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A RAPID AND VALIDATED REVERSE PHASE LIQUID CHROMATOGRAPHIC METHOD FOR DETERMINATION OF BROMAZEPAM AND RELATED IMPURITIES FROM TOPICAL TABLET FORMULATIONS

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ABSTRACT

A simple reverse phase HPLC method was developed and validated for the determination of Bromazepam and his decomposition products present in pharmaceutical dosage form. A Lichrospher 100 RP 8 column (250 × 4.0 mm, 5µm) is used as stationary phase. An isocratic mode with mobile phase consisting of methanol and Potassium dihydrogen phosphate buffer (KH_2PO_4) (pH 7.0; 0.02M) in ratio of 55:45 (v/v) at a flow rate of 0.9 ml/min and effluent was monitored at 238 nm. Chromatogram showed a peak of Bromazepam (BZP) at retention time of 8.36 ± 0.1 min. The linearity range was found to be 84–156 µg/ml of Bromazepam with correlation coefficient of 0.9997. The method was validated for linearity, accuracy, precision, limit of quantitation, limit of detection and robustness. Recovery of BZP was found to be in the range of 98.6–101.2%. The limit of detection and limit of quantitation for estimation of BZP was found to be 0.15µg/ml and 0.51µg/ml, respectively. This showed that proposed method is rapid, simple, precise, linear, robust, and accurate which is useful and economic for routine analysis of BZP in pharmaceutical dosage forms.

KEYWORDS

Bromazepam, HPLC, Pharmaceutical dosage form, Validation

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INTRODUCTION

Bromazepam (3-dihydro-5 (2-pyridinyl)-1H-1, 4-benzodiazepine-2-one), Figure. No.1 is a benzodiazepine (BZD) generally used for a number of medical reasons. Chemically it is 7-Bromo-2.The molecular formula is $C_{14}H_{10}BrN_3O$ and molecular weight is 316.153. The Bromazepam is an intermidiate- acting tranquiliser, prescrebed for the treatment of moderate to severe anxiety and panic attacks for the short-term treatment of insomnia[1,2,3,4].

Several methods for the analysis of BZDs have been reported.[5,6] A number of chromatographic methods, such as UV Spectroscopy[7] , gas chromatography[8], gas chromatography–mass spectrometry (GC–MS),[9] and high-performance liquid chromatographic –mass spectrometry (LC–MS)[10,] have been used in the analysis of Bromazepam and other 1, 4-benzodiazopines. Several high-performance liquid chromatographic (HPLC) methods have also been reported for the determination of Bromazepam and other BZDs[11,12]. However, all of these methods have limitations such as long run times and/or expensive. The present study focused on minimizing these limitations and to develop a simple precise accurate and economic method for estimation of Bromazepam in tablet dosage form.

MATERIAL AND METHOD

Chemicals and reagents

An analytically pure sample of Bromazepam was procured as gift sample from Laboratory pharmaceutical (Morocco). Methanol (HPLC grade) was procured from Merck Specialist. Ultra-pure water (HPLC-grade) was obtained from Merck. Potassium dihydrogen phosphate (AR grade, purity 99.6%) was procured from Merck. Tablet formulations (Laboratory in Morocco.) were procured from a local pharmacy with labeled amount 6 mg per tablet. Other chemicals used were of analytical grade.

Instrumentation & chromatographic conditions

The system used for quantification of Bromazepam consisted of a LaChrom L-7100 Merck Hitachi Pump, LaChrom L-7200 Merck Hitachi Autosampler and LaChrom L-7400 Merck Hitachi UV Detector. The chromatogram peaks were quantified by means of PC Multi- System Manager Software (Merck- Hitachi Model D-7000). Chromatography separation for analyte was achieved on Lichrospher 100 RP 8 analytical column with 250 × 4.0 mm i.d. and 5 μm particle size maintained at ambient temperature. The mobile phase consist of Methanol, KH_2PO_4 (pH 7.0; 0.02M) in ratio of 55:45 (v /v) that was set at a flow rate of 0.9 ml/min. The injection volume was 20 μl, and a chromatographic peak was detected at 238 nm.

Preparation of mobile phase

Mobile phase was a mixture of 550 ml of Methanol and 450ml of Potassium dihydrogen phosphate 0.02M adjusted to pH 7.0 with KOH 0.5M. Filtered through a 0.45 μm nylon filter and degassed for 5 min using an ultrasonicator.

