AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Method Development and Validation of Clobazam In Bulk and Pharmaceutical Dosage Forms by Using RP-HPLC Method

Senthilkumar. GP[1]*., Parag. S. Mahadik[1], S. Parthiban[1], T. Tamizhmani[1]
1.Department of Pharmaceutical Chemistry, Bharathi college of Pharmacy, Bharathinagara, Mandya- Dist, Karnataka, 571422, India.

ABSTRACT

Clobazam is an antiepileptic drug. There have been very less number of analytical methods developed for estimation of Clobazam in pure bulk form and in dosage form. In the present research a simple, accurate, precise and cost effective High performance liquid chromatographic method for the estimation of Clobazam, in bulk and pharmaceutical dosage form was carried out. HPLC method was developed on a Symmetry C-8 (4.6×150mm), 3.5µm particle, reversed-phase column. The mobile phase was acetonitrile: phosphate buffer (0.05M, pH- 4.5), 60:40 (v/v) at a flow rate of 0.8ml/min. The eluate was monitored at 231 nm. The method was validated reaching satisfactory results for selectivity, precision and accuracy. The retention time of the drug was found to be 3.38min in the mobile phase, acetonitrile: 0.05M potassium dihydrogen orthophosphate buffer (pH-4.5) 40: 60 (v/v). A linear response was observed in the range of 20-60µg/ml with a regression coefficient of 0.999. Validation parameters were carried out as per the guidelines of International Conference for Harmonization (ICH). This method can be used in the industries for determination of Clobazam to analyze the quality of formulation without interference of the excipients.

Keywords: Clobazam, Anti-epileptic, High performance liquid chromatography, ICH.

*Corresponding Author Email: drsenn78@yahoo.com
Received 14 May 2016, Accepted 25 May 2016

Please cite this article as: Senthil GP *et al.*, Method Development and Validation of Clobazam In Bulk and Pharmaceutical Dosage Forms by Using RP-HPLC Method. American Journal of PharmTech Research 2016.

INTRODUCTION

Clobazam is an antiepileptic drug belonging to the benzodiazepine series coming under the class of Anticonvulsant drugs and chemically called as 7-chloro-*ter*-methyl-5-phenyl-1H-1-5-benzodiazepine. Several HPLC methods have been reported for the determination of clobazam and its metabolite in human serum and plasma[1-6]. Some of the HPLC method and colorimertic methods has been developed for the determination of clobazam in tablet dosage forms [7,8]. Hence, it is important to develop an accurate, rapid and specific stability indicating analytical method, which is suitable for routine quality control analysis of clobazam in pharmaceutical dosage forms. In present study, reversed-phase HPLC method is proposed for the determination of clobazam in bulk drug and pharmaceutical dosage forms according to the International Conference on Harmonization (ICH) guidelines.

MATERIALS AND METHOD

Instruments

Chromatography was performed, under ambient conditions, with Waters alliance 2695 seperation module (Waters Corporation, Milford, USA) equipment comprising a variable wavelength Waters 2487 dual λ absorbance UV detector with empower-2 software was used for the analysis. Samples (20µl) were injected by means of a Rheodyne injector fitted with a 20µl loop. Compounds were separated on a Symmetry C-8 (4.6×150mm), 3.5µm particle, reversed-phase column. The mobile phase was acetonitrile: phosphate buffer (0.05M, pH- 4.5), 60:40 (v/v) at a flow rate of 0.8ml/min. The eluate was monitored at 231 nm.

Chemicals

Clobazam was obtained from Sanofi-Aventis, Ltd. Goa, India. HPLC-grade Acetonitrile was purchased from Qualigens fine chemicals, India. High-purity water was prepared using Millipore purification system. Other chemicals and reagents were of AR-grade.

Preparation of Solution

Preparation of working standard stock solution

About 10mg of Clobazam was weighed accurately and dissolved in 50ml of acetonitrile present in a 100ml volumetric flask. It was diluted up to the mark with acetonitrile to get the concentration of 100µg/ml. Resultant solution was filtered through Whatman filter paper No. 41.

