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RESEARCH ARTICLE

Zero Order and First Order Derivative Spectrophotometric Methods for Determination of Clonazepam in Pharmaceutical Dosage Form

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ABSTRACT

A simple, precise and economical spectrophotometric method has been developed for estimation of clonazepam in pharmaceutical dosage form using distilled water as a solvent. The quantitative determination of the drug was carried out using the zero order derivative values measured at 307nm and the first order derivative values measured at 330nm. The method was found to be linear and obeys Beer's law in concentration range 2-22µg/ml with a coefficient of correlation values for zero and first order derivative method 0.9994 and 1.0 respectively. The percent mean recovery for zero and first order derivative was found to be 100.5% and 97.9% respectively. The mean percentage drug content for zero and first order derivative methods was found to be 99.75% and 102% respectively, and %RSD value was found to be less than 2 which shows the precision of the method. The limit of detection and limit of quantitation for zero order derivative was found to be 0.6739µg/ml and 2.042µg/ml respectively. The developed method was validated according to ICH guidelines and found to be accurate and precise. Thus the proposed method can be successfully applied for the determination of clonazepam in the pharmaceutical dosage form.

KEYWORDS

Clonazepam, Zero order derivative spectrum, First order derivative spectrum, Validated

INTRODUCTION

Clonazepam, a benzodiazepine, is used primarily as an anticonvulsant in the treatment of absence seizures, petit mal variant seizures, akinetic and myoclonic seizures. It enhances the activity of gamma-aminobutyric acid (GABA), which is a major inhibitory neurotransmitter in the central nervous system[8]. A literature review revealed that only very few analytical methods are reported so far for the determination of clonazepam in pharmaceutical formulations and bulk drugs.

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Four HPLC methods[1,2,3] and two UV spectrophotometric methods[4,5,6] that is first order derivative method and AUC method using methanol as a solvent have been reported .Therefore the aim of the present study was to develop a new approach which is simple, rapid, economical and suitable for the routine determination of clonazepam in pharmaceutical dosage form using distilled water as a solvent.

MATERIAL & METHODS

Instrumentation

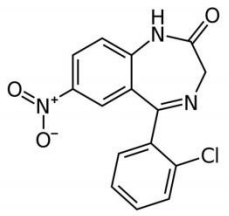
The present work was carried out on Shimadzu UV-2700 UV/VIS Spectrophotometer, with a pair of 1.0 cm matched quartz cells.

Reagents and Chemicals

Pharmaceutically pure samples of clonazepam were obtained from Suraksha pharmaceuticals, Roorkee, Uttarakhand. Tablet dosage formulation as Clonotril-2 was obtained from Salcette pharmacy, Goa. All Chemicals were of analytical grade.

Structure and chemical name of Clonazepam[8]

5-(2-chlorophenyl)-7-nitro-1,3-dihydro-1,4-benzodiazepine-2-one



Preparation of Standard Solution

Preparation of Stock Solution

Accurately weighed the 10mg quantity of clonazepam was transferred into a 100ml volumetric flask and add 20ml of methanol and sonicated for about 10 min and diluted up to the mark with methanol to obtain a stock solution of 100µg/ml for zero and first order derivative spectrophotometric analysis.

Preparation of Working Standard Solution

The standard solution was further diluted with distilled water to get working standard solution of 10µg/ml for zero and first order derivative spectrophotometric methods.

Determination of Maximum Wavelength

The standard working solution of clonazepam (10µg/ml) was scanned in the wavelength range of 200-400nm, and the spectrum was derivatized in first order at N=5 smoothening factor. An absorption maximum was found to be 307nm. Therefore analytical wavelength was fixed at 307nm for the analysis of clonazepam.

Stability of Clonazepam in the Selected Solvent

The stability of clonazepam in selected solvent was determined by measuring the absorbance of the drug solution for 6 hours. The solutions were found to be stable. The stability study data are tabulated in table 1.

