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RESEARCH ARTICLE

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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF CLOBAZAM BY USING UV SPECTROPHOTOMETRIC METHOD

ABSTRACT

A simple, accurate and cost efficient spectrophotometric method has been developed for the estimation of Clobazam in tablet dosage form. The optimum conditions for the analysis of the drug were established. The maximum wavelength (λ max) was found to be 230nm in 1N NaOH: Methanol (80:20). The percentage recovery of Clobazam was in the range of 99.50 -100.00%. Beers law was obeyed in the concentration range of 30-90µg/ml. Calibration curves shows a linear relationship between the absorbance and concentration. The line equation y=0.024144x-0.04595 with correlation coefficient of 0.99924 was obtained. Validation parameters were carried out as per the guidelines of International Conference for Harmonization. This method can be used in the industries for determination of Clobazam to analyze the quality of formulation without interference of the excipients.

KEY WORDS: Clobazam, Anti-epileptic, λmax, ICH, UV-Vis Spectroscopy.

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.

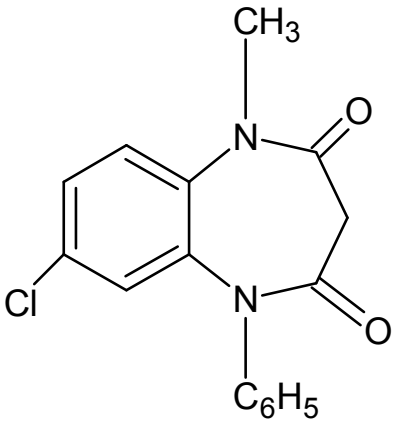

Fig 1 Chemical Structure of Clobazam

OBJECTIVE

The aim of present work is to find out a simple, sensitive, specific, spectrophotometric method for the detection of Clobazam in pharmaceutical dosage form.

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INSTRUMENTATION

UV-Visible double beam spectrophotometer (UV-1700, Pharmaspec, SHIMADZU Limited, Japan) with 1cm matched quartz cells and Digital balance (Citizen Co.)

CHEMICALS AND REAGENTS

Methanol (Merck), NaOH ,Water.

OPTIMIZATION

Scanning and determination of maximum wavelength (λmax)

In order to ascertain the wavelength of maximum absorption (**λmax**) of the drug solution (20µg/ml) were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400nm against reagent blank. The resulting spectrum was presented in Fig 2. and the absorption curve showed characteristic absorption maximum at 230 nm for Clobazam.

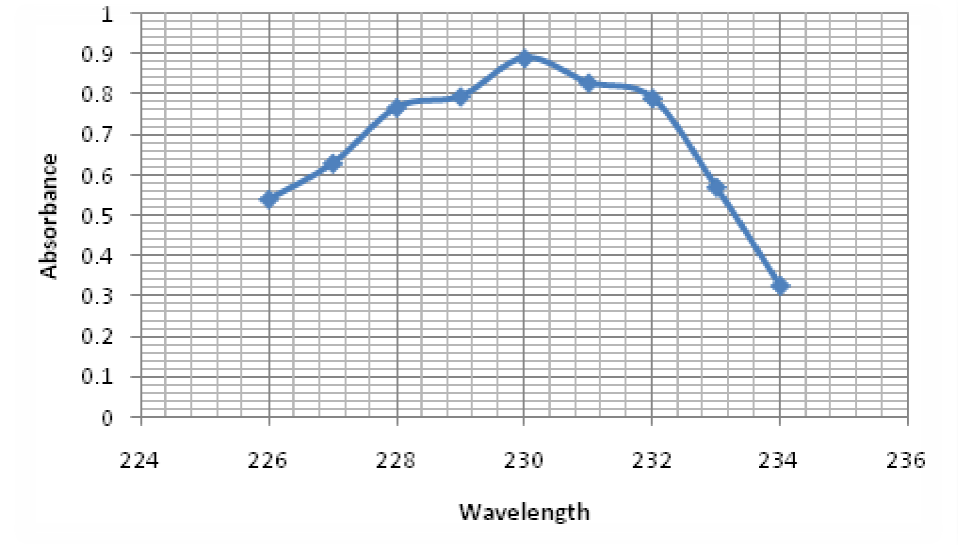

Fig.2. Absorbance spectrum of Clobazam (20µg/ml).

Preparation of standard stock solutions:

Weigh accurately 10 mg of Clobazam into a10 ml volumetric flasks. Then add sodium hydroxide and methanol in the ratio of 80:20 to dissolve the drug and then volume was made up to 10 ml with mobile phase. The concentration of standard stock solution is 1 mg/ml.

Preparation of working standard solution:

Transferred 5 ml from the above standard stock solutions in to 50 ml volumetric flasks and diluted up to the mark with mobile phase to get working standard solution of concentration 0.1 mg/ml. From this 2ml is diluted to 10 ml to get 20µg/ml.