Preparation of sample solution

Twenty tablets were accurately weighed and finely powdered. Tablet powder equivalent to 10mg of Clobazam was taken in 100ml of volumetric flask containing acetonitrile (approximately 50ml) and

was sonicated occasionally to dissolve the drug. The solution was filtered through Whatman filter paper (No. 41). The filter paper was washed with more solvent to obtain the concentration of 100µg/ml. The resulting solution was filtered through Whatman filter paper (No. 41).

Method Validation [9, 10]

The optimized chromatographic method was completely validated according to the procedures described in ICH guidelines Q2 (R1) and Q1 A (R2) for the validation of analytical methods and Stability Testing of New Drug, respectively.

Linearity

Appropriate aliquots of standard Clobazam stock solutions (100µg/ml) were taken in different 10ml volumetric flasks and resultant solution was diluted up to mark with diluents to obtain final concentration of drug solution. Calibration curve of Clobazam was constructed by plotting peak area *vs*. applied concentration of Clobazam. The slope, intercept and correlation coefficient were also determined.

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. To study the accuracy, twenty tablets of formulation were weighed and powdered and analysis of the same was carried out. Recovery studies were carried out by standard addition method by adding known amount of standard drug solution (50, 100 and 150%) to the sample solution and % Recovery was calculated.

Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra-day studies, six repeated injections of standard solution were made and the response factor of drug peak and % RSD were calculated. In the inter-day variation studies, six repeated injections of standard solution were made for six consecutive days and response factor of drug peaks was observed. From the data obtained, the developed method was found to be precise.

Detection (LOD) and Quantification (LOQ) Limits

The limit of detection (LOD) of the developed method was determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3:1). Based on the LOD strength, the LOQ values were calculated by multiplication with three times.

Ruggedness

Clobazam samples equivalent to 10mg were weighed and dissolved in a 100ml volumetric flask containing mobile phase (50ml), sonicate for 30min, and the final volume was made with mobile

phase. From the standard stock solution 4ml was pipette out and was placed into 10 ml volumetric flask and volume was made up to the mark with mobile phase. The samples were injected into the column.

Robustness

The samples were analyzed separately by slightly changes in the analytical methods such as by changing flow rate of mobile phase ±0.1ml and by changing ratio of organic composition of the mobile phase *i.e.* acetonitrile: buffer from ± 10%, the chromatograms were recorded. The retention time values were observed.

System-Suitability

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (t_R), number of theoretical plates (N) and tailing factor (T) were evaluated for six replicate injections of the drug at a concentration of 10µg/ml.

Analysis of Clobazam in Tablet

To determine the Clobazam content of the tablet (Frisium-5, Label claim-5mg), 240mg powdered tablet was dissolved with mobile phase to furnish 100ml stock solution. This solution was sonicated for 10min, then analyzed for drug content.

RESULTS AND DISCUSSION

Method development

Of several solvents and solvent mixtures investigated acetonitrile: phosphate buffer (pH- 4.5) 60: 40 (v/v) was found to furnish sharp, well-defined peaks with very good symmetry (1.5) and the t_R was 3.386 min (Figure. 1.). With methanol: phosphate (pH- 4.3) 50: 50 (v/v) as mobile phase t_R was 8.5min and peak shape and sensitivity were poor. Acetonitrile: triethylamine (pH- 8) 50: 50 (v/v) did not furnish sharp well defined peaks and other mobile phases tried either resulted in much lower sensitivity or did not give well defined peaks in a short time, and so were not considered.

Final decision on mobile phase composition and flow rate was made on the basis of peak shape (peak area, asymmetry, tailing factor), baseline drift, time required for analysis, and cost of solvent, and acetonitrile: phosphate buffer (pH- 4.5) 60: 40 (v/v) was selected as the optimum mobile phase. Under these conditions the retention time and tailing factor were 2.9 and 1.5, respectively.

