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Research Article

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NEW VALIDATED HPLC METHOD FOR THE ESTIMATION OF BROMAZEPAM IN PHARMACEUTICAL FORMULATION

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ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for Bromazepam in Bulk and Pharmaceutical tablet Formulation. Isocratic elution at a flow rate of 1ml/min was employed on symmetry Kromasil C_{18} column 250 x 4.6 mm ID with 5 µ at ambient temperature. The mobile phase consisted of Acetonitrile: 1% Acetic acid : Water(25:45:30 v/ v/v). The UV detection wavelength was 238nm and 20 µl sample was injected. The run time for Bromazepam is 6 min. The flow rate was found to be 1.0ml/min. The percentage recovery of the method was found to be 109%. The LOD and LOQ for Bromazepam were found to be 5µg/ml and 15µg/ml respectively. The method was successfully applied for routine quality control analysis of pharmaceutical formulation. The HPLC method can be successfully applied for the routine quality control analysis of Bromazepam formulations, which could be the better choices compared to the reported methods of literature.

Key Words: Bromazepam, Rp- HPLC, UV detection, Recovery, Precise.

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1. Introduction:

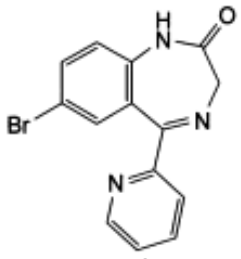

Fig:1 Structure of Bromazepam

Bromazepam [1] is a benzodiazepine derivative drug, patented by Roche in 1963[2] and developed clinically in the 1970s.[3][4] Various studies report impaired memory, visual information processing;[5][6][7] however, less

pronounced than other benzodiazepines such as lorazepam.[8] On occasion, benzodiazepines can induce extreme alterations in memory such as anterograde amnesia and amnesic automatism, which may have medico-legal consequences. Such reactions occur usually only at the higher dose end of the prescribing spectrum.[9] Hasan al-Hawasi et.al.,[10] proposed a rapid, sensitive and validated HPLC method for the separation and analysis of a Bromazepam, Medazepam and Midazolam mixture. The benzodiazepine compounds were separated on a reversed-phase C18 column at 50 °C using a mobile phase containing 25% acetonitrile, 45% methanol and 30% ammonium acetate (0.05 M). The pH was adjusted to pH=9. The samples were detected using a UV detector at 240 nm. The linear range of Bromazepam and Midazolam was between 0.12 and 0.18 mg/mL, while that of Medazepam was between 0.08 and 0.12 mg/mL. The RSD for precision was less than 2%. Recoveries from serum samples ranged between 91.5% and 99.0%. Gregory Podilsky et.al.,[11] proposed a HPLC method with UV detection (HPLC-UV) involves solid phase extraction of 2 mL plasma samples. Linearity was demonstrated in a concentration range of 5–100 ng/mL and 20–2000 ng/mL for bromazepam and omeprazole, respectively. The lower limit of quantification was 5 ng/mL and 20 mg/mL for bromazepam and omeprazole, respectively. Mandava V. Basaveswara Rao et.al.,[12] proposed the chromatographic separation of Diacerein was performed by using a Chromosil C18 column (250 x 4.6mm, 5 µm) as stationary phase with a mobile phase comprising of Methanol : Water 80:20 (v/v) at a flow rate of 0.5mL min-1 and UV detection wave length at 250nm and 20µL sample was injected. The retention time for Diacerein was 8.29min. The percentage RSD for precision and accuracy of the method was found to be 0.399%. Results of recovery studies are shown range 99.00-101.45%. The limit of detection for Diacerein was found to be 0.06.

MATERIALS AND METHODS

Preparation of solutions

Standard Solution

Accurately weighed 66.7 mg Bromazepam standard into a 100 ml volumetric flask, dilute to volume with methanol and mix well. Dilute 10 ml of this solution to 100 ml with dissolution medium. Dilute a further 10 ml of this solution to 100 ml with dissolution medium.