Preparation of standard solution

Accurately weighed about 120 mg of Bromazepam standard was taken in a 100 ml volumetric flask and was dissolved in 20ml with mobile phase. About 75 ml diluent was added and mixture was dissolved by sonication and it was diluted up to mark with mobile phase. 5 ml of this solution was further diluted to 50 ml with mobile phase.

Preparation of sample solution

Five tablets of Bromazepam hydrochloride were weighed and transferred into a 250 ml volumetric flask and was dissolved with mobile phase. After 10 min, 150ml of mobile phase was added and the mixture was sonicated for 30 min with intermittent shaking and then cooled at room temperature. The resulting solution was diluted with mobile phase up to the mark. The solution thus prepared was filtered through 0.45 μm membrane filter and the resulting filtrate was sonicated for 10 min.

After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the sample solution was loaded in the 20 μl fixed – sample loop of the injection port.

METHOD VALIDATION

Specificity

Specificity of proposed method was determined by checking blank and placebo interference at the retention time of Bromazepam peak. Identification of Bromazepam peak in sample solution was confirmed by comparing retention time of Bromazepam peak with retention time of solution standard of Bromazepam. Also Bromazepam peak was checked for peak purity using Photo diode array detector (PDA).

Linearity

Linearity of the method was evaluated by using 5 linearity solutions of different concentrations. Accurately measured aliquots of solution standard were taken in five different 100 mL volumetric flask and diluted up to the mark with the mobile phase such that the final concentrations of Bromazepam were 84 μg ml^{-1}, 102 μg ml^{-1}, 120 μg ml^{-1}, 138 μg ml^{-1} and 156 μg ml^{-1}. A 20 μl aliquot of each linearity solution was injected in Triplicate[13].

Instrumental precision

The instrumental precision was checked by injecting six replicates of solution standard containing Bromazepam (120.0 μg ml^{-1}) and calculated the percentage RSD of retention time and area responses of Bromazepam.

Method precision (repeatability)

The method precision of the proposed method was determined by preparing six different sample solutions of same batch and analyzed against standard solutions. Assay values of these all six samples were calculated.

Accuracy

The accuracy of the method was determined by calculating recoveries of Bromazepam by the standard addition method. Known amount of standard of Bromazepam was spiked to placebo in three different levels (70%, 100% and 130% of sample concentration) and prepared three spiked samples of each level (Total 9 determinations as per ICH guideline.) These spiked samples were analyzed against solution standard and the amount of Bromazepam recovered in three different levels was calculated.

Intermediate precision (reproducibility)

The intermediate precision of the proposed method was evaluated by preparing six different sample solutions of same concentrations as prepared in method precision and analyzed against standard solutions on different days. Assay values of all the six samples were calculated.

Robustness

Robustness of method is its ability to remain unaffected by small changes in method parameters. Robustness of proposed method was demonstrated by making slight changes in method parameters like flow rate (±5%), column temperature (±2 °C), detection wavelength (±5 nm), mobile phase composition (±5% organic phase) and used different lot of column.

Solution stability

The solution stability of sample solution and standard solution were evaluated by comparison of assay value of freshly prepared samples and stored samples (at room temperature for 48 h). Standard solution and sample solution were prepared as mentioned in chromatographic conditions. Sample solution was analyzed and assay value was calculated against standard solution. Both the solutions (standard and sample solution) were kept at room temperature for 12 h. After 12 h these stored samples were reanalyzed against freshly prepared standard solution and the assay values were compared[14].

RESULTS AND DISCUSSION

To optimize chromatographic parameters several mobile phase compositions were tried in this method. A satisfactory separation, good peak symmetry and to achieve good retention time was obtained with mobile phase consisting a mixture of Methanol, Potassium dihydrogen phosphate buffer (KH_2PO_4) (pH 7.0; 0.02M) in ratio of 55: 45 (v/v) that was set at a flow rate of 0.9 ml/min was found to be optimum and further optimized by adjusting pH 7.0 by adding KOH 0.5M. The suitability of the mobile phase decided on the basis of the sensitivity of the assay, time required for the analysis, ease of preparation, and use of readily available cost effective solvents. The composition of Methanol, Potassium dihydrogen phosphate buffer (pH 7.0; 0.02M) in ratio of 55: 45 (v/ v) gave the best results. The proposed method was validated as per ICH guidelines with respect to specificity, linearity, accuracy, precision, robustness, solution stability and filter paper compatibility. All results of validation parameters meet the limits of ICH guidelines.

