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# UV SPECTROPHOTOMETRIC METHOD FOR IDENTIFICATION AND ESTIMATION OF CLONAZEPAM IN TABLET DOSAGE FORM

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**Abstract:** The present research work describes the spectrophotometric method for the identification and estimation of Clonazepam in bulk and pharmaceutical dosage form. Identification test is proposed based on distinctive UV spectra of Clonazepam under acidic and alkaline condition. The drug exhibited UV absorption maximum at 271 nm in 0.01 N HCl which underwent Hyper chromic and Blue shift to 263 nm when sample was heated in boiling water bath for 2 hours. The drug exhibited UV absorption maximum at 364 nm in 0.01 N NaOH which underwent Hypo chromic and Red shift to 387 nm when sample was heated in boiling water bath for 2 hours. For the quantitative estimation, the stock solution was prepared in methanol and the drug was determined at absorption maximum of 309 nm. Beers law was obeyed in the concentration range of 4-28 µg/ml having regression line equation y = 0.0377x + 0.0005 with correlation coefficient of 0.9998. Results of the analysis were validated statistically and recovery was found between 98.81-101.03%. The method can be used for routine quality control analysis of Clonazepam formulation.

**Keywords:** Clonazepam, Identification, estimation, UV spectrophotometric

## INTRODUCTION

Clonazepam (CLO; 5-(o-chlorophenyl) -1,3-dihydro-7-nitro-2H-1,4-benzodiazepin- 2-one), a benzodiazepine with prominent anticonvulsant, anxiolytic properties[Figure 1]. It has been most effective in the treatment of typical and atypical absence, myoclonic and akinetic seizures, and infantile spasms. Co-administration of a barbiturate may exacerbate the drowsiness caused by clonazepam.[1, 2]

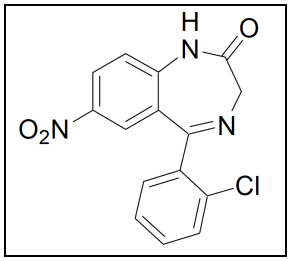


**Figure 1: Chemical structure of Clonazepam**

A literature survey revealed that clonazepam is official in the Indian Pharmacopeia, British Pharmacopeia, United States Pharmacopeia, European Pharmacopeia and Japanese Pharmacopeia.IR spectroscopy method have been reported for identification and potentiometric and HPLC methods have been reported for assay.[3-7] Different HPLC and GC methods has been reported for the determination of Clonazepam in biological fluids.[8-13]

No UV spectrophotometric analytical method is reported in literature for analysis of Clonazepam in tablet dosage form. This paper describes very specific UV spectral identification test and simple, precise and accurate UV Spectrophotometric method for estimation of clonazepam in tablet dosage form. The proposed method was validated in accordance with International Conference on Harmonization (ICH) guide-lines.[14]

## MATERIALS AND METHODS

### Experimental

#### *Instrument and materials*

Double beam UV-Visible Spectrophotometer (UV-1700, Shimadzu) was used. Clonazepam pure drug was obtained from Apostel Remedies, Vadodara as gift sample. Methanol was purchased from Finar Chemicals, India.

### Methodology

#### *Identification test*

Accurately weighed 50 mg quantity of Clonazepam API was transferred into 10

ml volumetric flask and dissolved in methanol using ultra sonication to give a stock solution having concentration of 5mg/ml. Two ml of this stock solution was diluted to 10 ml with 0.01 N HCL and 0.01 N NaOH and heated in water bath for 2 hour. Aliquots of 0.2 ml was taken at different time interval of 0, 10, 20, 30, 60 and 120 min & the volume was made up to mark with respective solvents. Spectra of the resulted solutions were scanned using respective solvent as blank.

***Preparation of standard stock solution***

Accurately weighed 10 mg quantity of Clonazepam API was transferred into 100 ml volumetric flask and dissolved in methanol using ultra sonication to give a stock solution having concentration of 100µg/ml.

***Preparation of calibration curve***

Aliquots of 0.4-2.8 ml of stock solutions were transferred to series of 10 ml volumetric flasks, and volume made up to mark with methanol to give concentration range (4-28 µg/ml). The absorbance was measured at absorption maximum of 309 nm against methanol as blank and calibration curve was plotted.

***Preparation of sample solution***

The proposed method was applied to analyze commercially available clonazepam tablet. About 20 tablets were weighed and powdered, a quantity of tablet powder equivalent of 1 mg of Clonazepam was weighed accurately and transferred to a 10ml volumetric flask. The tablet powder was dissolved in methanol with aid of ultrasonication and filtered through a whatman filter paper. Dilute this solution to get final test concentration 10 µg/ml and absorbance was measured against methanol as blank. The drug content of the preparation was calculated using standard calibration curve. Amount of drug estimated by this method is shown in Table 3.

