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Research paper

Method development and validation of clobazam in bulk and pharmaceutical dosage forms by using high performance thin layer chromatographic method

Chaithra. R. [1], Senthil Kumar. G. P. [1] *, Poojashree. P. [1], Vidya. D. N.[1], Bhadresh. D. R. [1], Manojkumar. S. [1]

[1] Department of Pharmaceutical Chemistry, Bharathi College of Pharmacy, Bharathinagara, Tal- Madhur, Dist.- Mandya, Karnataka, 571422, India
**Corresponding author E-mail: drsenn78@yahoo.com*

Abstract

In the present research a simple, accurate, precise and cost-effective High-performance thin layer chromatographic method for the estimation of clobazam, in bulk and pharmaceutical dosage form was illustrated. The RF value of the drug was found to be 0.74 in the mobile phase, acetone: toluene: formic acid (1: 1: 0.05 v/v/v). A linear response was observed in the range of 100-700 ng 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; λ max; ICH; High Performance Thin Layer Chromatography.*

1. Introduction

Clobazam is an antiepileptic drug belonging to the benzodiazepine series coming under the class of Anticonvulsant drugs and chemically called as 7-chloro-1,5-dihydro-1-methyl-5-phenyl-1,5-benzodiazepine2,4(3H)-dione (Fig 1). Clobazam is a long-acting 1,5-benzodiazepine with uses similar to those of diazepam as 1,4-benzodiazepine. It is used in the treatment of epilepsy in association with other antiepileptics. It is also used in the short-term treatment of acute anxiety [1-2]. Clobazam belongs to the 1,5-benzodiazepine class with a pk_a value of 6.65. Clobazam bulk powder is a white crystal with molecular weight of 300.7. the drug is slightly soluble in water and soluble in organic solvents [3]. The reference of Clobazam is not found in majority of pharmaceutical and chemistry books. clobazam is official in British and Indian pharmacopoeia 2007 [4-5]. Today majority of marketed antiepileptic dosage forms are of clobazam e.g. Frisium, Urbanyl, etc., There are several research papers which illustrates the method for estimation of clobazam by colorimetry and HPLC in bulk and pharmaceutical dosage form [6-7]. Also, there are several bio-analytical methods developed for clobazam in biological fluids containing clobazam, like serum and plasma [8]. There have been very a smaller number of analytical methods developed for estimation of clobazam in pure bulk form and in dosage form. In the present study method development and validation was carried out by HPTLC method [9].

7-chloro-1-methyl-5-phenyl-1H-1,5-benzodiazepine-2,4(3H,5H)-dione

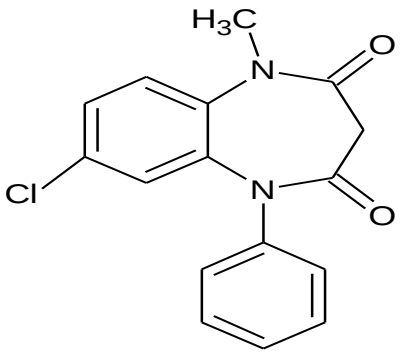

Fig. 1: Structure of Clobazam

2. Experimental

2.1. Chemicals

Clobazam was obtained from Sanofi-Aventis, Ltd. Goa, India. Acetone, toluene and formic acid were purchased from Qualigens fine chemicals, India. Chemicals and reagents were of AR-grade.

2.2. Chromatography

Analysis was performed on 10 × 10 cm aluminium backed silica gel F_{254} HPTLC plates (E-Merck, Darmstadt, Germany). Before using, the plates were predeveloped with methanol and then dried in an oven at 60 °C for 5 min. Standard solution and sample solution were applied to the plate as 6 mm bands by means of a Camag Linomat-V (Muttenz, Switzerland) sample applicator equipped with 100 µl syringe (Hamilton, Reno, Nevada, USA); the distance between the bands was 11.6 mm. Camag twin trough chamber used as development chamber previously saturated for 20 min with acetone: toluene: formic acid (1: 1: 0.05 v/v/v) as mobile phase. The average development time is 20 min. After development, the plate was dried at 110 °C in an oven for 10 min. Densitometric scanning at 254 nm was then performed with a Camag TLC Scanner equipped with Win-Cat software, version 1.3.0 using a Deuterium light source. The slit dimensions were 6.00 mm × 0.20 mm.