Validation of the Proposed Method

The method was validated as per ICH guidelines. The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, system suitability, limit of detection, limit of quantification, and solution stability.

Specificity

The chromatograms of blank, placebo of Bromazepam tablet and Bromazepam are shown in Figure No.2 respectively and it were observed that there was no interference from blank and placebo at the retention time of Bromazepam peak. Retention time of Bromazepam peak in sample solution matches the retention time of Bromazepam peak in standard solution. These results indicate that proposed method gives uniform and pure peak of Bromazepam.

Linearity

A excellent linearity was successfully achieved in the concentration range of 84µg/ml to 156 µg/ml The regression equation and correlation coefficient was found to $y = 112448\mathbf{x} + 171267$ and $R^2 = 0.9997$. Linearity curve of Bromazepam is shown in Figure No.3.

Instrumental precision

The percent relative standard deviation (RSD) for six replicate of standard solution was found to be 0.12% and 1.01% for retention time and area response respectively. Results are shown in Table No.1 and Chromatogram of injection of standard solutions of Bromazepam are shown in Figure No.4.

Method precision

Percent relative standard deviation (RSD) of Assay values for six samples were found to be 0.77%. The low RSD values indicate that the proposed method is precise or repeatable. Results of Method precision are shown in Table No.2 and chromatogram of injection of sample solutions are shown in Figure No.5.

Accuracy

The percentage recoveries of Bromazepam from tablet samples were calculated. Recovery ranged between 98.6% and 101.2%. Results of recovery experiment are shown in Table No.3.

Intermediate precision or reproducibility

%RSD of assay values of 12 samples (method and intermediate precision sample) were found to be 0.90%. The closeness of assay results and percent RSD values indicate that the proposed method is reproducible.

LOD and LOQ

LOD and LOQ for Bromazepam were estimated by injecting a series of dilute solutions with known concentration[15]. The parameters LOD and LOQ were determined on the basis of peak response and slope of the regression equation. The LOD and LOQ of the drug were found to be 0.15µg/ml and 0.51µg/ml respectively.

Robustness

It was observed that by making changes in chromatographic parameters, absolute difference between percent assay under altered condition and mean percent assay obtained during repeatability was not more than 2.0%. %RSD of area response and retention time was below 2%. The results of Robustness evaluation are shown in Table No.4.

Solution stability

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 48 h at an interval of 12 h at room temperature. The results show that for solutions, the retention time and peak area of Bromazepam hydrochloride remained unchanged and no significant degradation within the indicated period, this indicates that both solutions were stable for 24h.

Table No.1: Instrumental precision results of Bromazepam

S. No	Concentration (µg/ml)	Retention time (min)	Area response
1	126.5	8.38	13811715
2	126.5	8.37	14105656
3	126.5	8.35	13783168
4	126.5	8.36	13719172
5	126.5	8.36	13765206
6	126.5	8.36	13773083
Mean		8.363	13826333.3
%RSD		0.12	1.01

Table No.2: Method Precision results of Bromazepam

S .No	Concentration (µg/ml)	Area response
1	117.65	12859161
2	118.58	12960799
3	119.95	13110557
4	119.24	13032327
5	120.14	13131176
6	119.27	13036621
Mean		13021773.5
%RSD		0.77

Table No.3: Recovery results of Bromazepam

S. No	Accuracy level	Sample preparations	Added amount of Bromazepam (mg ml^{-1})	Recovered amount of Bromazepam (mg ml^{-1})	%Recovery	Mean % recovery	% RSD
1	Accuracy (70%)	Preparation-1	0.0845	0.0838	99.17	99.52	0.72
		Preparation-2	0.0858	0.0861	100.35		
		Preparation-3	0.0842	0.0834	99.05		
2	Accuracy (100%)	Preparation-1	0.1245	0.1251	100.48	99.52	0.97
		Preparation-2	0.1250	0.1232	98.56		
		Preparation-3	0.1271	0.1265	99.53		
3	Accuracy (130%)	Preparation-1	0.1562	0.1581	101.22	99.65	1.37
		Preparation-2	0.1538	0.1523	99.02		
		Preparation-3	0.1547	0.1527	98.71		

