Research Article

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RP – HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ANALYSIS OF CLOBAZAM IN PHARMACEUTICAL DOSAGE FORMS.

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Abstract:

A simple, precise and accurate RP-HPLC method was developed and validated for rapid assay of clobazamin tablet dosage form. Isocratic elution at a flow rate of 1ml min^{-1} was employed on a symmetry C18 column at ambient temperature. The mobile phase consisted of Methanol:water:OPA(0.1%) 35:40:25 (v/v/v). The UV detection wavelength was at 239nm.Linearity was observed in concentration range of 4-16ppm. The retention time for Clobazam was 4.71 min. 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.

Key Words:

CLOBAZAM, HPLC, Linearity, Precision, 239nm.

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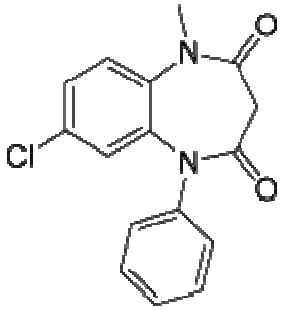
Introduction:

Clobazam[2] is a drug which is a benzodiazepine derivative. It has been marketed as an anxiolytic since 1975[3] and an anticonvulsant since 1984[4] It has also been used incorrectly in combination with several injectable contraceptives. As of 2005, clobazam (Frisium) is approved in Canada for adjunctive use in tonic-clonic, complex partial, and myoclonic seizures[5].



Clobazam (Urbanyl[6]) is approved for adjunctive therapy in complex partial seizures[7] certain types of status epilepticus, specifically themyoclonic, myoclonic-absent, simple partial, complex partial, and tonic varieties,[8] and non-status absence seizures.[9] It is also approved for treatment of anxiety. In India, clobazam (Frisium, Aventis Pharma India, Ltd.) is approved for use as an adjunctive therapy in epilepsy and in acute and chronic anxiety.[10] In Japan, clobazam (Mystan[11]) is approved for adjunctive therapy in treatment-resistant epilepsy featuring complex partial seizures[12]. In New Zealand, clobazam is marketed as Frisium[13] In the United Kingdom clobazam (Frisium) is approved for short-term (2–4 weeks) relief of acute anxiety in patients who have not responded to other drugs, with or without insomnia and without uncontrolled clinical depression[14]. It was not approved in the US until October 25, 2011, when it was approved for the treatment of seizures associated with Lennox-Gastaut Syndrome[15].

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. It is not recommended for use in children between the ages of six months and three years, unless there is a compelling need.[14] In addition to epilepsy and severe anxiety, clobazam is also approved as a short term (2–4 weeks) adjunctive agent in schizophrenia and other psychotic disorders to manage anxiety or agitation.[14]

Clobazam is sometimes used for refractory epilepsies. However, long-term prophylactic treatment of epilepsy has considerable drawbacks; most importantly loss of antiepileptic effects due to tolerance which may render long-term therapy ineffective.[16] other antiepileptic drugs may therefore be preferred for the long term management of epilepsy. Furthermore, benzodiazepines have the drawback, particularly after long-term use, of causing rebound seizures upon abrupt or over-rapid discontinuation of therapy forming part of the benzodiazepine withdrawal syndrome.Clobazam is available in oral form only, due to its insolubility in water.Benzodiazepines require special precaution if used in the elderly, during pregnancy, in children, alcohol or drug-dependent individuals and individuals with comorbid psychiatric disorders. In humans tolerance to the anticonvulsant effects of clobazam frequently occurs and withdrawal seizures can occur during abrupt or overrapid

withdrawal. Clobazam as with other benzodiazepine drugs can lead to physical dependence, addiction and what is known as the benzodiazepine withdrawal syndrome. Withdrawal from clobazam or other benzodiazepines after regular use often leads to withdrawal symptoms which are similar to those seen during alcohol and barbiturate withdrawal. The higher the dose and the longer the drug is taken for, the greater the risk of experiencing unpleasant withdrawal symptoms. Withdrawal symptoms can however occur from standard dosages and also after short term use. Benzodiazepine treatment should be discontinued as soon as possible via a slow and gradual dose reduction regime.Clobazam is an anticonvulsan Clobazam is a 1,5-benzodiazepine, meaning that its diazepine ring has nitrogen atoms at the 1 and 5 positions (instead of the usual 1 and 4). Like other 1,5-benzodiazepines (e.g., arfendazam, lofendazam, CP-1414S), it has less affinity for the ω_1-allosteric binding site on the $GABA_A$ receptor compared to the 1,4-benzodiazepines. It has selective affinity for the ω_2 site, where it has agonistic activity. [11]

