RP-HPLC Method Development And Validation For The Estimation Of Clobazam In The Tablet Dosage Form

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Abstract:

A simple, precise and accurate RP-HLC method was developed and validated for rapid assay of Clobazam in tablet dosage form. Isocratic elution at a flow rate of 1ml/min was employed on Zodiac c18 column at ambient temperature. The mobile phase consists of methanol: water 95:05 (v/v). The UV detection wavelength was at 211nm.Linearity was observed in concentration range of 40-100µg/ml. The retention time for Clobazam was 3.5min. The method was validated as per the ICH guidelines. The proposed method can be successfully applied for the estimation of Clobazam in pharmaceutical dosage forms.

Keywords: Clobazam, HPLC Method, Linearity, Precision, Recovery, Robustness,211 nm.

Introduction:

Clobazam is in a class of medications called benzodiazepines. It has been marketed as an anxiolytic since 1975[1] and an anticonvulsant since 1984 [2]. Clobazam is usually prescribed for epilepsys. It is uncleaClobazam is in a class of medications called benzodiazepines. It has been marketed as an anxiolytic since 1975 and an anticonvulsant since 1984. Clobazam is usually prescribed for epilepsys. It is unclear if there are any benefits to clobazam over other seizure medications for children with Rolandic epilepsy [3]. Clobazamis approved for adjunctive therapy in myoclonic seizures.[4] , complex partial seizures[5] certain types of status epilepticus, specifically the myoclonic, myoclonic-absent, simple partial, complex partial, and tonic varieties,[6] and non-status absence seizures. It is also approved for treatment of anxiety. It is also approved for adjunctive therapy for epilepsy in patients who have not responded to first-line drugs and in children who are refractory to first-line drugs. Clobazam is also approved as a short term (2–4 weeks) adjunctive agent in schizophrenia and other psychotic disorders to manage anxiety or agitation.[7] Clobazam is used with other medication(s) to control seizures in adults and children 2 years of age and older who have Lennox-Gastaut syndrome (a disorder that causes seizures and often causes developmental delays). Clobazam is sometimes used for refractory epilepsies. It works by decreasing abnormal electrical activity in the brain.

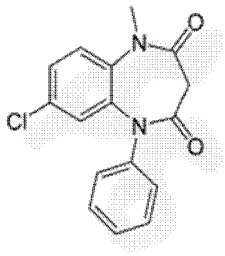

Figure:1 Structure of Clobazam:

Clobazam is available in oral form only, due to its insolubility in water. It is not recommended for use in children between the ages of six months and three years, unless there is a compelling need [7]. In addition to epilepsy and severe anxiety. However, long-term prophylactic treatment of epilepsy has considerable draw backs, most importantly loss of antiepileptic effects due to tolerance which may render long-term therapy ineffective [8]. Common side effects

with the use of drug includes Ataxia, Somnolence, Diplopia, Dysarthria. Literature review reveals that very few RP-HPLC methods are described for analysis of Clobazen [9-12].

Experimental:

Chemicals and Reagents:

Methanol, Acetonitrile and water used were HPLC grade and were purchased from Merck Specialties Private Limited, Mumbai, India.

Instrumentation:

To develop a High Pressure Liquid Chromatographic method for quantitative estimation of clobazam isocratic PEAK HPLC instrument with Zodiac C18Column (250 mm x 4.6 mm, 5µ) was used. The instrument is equipped with an Lc 20AT pump for solvent delivery and variable wavelength programmable LC-7000 UV-detector. A 20µ L Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software.

Preparation of standard stock solution of clobazam:

10mg of the standard drug (Clobazam) was weighed accurately and was dissolved in 10ml methanol separately to get a concentration of 1000µg/ml of both Clobazam separately. It was sonicated to dissolve completely. Then it was filtered through membrane filter paper. This standard stock solution used to prepare necessary concentrations to construct calibration curve by proper dilution.