Table 1: Data of Stability Studies

Sr. No	Time (h)	Absorbance at 307nm
1	0	0.328
2	2	0.331
3	4	0.330
4	6	0.332

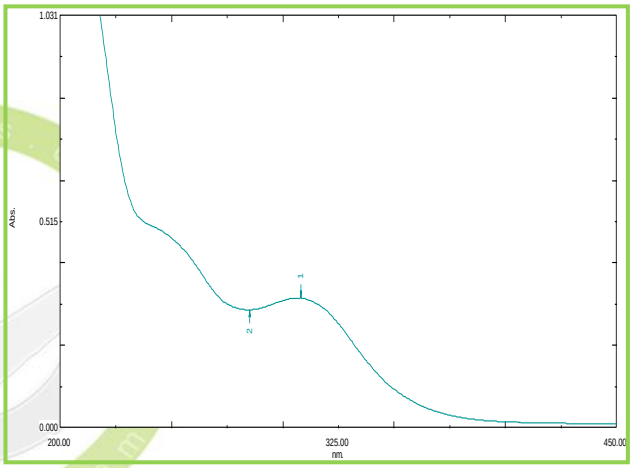


Figure 1: Zero Order Derivative Spectrum of Clonazepam

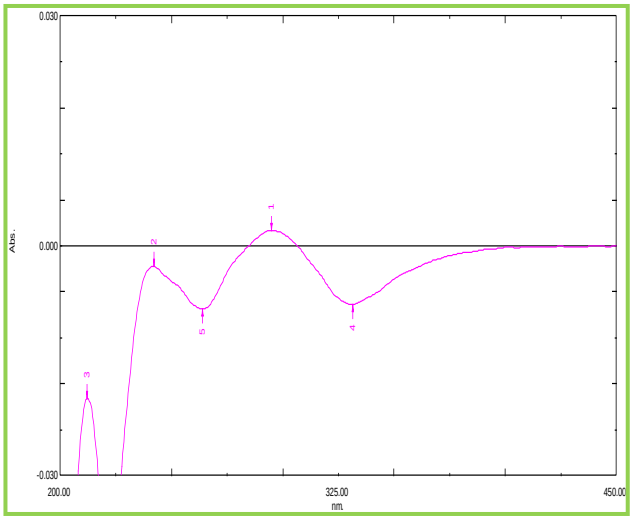


Figure 2: First Order Derivative Spectrum of Clonazepam

Assay

10 tablets of clonazepam were weighed, and their average weight was determined. An accurately weighed quantity of powder equivalent to 10mg of the drug was transferred to 100ml calibrated volumetric flask and extracted with 75ml of methanol for 20 minutes, sonicated and then the volume was made up to the mark with methanol to get the concentration of 100mcg/ml. The resulting solution was filtered through Whatman filter paper. From the filtrate 1ml of the solution was transferred to 10ml flask and diluted with distilled water to obtain 10mcg/ml concentration for zero and first order spectrophotometric methods. The assay results for zero order and first-order derivative methods are tabulated in table 2.

Table 2: Assay Results for Zero Order and First Order Derivative Methods

Parameters	Zero order derivative	First order derivative
Label claim	2mg	2mg
% Assay	99.75%	102%
Standard deviation	0.4176	0
% RSD	0.4178%	0%

Method Validation[7]

The method was validated by establishing Linearity, accuracy, repeatability study and intermediate precision, LOD (Limit of Detection) , LOQ (Limit of quantitation).

Linearity

The linearity was evaluated by analyzing the different concentration of the standard solution of clonazepam. The Beer Lambert's law was obeyed in the concentration range of 2-22mcg/ml. calibration curves (n=6) were plotted between the concentration of solution and absorbance of the drug. The data for linearity studies are shown in table 3 and calibration curve are shown for zero order and first-order derivative methods in figure 3 and 4 respectively.

Table 3: Data for Linearity Studies

Parameters	Zero order derivative	First order derivative
Max Wavelength	307nm	330nm
Linearity Range	2-22µg/ml	2-22µg/ml
Correlation coefficient (r^2)	0.9994	1
Slope	0.0311	0.0008
Intercept	0.0176	0.0005

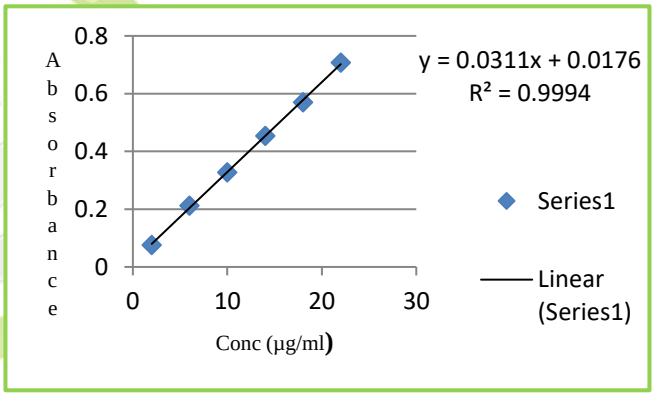


Figure 3: Calibration Curve of Clonazepam for Zero Order Derivative

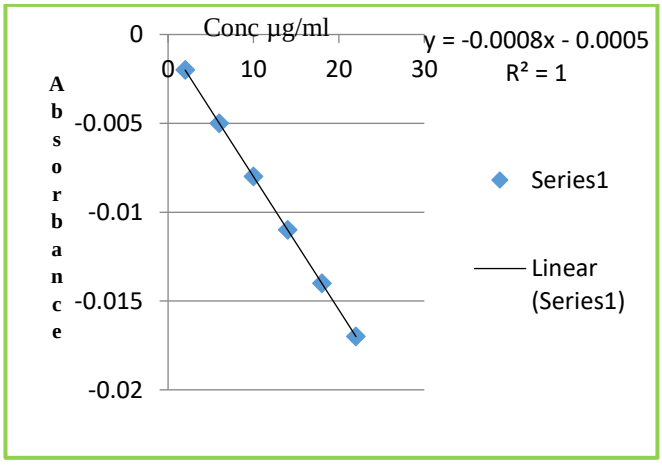


Figure 4: Calibration Curve of Clonazepam for First Order Derivative

Accuracy

The recovery studies were carried out to study the interference of the excipients and other interference at three different concentrations by standard addition method.