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S.No	Concentration	Absorbance
1	30	0.645
2	40	0.887
3	50	1.144
4	60	1.398
5	70	1.652
6	80	1.904
7	90	2.143
	CC	0.99924
	Slope	0.024144
	Intercept	-0.04594

Table 1. Linearity table of Clobazam at 230nm

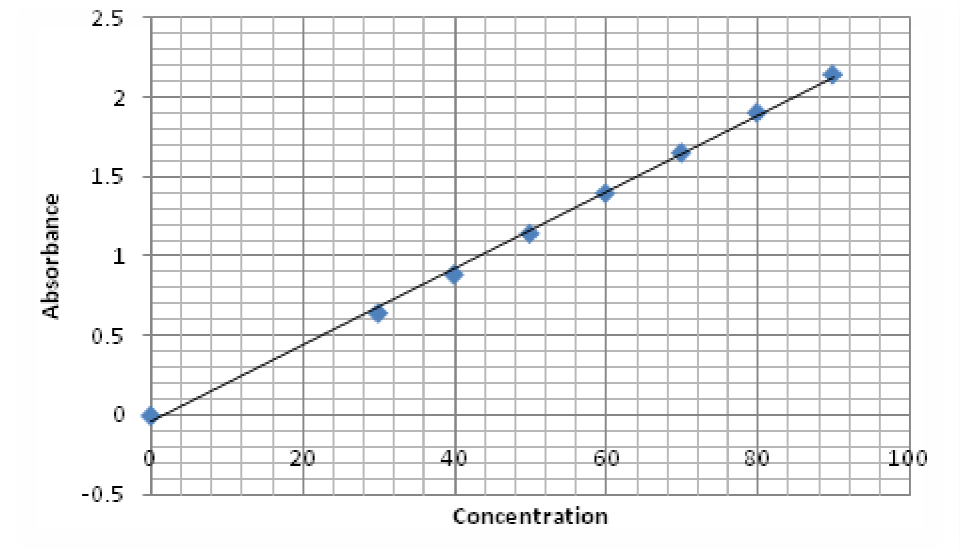

Fig.3. Linearity graph of Clobazam (30 – 90 µg/ml).

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VALIDATION OF METHOD PARAMETERS

LINEARITY

The aliquots of concentration ranging 30-90 µg/mL were prepared. The linearity results are tabulated in table 1.

Table: 1: Optical Characteristics.

Parameters	
λ (nm)	230 nm
Beer's Law limit (µg/ml)	30 – 90 µg/ml
Correlation coefficient	0.99924

ACCURACY

The accuracy, specificity, suitability and validity of the proposed methods were satisfied by conducting recovery studies. A known quantity of the drug was added to the pre analyzed sample formulation at 25%, 50% and 100% levels. The percentage recovery was calculated and given in table no:2

S.NO	Recovery	Concentration	Absorbance	Amount found	% of Recovery
1	25%	50ppm	1.142	49.91	99.82
		50ppm	1.14	49.82	99.64
		50ppm	1.143	48.57	99.9
2	50%	60ppm	1.395	59.87	99.78
		60ppm	1.397	59.95	99.91
		60ppm	1.397	59.95	99.91
3	100%	80ppm	1.9	79.83	99.91
		80ppm	1.901	79.87	99.83
		80ppm	1.903	79.95	99.93

Table 2. Accuracy Data of Clobazam.

PRECISION

Repeatability:

The repeatability of the method was studied by measuring the absorbance at 230 nm of standard solutions of six replicate samples and measured the absorbance at 230 nm.

Repeatability of assay:

Intraday precision:

This was done by analyzing formulation in same day for six times of individual preparation. The results are summarized in table no:3

S.NO	Concentration(µg/ml)	Absorbance at 230nm	Statistical analysis (n=6)
1	60	1.398	Mean=1.402
2	60	1.391	
3	60	1.405	
4	60	1.409	%RSD=0.47
5	60	1.404	
6	60	1.406	

Table no 3. Intraday precision of Clobazam

Interday precision: This was done by analyzing formulation by same analyst but for six days subsequently. The results are summarized in table no:4

s.no	Concentration(µg/ml)	Day	Absorbance at 230nm	Statistical analysis at 255nm
1	60 µg/ml	1	1.385	Mean=1.392
2		2	1.392	
3		3	1.391	
4		4	1.401	%RSD=0.37
5		5	1.39	
6		6	1.392	

Table no 4. Interday precision of Clobazam

ESTIMATION OF CLOBAZAM IN COMMERCIAL DOSAGE FORM

Twenty tablets were weighed accurately and the average weight of each tablet was calculated. The tablets were powdered well with the help of glass mortar and pestle. Tablet powder equivalent to 1 tablet weight was weighed accurately and transferred to a 10 ml volumetric flask. Then add small quantity of methanol: 1 NaOH and sonicate it for 30 min to dissolve the drugs completely and then the volume was made up to the mark with the diluent and filtered through 0.45 µm membrane filters. From this, 2 ml was taken and diluted to100 ml with diluents to get 20 µg/ml concentration. The absorbance of this solution was measured at 230 nm against diluent as a blank. This procedure was repeated 3 times. The results are summarized in table no:5

Brand Name	Label claim	Concentration	Absorbance	Average	% Assay
Frisium	10mg	60ppm	1.397		
Frisium	10mg	60ppm	1.396	1.396667	**99.92**
Frisium	10mg	60ppm	1.397		

Table no 5. %Assay of Clobazam

RESULTS AND DISCUSSION

From the optical characteristics of the proposed method, it was found that Clobazam obeys linearity within the concentration range of 30 - 90 µg /ml. From the results shown in precision table-3 & 4, it was found that the % RSD is less than 2%, which indicates that the method has good reproducibility. From the results shown in accuracy table-5, it was found that the percentage recovery values of pure drug to the Placebo were in between 99.50 – 100.00 %, which indicates that the proposed method is accurate and also reveals that the commonly used
excipients and additives in the pharmaceutical formulations were not interfering in the proposed method.

CONCLUSION

The proposed method was simple, sensitive and reliable with good precision and accuracy. The proposed method is specific while estimating the commercial formulations without interference of excipients and other additives. Hence, this method can be used for the routine determination of Clobazam in Bulk and Pharmaceutical formulations

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