Preparation of sample solution:

A 100mg tablet containing clobazam was taken for assay estimation of clobazam. It was grinded till to get a fine powder and homogenously mixed using mortar and pestle. From the powder, an amount of the powder equivalent to 10mg of clobazam was weighed and was dissolved in 10ml of Methanol. The solution was sonicated for 10min complete extraction of drug in Methanol. The solution was centrifuged at 400rpm for 10min; the clear supernatant was collected and was filtered through 0.45mu nylon membrane filter paper. From this solution selected concentration of 10mg/ml was prepared by proper dilution. Similar procedure was followed for the preparation of remaining branded tablets separately. The prepared solutions were used for the assay of clobazam.

Method validation

Linearity:

The developed method has been validated valid as per ICH guidelines (ZucmanD, 2007.)Working standard solutions of Clobazam in the mass concentration range of 40 to 100µg/ml were injected into the chromatographic system. The chromatogram was developed and the peak area was determined for each concentration of the drug solution. Calibration curve of Clobazam was obtained by plotting the peak area ratio versus the applied concentrations of Clobazam. The linear correlation coefficient was found to be 0.99.Results were shown in Table 1. Linearity graph was shown in Figure.2.

Precision:

The intraday and interday precision of the proposed methods were performed by analyzing the corresponding responses six times on the same day and on three different days over a period of one week for three different concentrations of standard solutions of clobazam .The results were reported in terms of relative standard deviation (RSD) Results were shown in Table 2 and Table.3.

Recovery (%Accuracy)

Accuracy of the method was determined by calculating recovery of Clobazam (4, 8, 12µg/ml) by the standard addition method. The Known amount of Clobazam was added to a pre quantified sample solution and the amount of Clobazam was estimated by measuring the peak area with standard area. The recovery studies were carried out three times over the specified concentration range. From the above determination, percentage recovery and standard deviation of percentage recovery were calculated. Results were shown in Table 5 Recovery (%Accuracy).

Ruggedness:

To determine the ruggedness of the method standard solution was injected for six times by other chemist for study of person to person variation. Results were shown in Table.4

Robustness:

To determine the robustness of the method, the optimized chromatographic conditions (mobile ohase ratio, wavelength of the detector and Mobile phase pH) were varied**.** Results were shown in Table.5

Specificity:

The specificity of the method was determined by comparing test results obtained from analysis of sample solution containing excipients with that of test results those obtained from standard drug. The standard chromatogram was given in figure 3.

LOD and LOQ:

Limit of detection (LOD) and limit of quantification (LOQ) were found 1.5µg/ml and 5µg/ml respectively as per ICH guide-lines. Results were shown in Table 6.

System Suitability Parameter:

System suitability test was performed by freshly prepared standard stock solutions of Clobazam. The tailing factor and theoretical plates of the chromatographic peak were within the limit. Results were shown in Table 7.

Result and Discussion

The nature of the sample, solubility decides the proper selection of the stationary phase. The drug Clobazam being non-polar is preferably analyzed by reverse phase columns and accordingly C18 column was selected. So the elution of the compound from the column was influenced by polar mobile phase. The concentration of the methanol and Acetonitrile were optimized to give symmetric peak with short run time based on asymmetric factor and peak area obtained. Different mobile phases performed and optimized but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase Methanol: Acetonitrile 95:5 (v/v). The retention time of Clobazam was found to be 3.50min, which indicates a good base line. The RSD values for ruggedness and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The system suitability and validation parameters are given in Table 7. The high percentage of recovery of Clobazam was found to be 99.05 indicating that the proposed method is highly accurate. Proposed liquid chromatographic method was applied for the determination of Clobazam in tablet formulation. The result for Clobazam was comparable with a corresponding labeled amount .The absence of additional peaks indicates no interference of the excipients used in the tablets.

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. The method was proved for analysis of clobazem in tablet dosage form with good recovery. Therefore, it is suitable for the routine analysis of Clobazam in pharmaceutical dosage form.