In a double-blind placebo-controlled trial published in 1990 comparing it to clonazepam, 10 mg or 20 mg of clobazam was shown to be much less sedating than either 0.5 mg or 1 mg of clonazepam.The ω_1-receptor, which is found on the α_1 subtype of the $GABA_A$ receptor, was shown to be responsible for the sedative effects of diazepam by McKernan et al. in 2000, who also showed that its anxiolytic and anticonvulsant properties could still be seen in mice whose α_1 receptors were insensitive to diazepam.It would seem, then, that the anticonvulsant properties of clobazam are due to its selective affinity for ω_2.In 1996, Nakamura et al. reported that clobazam and its active metabolite, N-desmethylclobazam (norclobazam), work byenhancing GABA-activated chloride currents at $GABA_A$-receptor-coupled Cl^-channels. It was also reported that these effects were inhibited by the GABA antagonist flumazenil, and that clobazam acts most efficiently in GABA-deficient braintissue.

Experimental

Chemicals and reagents

All HPLC SOLVENTS used like Acetonitrile, Methanol,Orthophosphoric Acid which are of HPLC grade were purchased from E.Merck,

Instrumentation and analytical conditions

The analysis of the drug was carried out on Shimadzu HPLC model (VP series) containing LC-10AT (VP series) pump, variable wave length programmable UV/visible detector SPD-10AVP and rheodyne injector (7725i) with 20µl fixed loop. Chromatographic analysis was performed using Inertsil ODS C-18 column with 250 x 4.6mm internal diameter and 5µm particle size. Shimadzu

electronic balance (AX-200) was used for weighing. Isocratic elution with Methanol: water: OPA(0.1%) 35:40:25 (v/v/v) was selected with a flow rate of 1.0 ml min^{-1}.The detection wavelength was set at 239nm with a runtime of 10 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 Stock, working standard solutions and Sample solutions

100mg of Clobazam was weighed and transferred (working standard) into a 100ml volumetric flask. The diluent methanol was added and sonicated to dissolve it completely and made up to the mark with the same solvent. Further 1ml of the above stock solution was pipetted into a 10ml volumetric flask and diluted up to the mark with diluent. The contents were mixed well and filtered through Ultipor N_{66} Nylon 6, 6 membrane sample filter paper. The calibration curve was plotted with the concentrations of the 4 to 16ppm working standard solutions. Calibration solutions were prepared and analyzed immediately after preparation.

The formulation tablets of Clobazam were crushed to give finely powdered material. Powder equivalent to 25 mg of drug was taken in 10 ml of volumetric flask containing 5 ml of mobile phase and was shaken to dissolve the drug and then filtered through Ultipor N_{66} Nylon 6,6 membrane sample filter paper. Volume of the filtrate was adjusted to the mark with the same solvent to obtain concentration of 1mg/ml.

Method Validation procedure

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy, specificity, and limit of detection, limit of quantification, robustness and system suitability.

Linearity

The developed method has been validated as per ICH guidelines (Zucman D, 2007). Working standard solutions of Clobazam in the mass concentration range of 4 to 16ppm was injected into the chromatographic system. The chromatograms were 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.