2.3. Optimization and detection of UV wavelength

The sensitivity of HPTLC method that uses UV/VIS detection depends upon the proper selection of detection wavelength. An ideal wavelength is one that gives good response for the drug that is to be analyzed. In the present study, by appropriate dilution of each stock solution, various concentrations of clobazam were prepared. Each solution was scanned in the spectrum mode and their spectra were observed. The wavelength selected for the analysis was 254 nm at which clobazam showed significance absorbance.

2.4. Preparation of stock solutions

Standard clobazam 10 mg was weighed and transferred to a 10 ml volumetric flask. 5 ml of methanol was added to dissolve the drug. The flask was shaken and volume was made up to the mark with methanol to give a solution containing concentration of 1000 µg/ml (stock solution A). From this stock solution, pipette out 1 ml and place it in 10 ml volumetric flask. The volume was made up to mark with methanol to give a solution containing concentration of 100 µg/ml (stock solution B).

2.5. Method validation [10]

2.5.1. Linearity

Standard solution equivalent to 100, 200, 300, 400, 500, 600 and 700 ng per band of clobazam were applied to a pre-developed HPTLC plate. The plate was developed, dried and scanned as described above. The chromatograms were obtained and peak area was determined for each concentrations of drug solution. A calibration plot was constructed by plotting peak area against amount of clobazam (ng). The linearity of response for clobazam was assessed in the concentration ranges 100-700 ng per band; the slope, intercept, and correlation coefficient were also determined.

2.5.2. Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection (LOD) and limit of quantitation of clobazam were determined by using standard deviation of the response and slope approach as defined as in International Conference on Harmonization (ICH) guidelines.
The LOD and LOQ were calculated by using following formulae

LOD = 3.3 × S.D of intercept/ Average of slope.

LOQ = 10 × S.D of intercept/ Average of slope.

2.5.3. Precision

Precision was evaluated by using standard solutions containing clobazam at concentration covering the 100, 200 and 300 ng per band. The precision of the method in terms of intra-day precision (% R.S.D) was determined by analyzing clobazam standard solution in the range (100-300 ng per band) three times on the same day. The inter-day precision (% R.S.D) was assessed by analyzing these solutions (100-300 ng per band) on three different days over a period of one week.

2.5.4. Accuracy

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

2.5.5. Reproducibility

The reproducibility of sample application was assessed by spotting drug solution (4 µl) six times on a HPTLC plate, then development of plate and recording the peak AUC.

2.6. Analysis of the marketed formulation

Twenty tablets [Frisium 5, Sanofi-Aventis, Ltd.] were weighed and finely powdered. The powder equivalent to 10 mg of clobazam was accurately weighed and transferred to 10 ml volumetric flask containing 5 ml methanol. The flask was shaken and volume was made up to the mark with methanol. The solution was filtered through Whatmann filter paper (No. 41) to give a solution of concentration 1000 µg/ml. From the above solution pipette out 1 ml and make up the volume to 10 ml with methanol to give a solution containing 100 µg/ml. From this solution, appropriate volume was injected to the TLC plate. The analysis was carried out by two analysts.

3. Results and discussion

3.1. Optimization of the procedure

The pure drug was applied to the TLC plates and chromatographed with different mobile phases. Initially ethyl acetate: chloroform (4: 6 v/v) and acetone: toluene (1: 1 v/v) were tried. Addition of 0.05 ml formic acid to these mobile phases improved the characteristics of the bands. Finally, the mobile phase acetone: toluene: formic acid (1: 1: 0.05 v/v/v) was found to enable good resolution with a sharp and symmetrical peak of R_F 0.74. Well defined bands were obtained when the chamber was saturated with mobile phase for 20 min at room temperature (Fig.2)

