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RP-HPLC METHOD FOR QUANTITATIVE ESTIMATION OF CLOBAZAM IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A Simple, precise and accurate RP-HPLC method was developed and validated of clobazam in pharmaceutical dosage forms. Isocratic elution at a flow rate of 1ml min^{-1} was employed on a chromosil C18 column (250 mm x 4.6 mm, 5µ) at ambient temperature. The mobile phase consisted of Water: Methanol: Acetonitrile in the ratio of 55:25:20 (v/v/v). The UV detection wavelength was at 231nm. The retention time for clobazam was found to be 4.49min. Linearity was observed in the concentration range of 10-40ppm. The method was validated as per ICH guidelines.

KEY WORDS: Clobazam, Estimation, RP-HPLC, Validation.

INTRODUCTION

Clobazam, is a benzodiazepine derivative with chemical name 7-chloro-*ter*-methyl-5-phenyl-1H-1-5-benzodiazepine, (Figure 1) is a anxiolytic angent used in the treatment of epilepsy, anxiety and myoclonic seizures. Clobazam is available in oral form only due to its insolubility in water. Like other 1,5-benzodiazepines it has less affinity for the ω1-allosteric binding site on the $GABA_A$ receptor compared to the 1,4-benzodiazepines. It has selective affinity for the ω2 site, where it has agonistic activity. Clobazam binds to one or more specific GABA receptors at several sites within the CNS including the limbic system and reticular formation.

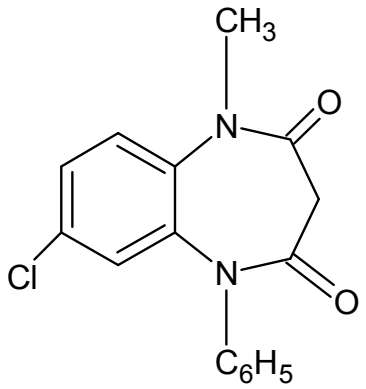

Figure 1 Chemical Structure of Clobazam

EXPERIMENTAL

Chemicals and solvents

HPLC grade Acetonitrile, HPLC grade Methanol and Orthophosphoric acid was used for mobile phase preparation. Pure sample of Clobazam was a gift sample from a local pharmaceutical industry. Commercial samples containing the drug Clobazam was purchased from the local pharmacy.

INSTRUMENTATION

The analysis of the drug was carried out with Peak HPLC LC 7000 series, 20AT pump and variable wavelength programmable UV-Visible detector, SPD-10AVP. A 20µL Hamilton syringe was used for injecting the samples. Chromatographic analysis was performed using Chromosil C18 column (250 mm x 4.6 mm, 5µ). A Denwar balance was used for weighing the materials. Isocratic elution with Water: Methanol: Acetonitrile in the ratio of 55:25:20 (v/v/v) was selected with a flow rate of 1.0 ml min^{-1}.The detection wavelength was set at 231nm with a runtime of 8 min. The mobile phase was prepared freshly and it was degassed by sonicating for 5 min before use. The column was equilibrated for at least 30min with the mobile phase flowing through the system. The column and the HPLC system were kept at ambient temperature.

PREPARATION OF SAMPLE

About 10 mg of clobazam was weighed accurately and transferred into a 100 ml volumetric flask and dissolved in 10 ml diluent. The solution was sonicated for 20 min and then the volume was made up with a further quantity of the diluent to get a 100ppm solution. Subsequent dilutions of this solution ranging from 10 to 40 ppm were made in 100 ml volumetric flasks with diluents. 20 µl of the solution was injected each time into the column, at a flow rate of 1ml/min. Each of the dilutions was injected 5 times into the column and the corresponding chromatograms were obtained. From these chromatograms, the retention times and the areas under the peaks of the drug were noted. The regression equation of the drug concentrations was computed. This equation was later used to estimate the amount of clobazam in pharmaceutical dosage forms.