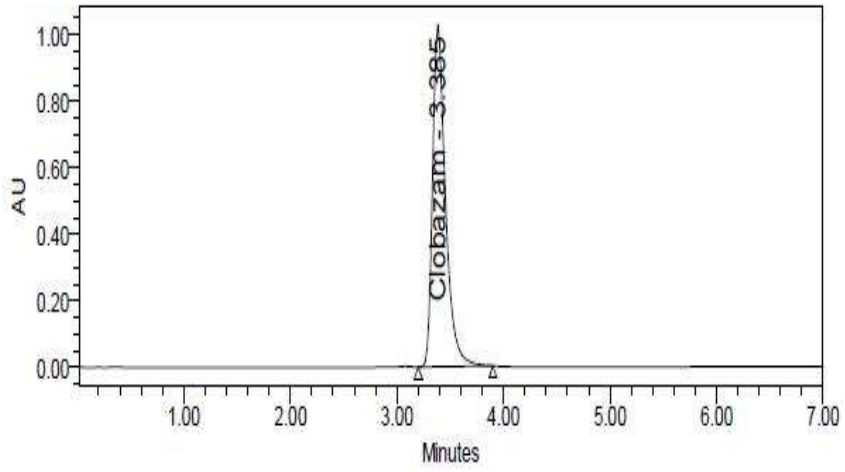

Figure 1: Chromatogram of Clobazam at 231 nm by RP-HPLC method in acetonitrile: phosphate buffer (pH- 4.5) 60: 40 (v/v)

Validation of the method

Linearity

The calibration curve was plotted as concentration against peak area and concentration range was maintained between 20-60µg/ml and results showed in Figure. 2. The linear regression equation was Y = 20038X +58343 and the regression coefficient was found to be 0.999 as mentioned in Table 1. The correlation coefficient was indicative of high significance.

Table 1: Linearity data for Clobazam with acetonitrile-phosphate buffer

Parameters	Clobazam
Linearity range	20-60µg/ml
Regression equation	y = 20038x + 58343
Correlation coefficient	0.999
Slope	20038
Intercept	58343

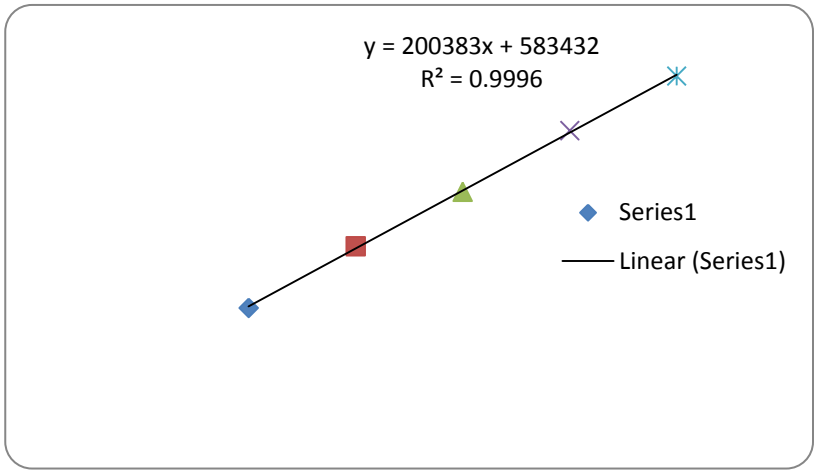

Figure 2: Calibration curve of Clobazam by HPLC with acetonitrile-phosphate buffer

Accuracy

The recovery of the method, determined by spiking a previously analyzed test solution with additional drug solution, was found between the acceptable limit of 98.00% and 102%. The values of recovery (%) and RSD (%) listed in table 2 indicate method is accurate.