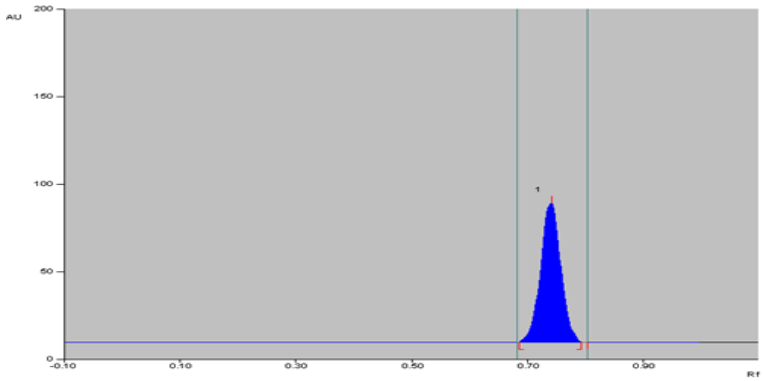

Fig. 2: Densitogram Obtained from Clobazam Standard by HPTLC Method.

3.2.1. Linearity

The calibration graph was linear, i.e. the system adhered to Beer's law, over the range 100-700 ng per band. Linearity was evaluated by duplicate analysis of seven standard working solutions equivalent to 100-700 ng per band of clobazam. The regression data showed linearity was good over the concentration range investigated; this was apparent from the high value of the correlation coefficient. Typical linearity data are given in Table 1.
The calibration curve (Fig. 3) and 3D view (Fig. 4) shows good correlation between clobazam concentrations and peak areas (Fig. 5).

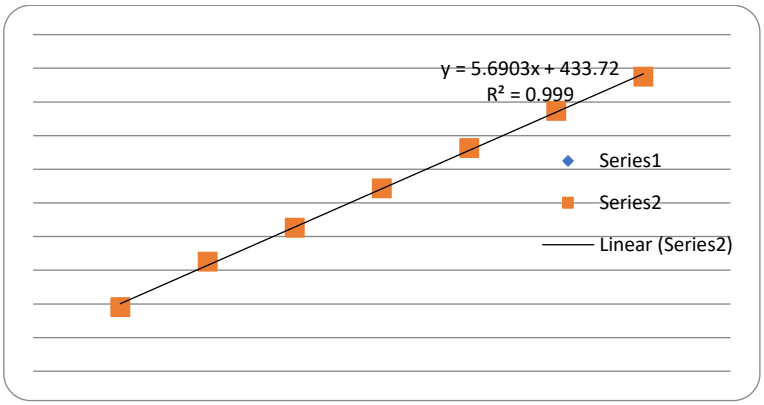

Fig. 3: Calibration Curve of Clobazam by HPTLC Method.

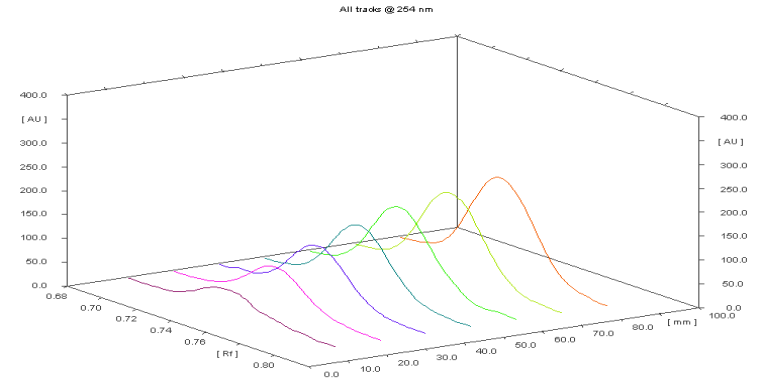

Fig. 4: 3D View of Clobazam by HPTLC Method.