Table No.4: Results of robustness study

Method parameter	Altered condition	%Assay	%RSD
Flow rate	0.945 ml min^{-1}	99.45	1.15
	0.90 ml min^{-1}	99.28	0.77
	0.855 ml min^{-1}	100.15	0.84
Temperature	27 °C	99.56	1.20
	25 °C	99.28	0.77
	23 °C	99.83	0.96
Wavelength (nm)	243 nm	98.95	0.98
	238 nm	99.28	0.77
	233 nm	99.82	1.26
Mobile phase composition (Methanol: KH_2PO_4 (pH 7.0; 0.02M) (v/v)	50: 50	99.34	1.32
	55: 45	99.28	0.77
Columns	60: 40	100.13	0.89
	Lot-1	99.28	0.77
	Lot-2	99.48	1.12

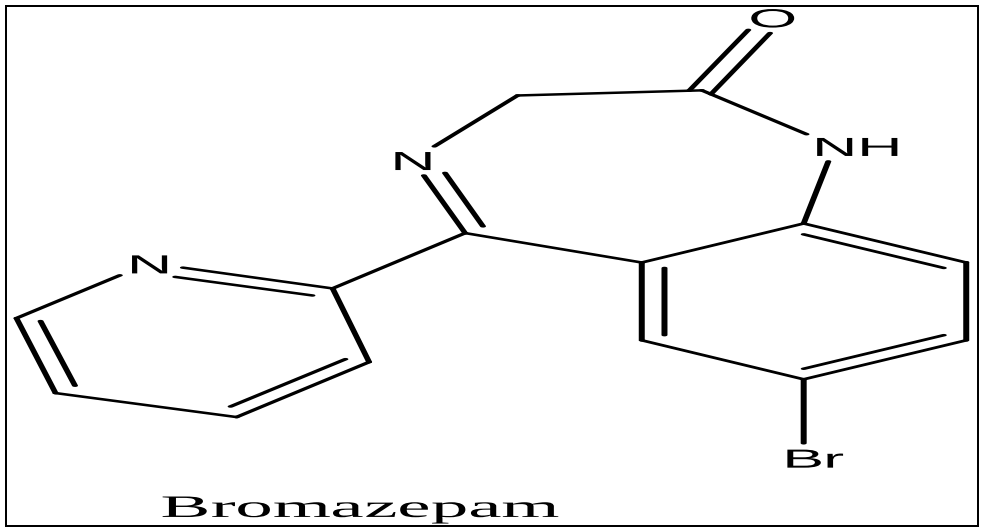

Figure No.1: Structure of Bromazepam

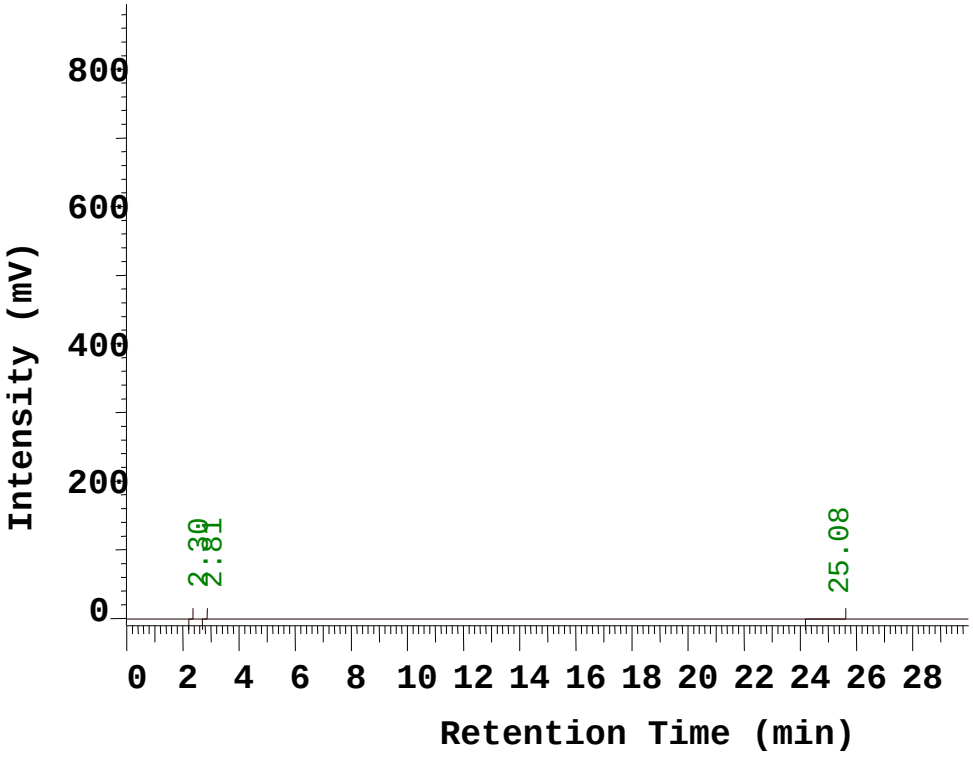

Figure No.2a: Chromatogram of blank

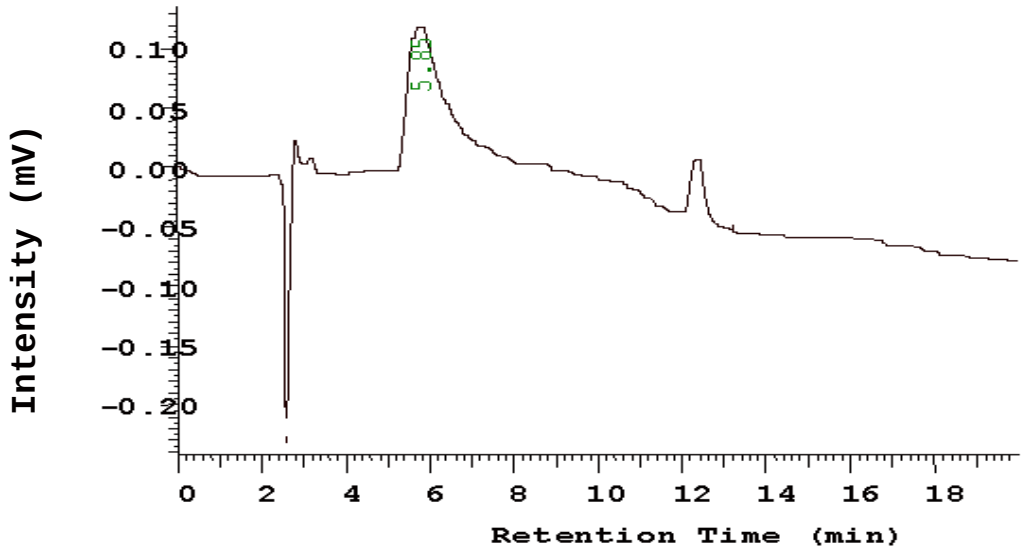

Figure No.2b: Chromatogram of placebo of Bromazepam tablet

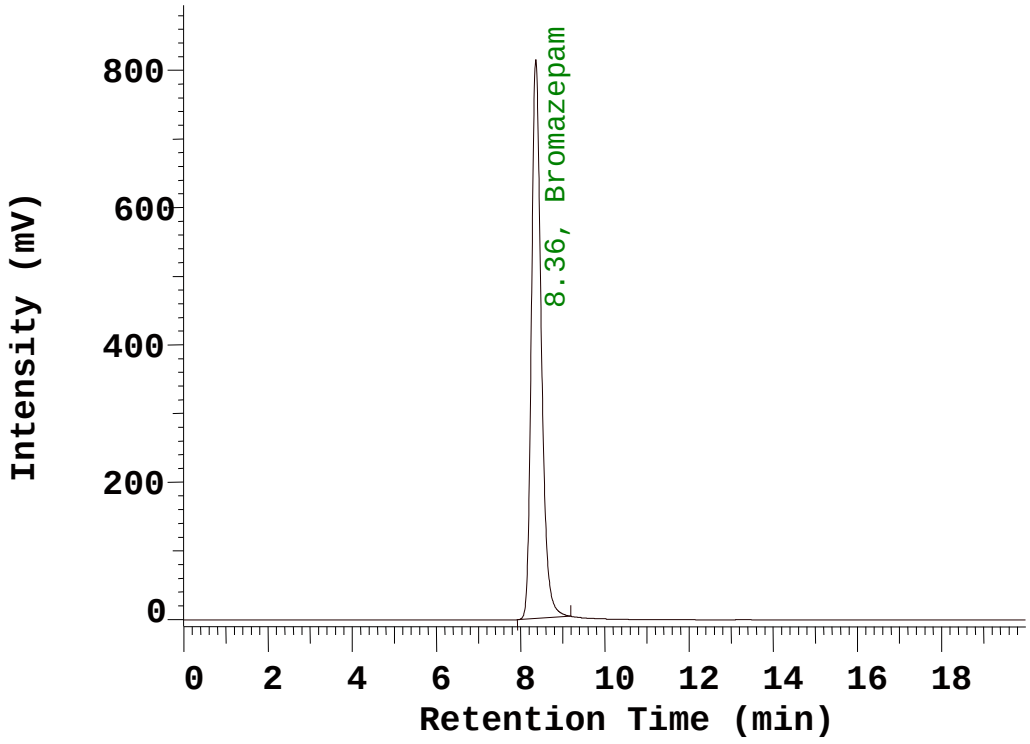

Figure No.2c: Chromatogram of Bromazepam