Sample Preparation

900 ml of dissolution medium was placed in the vessel of the apparatus. Assembled the apparatus and allow the temperature of the dissolution medium to equilibrate to 37 °C ± 0.5 ° C. Place one tablet into each of the baskets and immerse in the vessels, taking care to exclude air bubbles from the surface of the tablet, and immediately operate the apparatus at 100 rpm. At 30 minutes, withdraw a 10 ml aliquot from a zone midway between the surfaces of the dissolution medium and the top of the rotating basket, not less than 1 cm from the vessel wall. Filter the sample through a 0.45 µm filter, discarding first few mls of filtrate. Measure the absorbance of the resulting standard and sample solutions at 238 nm in a 1 cm cell using 0.1 M HCl as the reference.

Method Development

The following studies were conducted for this purpose:

Detection of wavelength

The spectrum of 10 ppm solution of brazepam was recorded separately on UV spectrophotometer. The peak of maximum absorbance wavelength 238nm was observed.

Choice of Stationary and Mobile phase

Finally the expected separation and peak shapes were obtained on Kromasil C_{18} column 250 x 4.6 mm ID with 5 µ particle size.

Flow rate

Flow rates of the mobile phase were changed from 0.5-1.5 ml/min for optimum separation. It was found from experiments that 1.0ml/min flow rate was ideal for elution of analyte.

Method development & Validation 0f the proposed method

The analytical performance of the method of analysis was checked for specificity, System suitability, detection limit, and method precision.

Specificity

The spectra of solutions listed below were run using the conditions specified in the method of analysis. The solvent and placebo solution spectra must not show any absorbance corresponding in wavelength to that of the active compound. The product does not to have any absorbance at specified wavelength corresponding to Bromazepam therefore it is considered spectrally pure.

Linearity

The correlation coefficient of the regression line for Bromazepam should be greater than or equal to 0.99. The Y-intercept of the line should not be significantly different from zero, i.e. the assessment value (z) falls within the specified limits only when + 5 > z > - 5. Five solutions containing 25, 50, 75, 100, and 125 % of Bromazepam, relative to the working concentrations, were prepared and read according to the method of analysis. A linear regression curve was constructed, and the correlation coefficients (R2) and assessment values calculated. The correlation coefficient (R2) for Bromazepam is 1.00.The plot is a straight line and the assessment value (z) is 3 for Bromazepam. Results are shown in the Table:1. And Linearity curve was shown in the Fig:2.