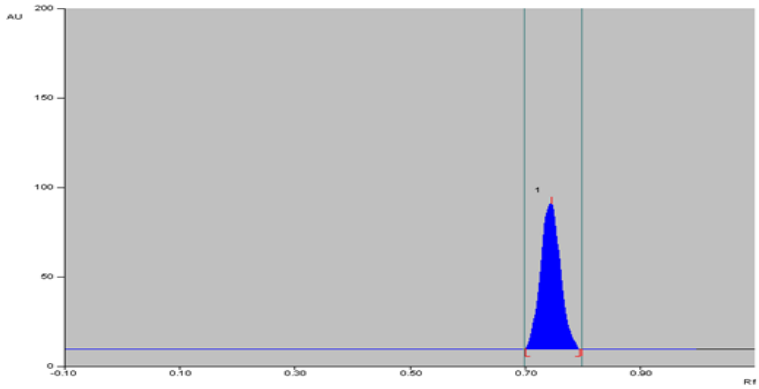

Fig. 5: Chromatogram of 400ng of Sample Solution of Clobazam by HPTLC Method.

Table 1: Linear Regression Data for the Calibration Plots

Regression data Value
Linear range 100-700 ng per spot
r^2 0.999
Slope 5.690
Intercept 433.7

3.2.2 Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ for the proposed method were found to be 20.98 and 63.56 ng per band, respectively.

3.2.3. Precision

Repeatability of sample application of peak area, as % R.S.D, was determined for concentrations 100, 200 and 300 ng. % R.S.D for interday and intraday analysis was <2%. The values are shown in Table 2.

Table 2: Results of the Measurement of Intra and Interlay Precision for Clobazam by HPTLC Method

Concentration (ng per spot)	Intra-day precision Mean ± S. D**	% R. S. D	Inter-day Precision Mean ± S. D**	% R. S. D
100	943.3 ± 15.42	1.635	945.26 ± 0.66	0.070
200	1618.6 ± 9.77	0.639	1658 ± 17.74	0.949
300	2199.3 ± 24.54	1.116	2215.4 ± 40.61	1.833

**Average of Three determinations.

3.2.4. Accuracy

When the method was used for subsequent analysis of clobazam in Pharmaceutical dosage form spiked with 50, 100 1nd 150 % extra drug, recovery was 98-102 % of clobazam as bulk and in dosage form (Table 3).

Table 3: Results of Accuracy Studies of Clobazam by HPTLC Method

Level of recovery (%)	Amount of drug added (mg)	Amount of drug recovered (mg)*	% Recovery ± S. D*
50	5	5.05	101.92 ± 0.479
100	10	9.95	99.55 ± 0.286
150	15	14.80	98.68 ± 0.917

*Average of three determinations

3.2.5. Reproducibility

The % R.S.D was calculated for peak area and R_F value with repeated determination for the same concentration. The studies were carried out using 400 ng as a target concentration. The reports are listed in Table 4.

Table 4: Reproducibility Results of Clobazam by HPTLC Method

Sl. No	Area	Rf
1	2749.8	0.74
2	2780.8	0.75
3	3 2734	0.74
4	2822.8	0.74
5	2839.8	0.74
6	2829.4	0.75
Mean	2792	0.74
S. D	44.49	0.0051
% R.S. D	1.593	0.6947

3.3. Analysis of marketed formulation

A single band at R_F 0.74 was observed in the densitogram of drug samples extracted from tablets. There was no interference from excipients commonly present in the tablets. The drug content was found to be 99.84% and 99.44 for analyst 1 and analyst 2, respectively. The results are reported in Table 5.

Table 5: Applicability of the Proposed HPTLC Method for Analysis of Commercial Tablets

Sample	Label claim (mg)	Analyst I		Analyst II	
		Amount found (mg)	% Recovery ± S.D**	Amount found (mg)	% Recovery ± S.D**
Frisium	5	4.99	99.84 ± 1.04	4.97	99.44 ± 1.271

**Average of three determinations

4. Conclusion

This HPTLC method for quantitative analysis of clobazam in Pharmaceutical formulations is cost effective, precise, accurate and reproducible without interference from the excipients. The method was validated in accordance with ICH guidelines. The method reduces analysis time compared with other methods and seems to be suitable for routine analysis of Pharmaceutical formulations in quality-control laboratories, where economy and speed are essential.

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