Table 2: Accuracy studies of Clobazam

Level of recovery (%)	Amount of drug added (mg)	Amount of drug Recovered (mg)	% Recovery ± SD*
50	5	5.06	101.38 ± 0.010
100	10	9.83	98.36 ± 0.038
150	15	14.89	99.31 ± 0.002

Precision

Precision was considered at two levels, *i.e.* intraday and interday, in accordance with ICH recommendations. Repeatability of sample injection was determined as intraday variation whereas intermediate precision was determined by measuring inter-day variation for six determination of Clobazam at 40µg/ml. Results from determination of intraday and interday precision, expressed as RSD (%), are listed in Table 3. The low values of RSD indicate the repeatability of the method.

Table 3: Intraday and interday precision studies of Clobazam

Sr. No	Concentration (µg/ml)	Intraday	Interaday
1	40	8597300	8393210
2	40	8598344	8325910
3	40	8595202	8242494
4	40	8598419	8362263
5	40	8600234	8344640
6	40	8591348	8382877
Avg.	-	8596808	8341899
S.D*	-	3140.86	54536.64
%RSD*	-	0.036535	0.0653768

Detection (LOD) and Quantification (LOQ) Limits:

The LOD and LOQ of the method, determined by signal to noise ratio and was found to be 0.005662 and 0.018873 µg/ml, respectively, which indicated the method can be used for detection and quantification of Clobazam over a wide range of concentrations.

Ruggedness

There was single peak observed at the retention time of Clobazam in tablet formulation. There was no interaction between Clobazam and excipients present in tablet. The RSD was found within the limit and are shown in Table 4.

Table 4. Ruggedness study of Clobazam

Sample	Label claim (mg)	Analyst I		Analyst II	
		Amt. found	% Recovery ± S.D**	Amt. found	% Recovery ± S.D**
Frisium	5	5.032	100.65 ± 0.0512	5.004	100.09 ± 0.08004

Robustness

The change in the retention time of Clobazam when the composition and flow rate of the mobile phase were changed was observed. It was found that there was no significance change in the retention time. The results are enlisted in Table 5a and 5b.

Table 5a. Robustness of the method by changing the composition of the mobile phase. (The concentration of the solution analyzed was 40µg/ml)

Sr. No	Change in organic composition	System suitability results		Retention time (min)
		Plate count	Tailing	
1	10% less	4317.2	1.5	3.829
2	*actual	4006.4	1.5	3.406
3	10% more	3656.9	1.4	3.079

Table 5b. Robustness of the method by changing the mobile phase flow rate. (The concentration of the solution analyzed was 40µg/ml)

Sr.No	Flow rate (ml/min)	System suitability results		Retention time (min)
		Plate count	Tailing	
1	0.7	4155.0	1.5	3.853
2	0.8*	4006.4	1.5	3.406
3	0.9	3793.8	1.5	2.992

System Suitability

The repeated change in the retention time, therotical plates and tailing factor for the same concentration was observed. The result of the system suitability is given in Table 6.

Table 6: System suitability results for Clobazam.

Parameters	Values	Required limits
Retention time (min)	3.079	RSD ≤ 1%
Theoretical plates (N)	3656.9	N > 2000
Tailing factor (T)	1.4	T ≤ 2

Analysis of Clobazam in Tablet formulation

A single peak was observed at the retention time of Clobazam when the tablet formulation was chromatographed. There was no interaction between Clobazam and excipients present in the tablet. The chromatogram of the tablet formulation is shown in Figure.3.