CALIBRATION CURVE: y = Bx + A, R = coeff. of determination

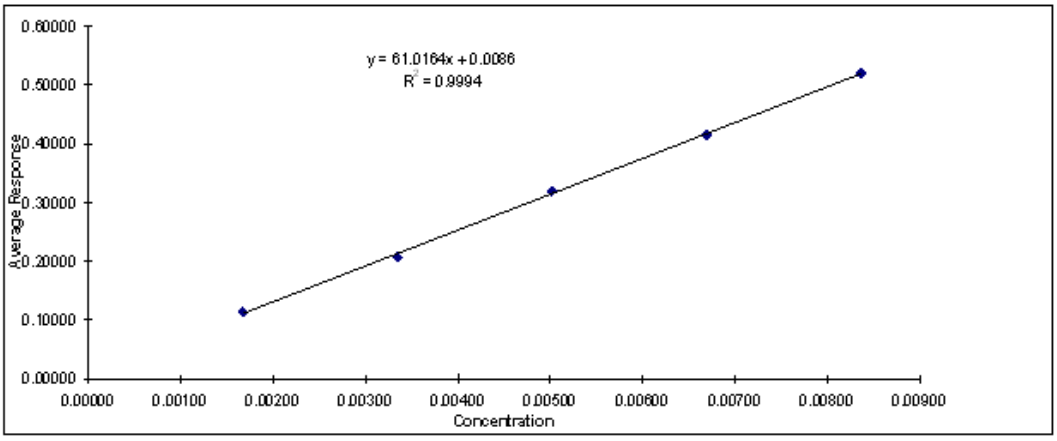

Fig:2 Calibraiton Curve

Table: 1 Linearity curve Results

Sample No	Concentration	Response 1	Response 2	Average Response
25%	0.00167	0.1130	0.1130	0.11300
50%	0.00335	0.2070	0.2070	0.20700
75%	0.00502	0.3200	0.3190	0.3195

100%	0.00669	0.4160	0.4130	0.4145
125%	0.00836	0.5190	0.5200	0.5195

Accuracy

The percentage recovery of the active compound, for each solution prepared, must be within 90.0 – 110.0 % of the actual amount. Sample solutions were weighed with known concentrations of Bromazepam to result in concentrations representing 25, 50, 75, 100, and 125 % of Bromazepam relative to the working concentration. The absorbance of the above samples was read in duplicate according to the method of analysis. From the accuracy results above, the percentage recovery values for Bromazepam satisfy the acceptance criteria for accuracy across the range of 25 - 125 %. Results are shown in the Table:2.

Table:2 Accuracy Results

Sample %	Theoretical Active	Absorbance	Actual Active	% Recovery	Average % Recovery
25	25.1	0.112	27.1	108.0	109.0
25		0.114	27.6	110.0	
50	50.2	0.209	50.6	100.8	100.5
50		0.208	50.3	100.2	
75	75.3	0.322	77.9	103.5	103.5
75		0.322	77.9	103.5	
100	100.4	0.416	100.6	100.2	100.0
100		0.414	100.2	99.8	
125	125.5	0.521	126.0	100.4	100.4
125		0.521	126.0	100.4	

Method Precision

The precision of an analytical procedure expresses the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of a homogenous sample.

Repeatability

This parameter determines the repeatability of assay results under the same operating conditions over a short period of time. The % RSD due to Bromazepam concentration for the six samples must be less than or equal to 5.0 %. Six separate sample preparations of batch 300023 were analysed according to the method of analysis. The % RSD due to Bromazepam concentration for the dissolution is 2.1 % and it meets the requirements. The results are shown in the Table: 3.

Table: 3 Repetability Results

Sample number	Results (%)Bromazepam
1 2 3 4 5 6	100.0 100.0 105.0 100..0 100.0 99.0
Mean	100.7
% RSD	2.1

4.4.2 Intermediate Precision

Intermediate Precision of an analytical procedure expresses intra-laboratory variations of the repeatability test performed:by a different analyst, on a different day, Using different reagents and solvents. The % RSD due to Bromazepam concentration for the six samples must be less than or equal to 5.0 %. The mean results obtained in the repeatability, and the intermediate precision must not differ by more than 5.0 %. Six separate sample preparations of batch 300023 were analysed according to the method of analysis. The % RSD for intermediate precision is 1.2 %. The intermediate precision and repeatability comply as they differ by 1.4 %. The results are shown in the Table 4.

Table: 4 Intermediate precision Results

Sample	Results (%)Bromazepam
1 2 3 4 5 6	100.3 100.1 97.9 97.6 98.4 98.1
Mean	98.7
% RSD	1.2
Sample	Results (%)Bromazepam
Repeatability Intermediate Precision	100.7 98.7
Mean	99.7
% RSD	1.4

Range

Range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. Based on the accuracy, and linearity results, the range for the dissolution of Brazepam 6mg tablets is 25 -125 % of Bromazepam, which represents 25 - 125 % of the working concentration.

Declaration on the Validity Of The Method

The method for the Dissolution of Brazepam 6mg tablets complies with the requirements for linearity, specificity, method precision and accuracy across the range of 25 % to 125 %. The method is therefore acceptable as valid.

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