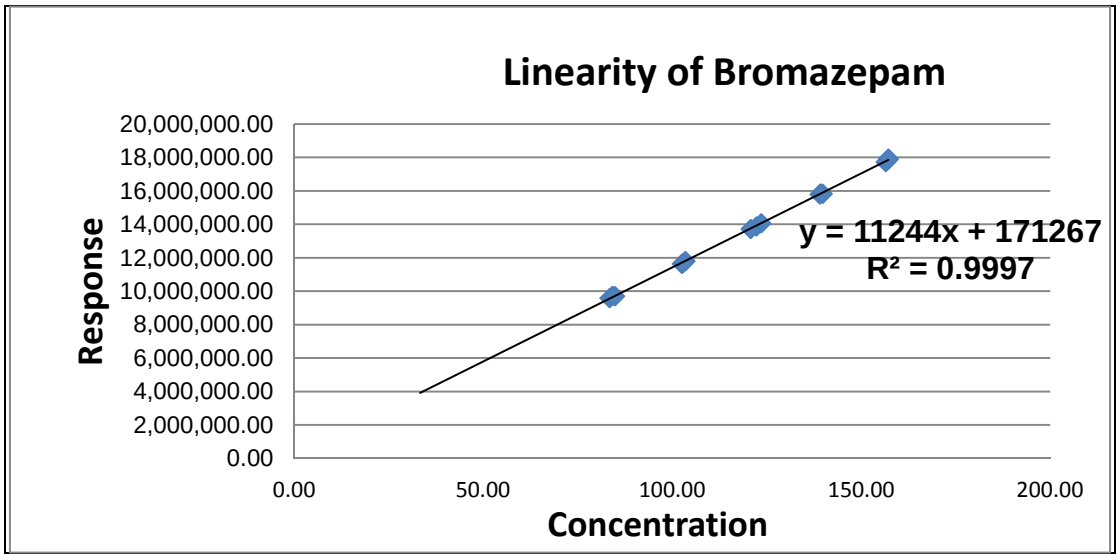

Figure No. 3: Linearity curve of Bromazepam

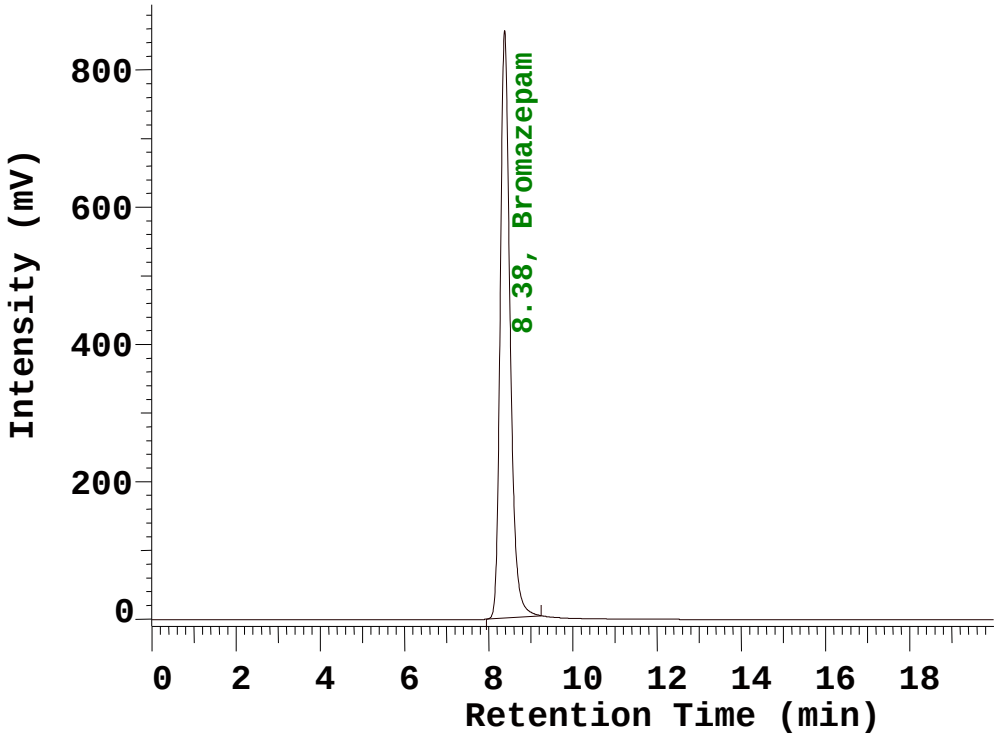

Figure No.4: Chromatogram of injection of standard solution of Bromazepam

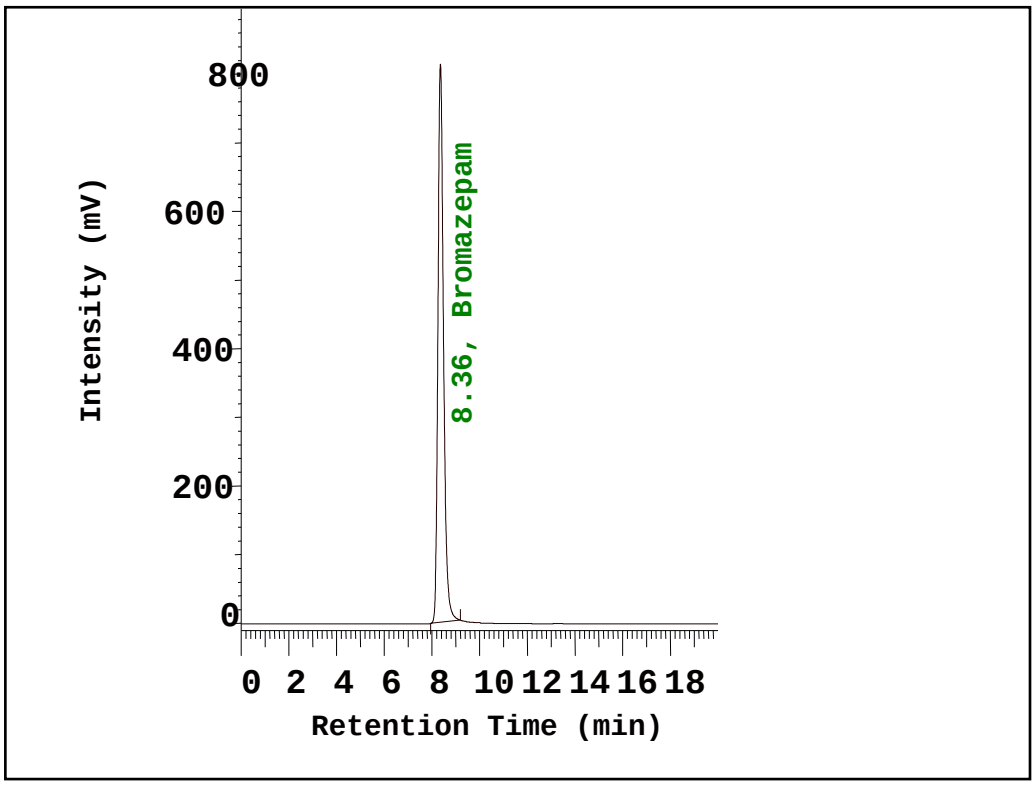

Figure No.5: Chromatogram of injection of sample solutions

CONCLUSION

A validated RP-HPLC method has been developed for the determination of Bromazepam in tablet dosage form. The proposed method is simple, rapid, accurate, precise, and specific without interference of excipients. Its chromatographic run time of 8.36 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of Bromazepam in pharmaceutical dosage forms. So it could be used for the rapid and reliable determination of Bromazepam in tablet formulations.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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