.4–1.0 for precise determination of bromazepam.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Concentration (µg/mL⁻¹)</th>
<th>Stock solution in (mL)</th>
<th>Final volume with 0.1 N Methanolic Sulphuric Acid (mL)</th>
<th>Abs.(nm)</th>
<th>R²</th>
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Table 1: Data of Calibration Curve

Figure 2: Calibration Curve of Bromazepam.
Table 2: Precision Data of the Proposed Method

<table>
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<tr>
<th>S. No.</th>
<th>Concentration (µg mL⁻¹)</th>
<th>Abs.(nm)</th>
<th>Avg. Abs.</th>
<th>SD</th>
<th>% RSD</th>
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<tr>
<td>6</td>
<td>20</td>
<td>0.536</td>
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Table 3: Method Accuracy from Recovery Assay

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample Identity</th>
<th>Conc. (µg mL⁻¹)</th>
<th>Abs.</th>
<th>Avg. Abs.</th>
<th>Amount Recovered in µg mL⁻¹</th>
<th>% Recovered</th>
<th>Average % Recovered</th>
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<tr>
<td>1</td>
<td>Accuracy 80 %</td>
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<td>0.436</td>
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<td>Accuracy 100 %</td>
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<td>0.536</td>
<td>20.002</td>
<td>100.010</td>
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<td>0.537</td>
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<tr>
<td>3</td>
<td>Accuracy 120 %</td>
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<td>24.0</td>
<td>0.635</td>
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CONCLUSIONS

A rapid, simple, least economic, fast and non-toxic UV spectrophotometric methods have been developed for the determination and quantification of bromazepam. The present methods also validated as per ICH guidelines for linearity, precision, accuracy, LOD and LOQ. The results of all these parameters were shows that the present UV-spectrophotometric methods found to be precise, linear, rapid, less time consuming and accurate and can be used for routine quality control analysis of bromazepam in API and tablet dosage formulation in any laboratory.

REFERENCES

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