S.No	Concentration in µg/ml	Clobazam peak area
1	40	340032
2	50	461823
3	60	561062
4	70	662643
5	80	749422
6	90	894110
7	100	963528
		Slope:10440.79 Intercept:- -69052.6 Correlation coefficient:0.998

Table.1. Standard curve for Clobazam

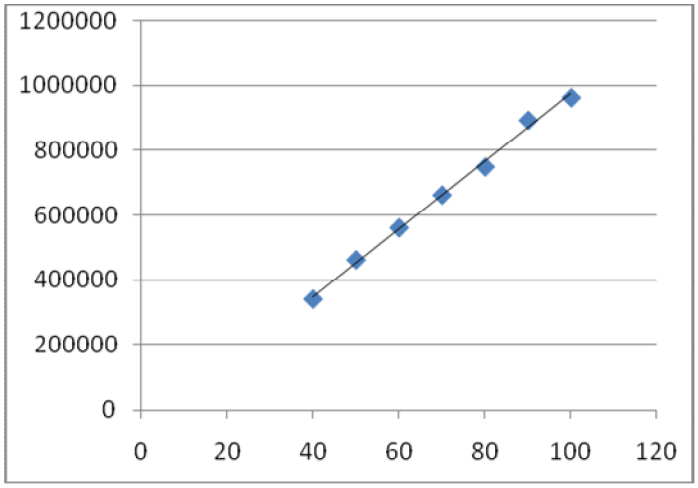

Figure:2 Linearity graph

S.NO	Concentration in µg/ml	Clobazam Peak Area
1	80	759695
2		765898
3		748929
4		743700
5		738931
6		738508
		RSD:1.50

Table:2 Inter day Precision

S.NO	Concentration in µg/ml	Clobazam Peak Area
1	80	759695
2		765898
3		748929
4		743700
5		738931
6		738508
		RSD:1.50

Table:3 Intraday precision

S.NO	Concentration in µg/ml	Clobazam Peak Area
1	**80**	**754122**
2		**748509**
3		**753117**
4		**726066**
5		**743478**
6		**743463**
		RSD:1.374

Table:4 Ruggedness

S.NO	Condition	Change	Clobazam Peak Area	% Change
1	Standard	……..	749422	…….
2	MP 1	+ 0.2% Mobile phase ratio	748309	-0.14851
3	MP 2	- 0.2% Mobile phase ratio	748074	-0.17987
4	WL 1	+detector wavelength	741013	-1.12206
5	WL 2	-detector wavelength	738418	-1.46833
6	pH 1	+MP PH	737906	-1.53665
7	pH 2	-MP PH	743447	-0.79728

Table:5 Robustness

Limit of Detection	**1.5 µg/ml**
Limit of Quantification	**5 µg/ml**

Table:6 LOD and LOQ

Drug	Conc.	R. Time	Area	TP	TF
Clobazam	**80 µg/ml**	**3.50min**	**749422**	**9701**	**1.17**

Table: 7 System Suitability Parameter

S.NO	Brand Name	Label claim	Amount prepared	Area	Amount found	%Assay
1	**Clobazam**	**25mg**	**90µg/ml**	**887018**	**89.28µg/ml**	**99.20**

Table:8 Formulation

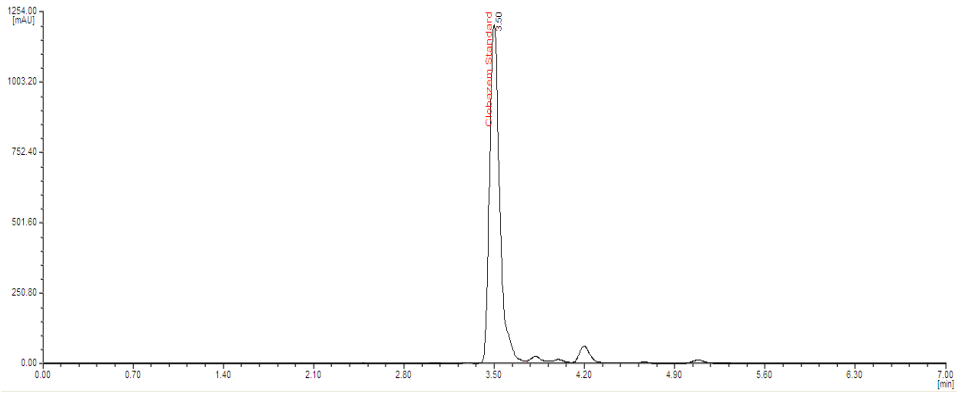

Figure 3: Standard Chromatogram of Clobazem

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