CONCLUSION

A validated RP-HPLC method has been developed for the determination of Clobazam in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 8 min allows the analysis of a large number 10 of samples in short period of time. The results indicate that the described method can be used for quantitative analysis of the compound.

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Calibration of the proposed method

Linearity range (ppm)	10-40(ppm)
r^2 ± S.D.	**0.9998**

Table 1

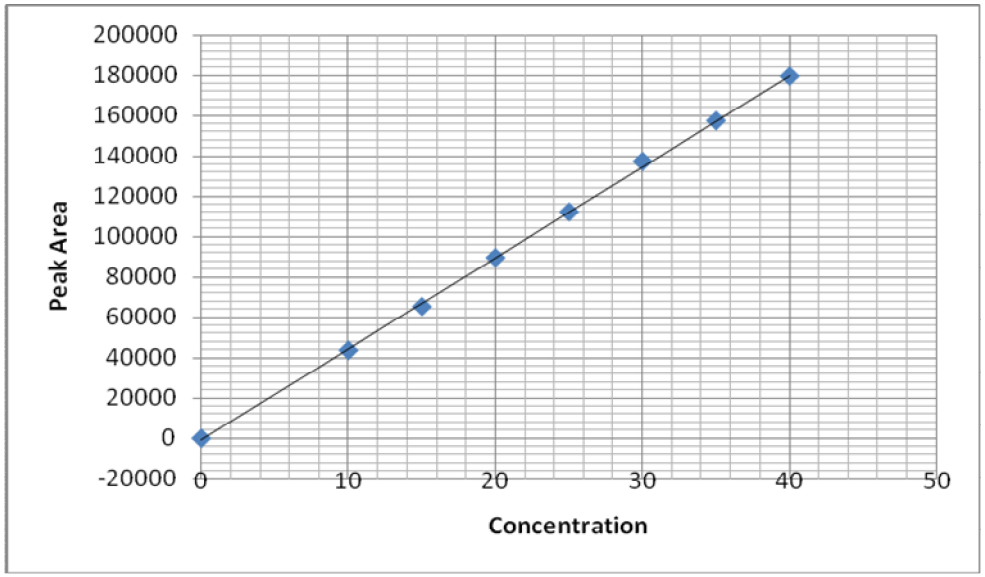

Figure 2 Linearity of clobazam

PRECISION

Injections	Concentration	Interday	Intraday
1	30 ppm	137921	137243
2	30 ppm	136825	137921
3	30 ppm	136681	137528
4	30 ppm	137925	137901
5	30 ppm	137029	137021
6	30 ppm	137718	137153
RSD		0.41	0.28

Table 2

ASSAY OF CLOBAZAM

Brand name	Dosage	Concentration	Amount found	% Assay
Frisium	10mg	30 ppm	29.78	99.27

Table 3

% RECOVERY OF CLOBAZAM

% Recovery	Clobazam				
	Target Conc., (ppm)	Spiked conc, (ppm)	Final Conc, (ppm)	Conc., Obtained	% of Recovery
50%	10	5	15	14.98	99.86
	10	5	15	14.89	99.27
	10	5	15	15.09	100.62
100%	10	10	20	19.83	99.15
	10	10	20	19.78	98.90
	10	10	20	20.06	100.32
150%	10	15	25	24.54	98.17
	10	15	25	25.39	101.57
	10	15	25	25.25	101.00

Table 4

ROBUSTNESS

S.NO	Parameter	Change	Area of Clobazam	% Change of Clobazam
1	MP	Water: Methanol: Acetonitrile 50:30:20 (v/v/v)	139001	1.60
		Water: Methanol: Acetonitrile 60:20:20 (v/v/v)	137982	1.33
2	WL	226nm	136935	0.98
		236nm	136825	0.30
3	Flow rate	0.8ml/min	136632	0.58
		1.2ml/min	137863	0.45

Table 5

LOD and LOQ

Parameters	Measured volume
Limit of Quantification	0.5ppm
Limit of Detection	0.15ppm

Table 6

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