S.NO	CONC of standard solution(ppm)	AREA
1	4	16745
2	6	25316
3	8	34925
4	10	42741
5	12	51228
6	14	60117
7	16	68274

Table 1: Linearity of Clobazam

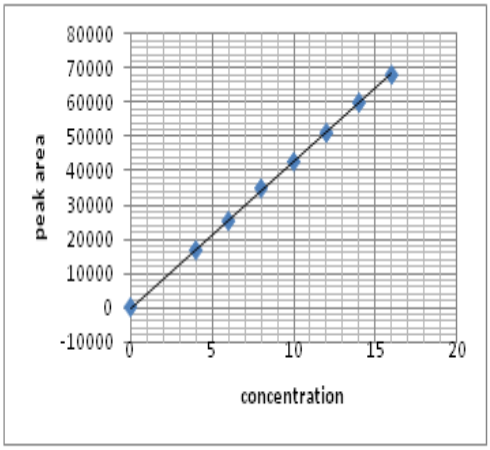


Figure 2: Calibration curve of Clobazam

Precision

Repeatability of the method was checked by injecting replicate injections of 10 ppm of the solution for six times on the same day as intraday precision study of Clobazam and the RSD was found to be 0.60.

INJECTION	CONCENTRATION	INTERDAY	INTRADAY
1	10ppm	42741	42179
2	10ppm	42541	42785
3	10ppm	42782	42698
4	10ppm	42119	42281
5	10ppm	42275	42265
6	10ppm	42388	42338
RSD		0.61	0.59

Table 3: Precision parameters of Clobazam

Accuracy

The accuracy of the method was determined by calculating recovery of Clobazam(4, 8, 012ppm) by the method of standard addition. Known amount of Clobazam was added to a pre quantified sample solution and the amount of Clobazam was estimated by measuring the peak area ratios and by fitting these values to the straight line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range and amount of Clobazam was estimated by measuring the peak area ratios by fitting these values to the straight line equation of calibration curve. From the above determination, percentage recovery and standard deviation of percentage recovery were calculated.

Recovey	Conc. of sample	Recovery	% of recovery
50%	4ppm	3.98	99.5
100%	8ppm	7.85	98.12
150 %	12ppm	12.09	100.75

Table 4: Accuracy results of Clobazam

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.

LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 50microgram/ml and 10microliter respectively as per ICH guide-lines

Parameter	Measured
LOD	0.8 ppm
LOQ	0.25 ppm

Table 5: LOD and LOQ

Ruggedness

Inter day variations were performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different days over a period of one week. Ruggedness also expressed in terms of percentage relative standard deviation.

Robustness

To determine the robustness of the method, two parameters from the optimized chromatographic conditions were varied.

Parameter	Modification	Peak Area	% of change
M.PHASE	Methanol:water:OPA(0.1%) 35:40:25 (v/v/v)	42229	0.98
PH	4.5	43081	1.007
WAVELENGTH	245	43152	1.009

Table 6 : Robustness Results.

System Suitability Parameter:

System suitability tests were carried out on freshly prepared standard stock solutions of Clobazam and it was calculated by determining the standard deviation of Clobazam standards by injecting standards in six replicates at 6 minutes interval and the values were recorded.

Parameters	Values
λ max (nm)	239
Beer's law limit (µg/ml)	4-16ppm
Correlation coefficient	0.999
Retention time	4.71min
Theoretical plates	157605
Tailing factor	0.94
Limit of detection	0.8 ppm
Limit of quantification	0.25 ppm

Table 5: System suitability parameters of Clobazam

Result and Discussion

Optimization of the chromatographic conditions

The nature of the sample, its molecular weight and 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 were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase Methanol:water:OPA(0.1%) 35:40:25 (v/v/v). The retention time of Clobazam was found to be 4.71min, which indicates a good base line. The RSD values for accuracy 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 4. The high percentage of recovery of Clobazam was found to be 98.28 indicating that the proposed method is highly accurate. Proposed liquid chromatographic method was applied for the determination of Clobazamin tablet formulation. The result for Clobazam was comparable with a corresponding labelled amount (Table 6). The absence of additional peaks indicates no interference of the excipients used in the tablets.

Formulation

Formulation	Tablet dosage	Sample concentration	Amount of drug estimated	% of drug estimated
FRISIUM CAPSULE	10mg	10 ppm	9.95 ppm	99.5 %

Table 6: Tablet estimation of Clobazam

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 10 min allows the analysis of a large number 10 of samples in short period of time. Therefore, it is suitable for the routine analysis of Clobazam in pharmaceutical dosage form.

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