The recovery studies were performed by adding known amounts of the drug solution to the tablet samples at three concentration levels 80%, 100% and 120% of clonazepam standard concentration by standard addition method. At each level, samples were prepared in triplicate. The solution was then analyzed and the mean percentage recoveries and % RSD values were calculated. The results are tabulated in Table 4.

Precision

The precision of the method was confirmed by repeatability and intermediate precision. The repeatability was confirmed by analysis of formulation for six times. The intermediate precision was confirmed by repeated analysis of formulation on two successive days. The amount of drug and % RSD was calculated. The repeatability and intermediate precision data are tabulated in table 5 and 6 respectively.

Sensitivity

The limit of detection (LOD) and limit of quantitation (LOQ) parameters were calculated using the following equations:

LOD= 3.3* SD/slope

LOQ=10* SD/slope

The data for LOD and LOQ are tabulated in table 7.

Table 4: Data of Clonazepam for Recovery Studies

% Recovery	% Mean recovery (%)		% RSD (%)	
	Zero order derivative	First order derivative	Zero order derivative	First order derivative
80%	100.16	95.75	1.147	0
100%	99.73	102.2	0.798	0
120%	100.26	95.83	0.913	0

Table 5: Data of Clonazepam for Repeatability Study

Parameters	% Assay	S.D	% RSD
Zero order derivative	99.85	0.5692	0.57
First order derivative	102	0	0

Table 6: Data of Clonazepam for Intermediate Precision

Parameters	% Assay	S.D	% RSD
Zero order derivative	99.75	0.7327	0.734
First order derivative	102	0	0

Table 7: Data of Clonazepam for LOD and LOQ

Parameters	LOD µg/ml	LOQ µg/ml
Zero order derivative	0.6739	2.042

RESULT AND DISCUSSION

The proposed method for estimation of clonazepam in pharmaceutical dosage form was found to be accurate, rapid and straightforward. Hence, the zero order derivative and first-order derivative methods can be used usefully for routine analysis of clonazepam in pharmaceutical dosage forms. There was no interference from tablet excipients was observed in these methods. The zero order and first order derivative spectra for clonazepam were recorded at 307nm and 330nm respectively. The calibration plot was found to be linear in the concentration range of 2-22mcg/ml at 307nm and 330nm for zero order and first order derivative spectra of clonazepam respectively. The newly developed method was validated as per the ICH guidelines. The developed method for the quantitative estimation of clonazepam was subjected to different validation parameters like linearity, accuracy, precision, LOD (Limit of Detection), LOQ (Limit of quantitation).

CONCLUSION

The proposed analytical UV spectrophotometric method was developed and validated for quantitative determination of clonazepam in the pharmaceutical dosage form. The present work describes a simple and cheap method for determination of clonazepam in its dosage form. The developed method was found to be simple, rapid, accurate, precise and economical, which can be easily applied to the pharmaceutical formulation.

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REFERENCES

1. Patil, P. M., Wankhede, S. B., & Chaudhari, P. D. (2015). A Validated Stability–Indicating HPLC Method estimation of Clonazepam In the bulk drug and Pharmaceutical Dosage Form. *Pharm Anal Acta*, 6(2).
2. Spell, J. C., & Stewart, J. T. (1998). Analysis of clonazepam in a tablet dosage form using smallbore HPLC. *Journal of pharmaceutical and biomedical analysis*, 18(3), 453-460.
3. Bares, I. F., Pehourcq, F., & Jarry, C. (2004). Development of a rapid RP–HPLC method for the determination of clonazepam in human plasma. *Journal of pharmaceutical and biomedical analysis*, 36(4), 865-869.
4. Ramaling Kotnal, Amruta Patil, Santosh Karajgi. (2016). Validated First Order Derivative UV Spectrophotometric Method for the Determination of Clonazepam, *International Journal of Pharmaceutical Science*, 39(1), 66-69.
5. Santosh Karajgi, Amruta Patil, Ramaling Kotnal. (2016). Area under Curve UV Spectrophotometric Method for the Determination of Clonazepam in Tablet, *International Journal of Pharmacy and Pharmaceutical Research*, 6(2).
6. Patel, V. B., Dave, J. B., Patel, F. M., & Patel, C. N. (2012). UV-spectrophotometric method for identification and estimation of Clonazepam in tablet dosage Form. *International Journal of Pharmaceutical Research and Bio-science*, 1(2), 62-70.
7. Guideline, I. H. T. (2005, November). Validation of analytical procedures: text and methodology Q2 (R1). In International Conference on Harmonization, Geneva, Switzerland (pp. 11-12).
8. http://en.m.wikipedia.org/wiki/clonazepam