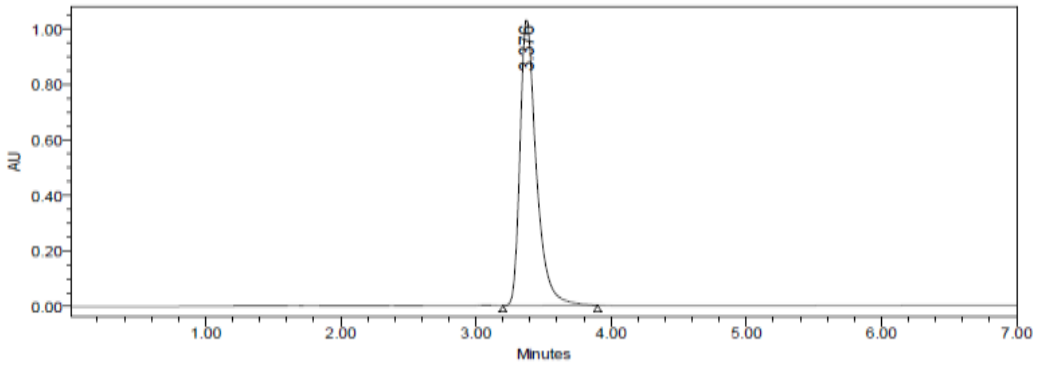

Figure 3: Chromatograph of the tablet formulation of Clobazam

CONCLUSION

For routine purposes it is always of interest to establish methods capable of analyzing a sample in a short period with due accuracy and precision. The main purpose of this study was to develop accurate, precise and economic method for the determination of Clobazam. High performance liquid chromatographic technique namely, was applied without using any prior chemical pretreatment. The proposed HPLC method is rapid, selective, simple, cost effective, fast and efficient. Finally, since there is only one Pharmacopoeial method for determination of Clobazam in bulk and pharmaceutical formulations have been reported yet, the proposed method could be useful and suitable for determination of Clobazam in bulk and pharmaceutical formulations.

ACKNOWLEDGEMENT

Authors wish to thank the Principal, Bharathi College of pharmacy, Bharathinagara for providing the facilities to carry out the research work.

REFERENCES

1. Knapp J, Boknik P, Gumbinger HG, Linck B, Luss H, Muller FU, Schmitz W, Vahlensieck U, Neumann J. Quantitation of clobazam in human plasma using high-performance liquid chromatography. J Chromatogr Sci 1999; 37:145-149.
2. Kunicki PK. Simple and sensitive high-performance liquid chromatographic method for the determination of 1, 5-benzodiazepine clobazam and its active metabolite N-desmethylclobazam in human serum and urine with application to 1, 4-benzodiazepine analysis. J Chromatogr B 2001; 750:41-49.
3. Bolner A, Tagliaro F, Lomeo A. Optimized determination of clobazam in human plasma with extraction and high-performance liquid chromatography analysis. J Chromatogr B 2001; 750:177-180.

4. Pistos C, Stewart JT. Direct injection HPLC method for the determination of selected benzodiazepines in plasma using a Hisep column. J Pharm Biomed Anal 2003; 33:1135-1142.
5. Rouini M, Ardakani YH, Hakemi L, Mokhberi M, Badri G. Simultaneous determination of clobazam and its major metabolite in human plasma by a rapid HPLC method. J Chromatogr B 2005; 823:167-171.
6. Mohammad A Abounassif, El-Rasheed. A, Gad Kareim and Hassan. Y. High performance liquid chromatographic determination of clobazam and one of its pharmaceutical formulations. J Pharmaceut Biomed 1987; 5(4): 431-434.
7. Gopalakrishnan S, Jeyashree B, Backialakshmi S, Chenthilnathan A. A new validated RP-HPLC method for the estimation of clobazam in tablet dosage form. Asian J Res Chem 2011; 4(6):882-886.
8. Parag S Mahadik, Senthilkumar GP, Devprakash Dahia, T Tamizh Mani, Priyanka K Gaikwad. Method development and validation of clobazam in bulk and pharmaceutical dosage forms by using spectrophotometric method. American Journal of Pharmtech Research 2011; 2(4): 248-257.
9. ICH Harmonised Tripartite Guideline, Q2(R1), Validation of Analytical Procedures: Text and Methodology. International Conference on Harmonization, Geneva. 2005; 1-13.
10. International Conference on Harmonization Guideline on Stability Testing of New Drug Substances and Products (2003) Q1 A (R2).