***Method validation***

The developed method was validated for parameters like linearity, precision, accuracy, LOD, LOQ. the data for which are presented in the Tables. The low value of R.S.D. value indicates that all the methods are precise and accurate.

## *RESULTS AND DISCUSSION*

Identification test is proposed based on distinctive UV spectra of clonazepam under acidic and alkaline condition. The drug exhibited UV absorption maximum

at 271 nm in 0.01 N HCl which underwent Hyperchromic and Blue shift to 263 nm when sample was heated in boiling water bath up to 2 hour (Figure 2). The drug exhibited UV absorption maximum at 364 nm in 0.01 N NaOH which underwent Hypo chromic and Red shift to 387 nm when sample was heated in boiling water bath up to 2 hour (Figure 3).

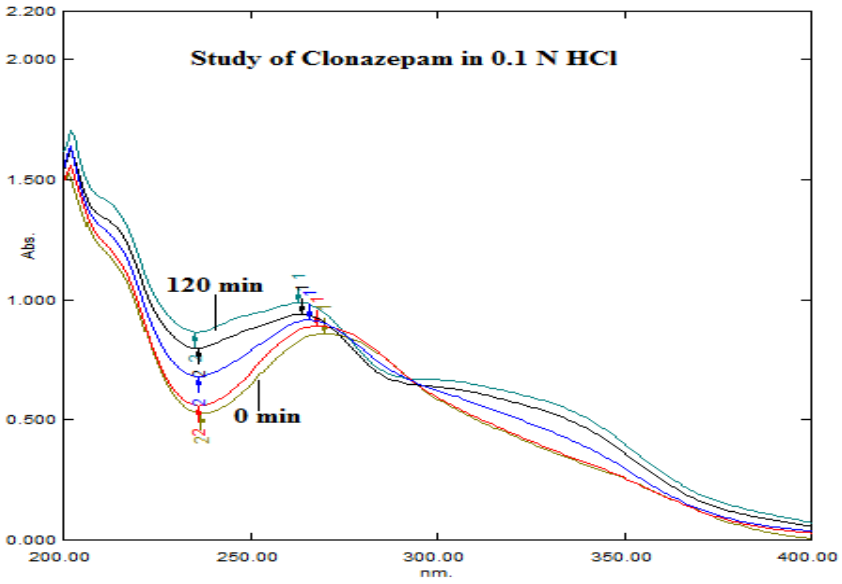


Figure 2. Identification spectra of clonazepam in 0.01 N HCl

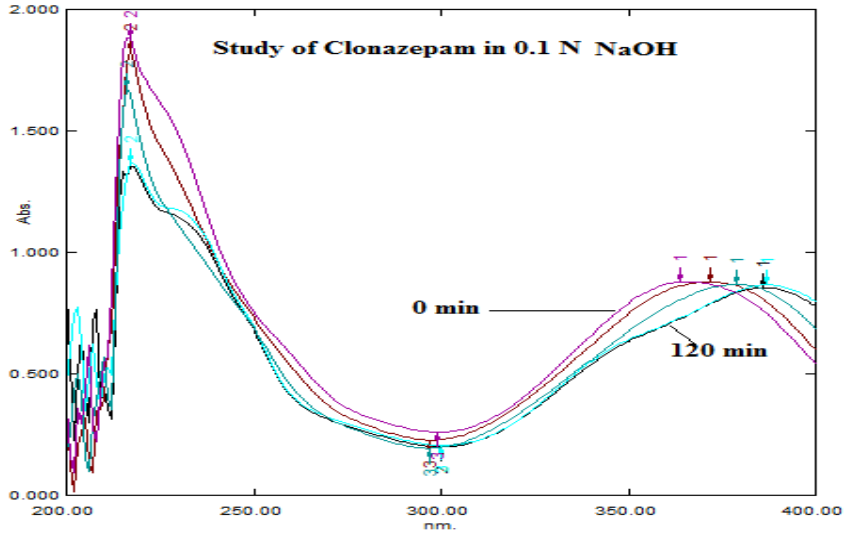


Figure 3.Identification spectra of clonazepam in 0.01 N NaOH

The zero order UV spectrum of Clonazepam in methanol has showed maximum absorbance at 309 nm (Figure 4). The calibration plots were found to be linear over the range of 4-28

µg/ml with correlation coefficients of 0.9998 (Figure 5). The LOD and LOQ were 0.22 and 0.66 µg/ml respectively. Precision of the method was confirmed by intraday and interday precision. The % RSD values for intraday and interday analysis was found to be 0.43 and 0.87 (Table 1). Analytical recovery experiments were carried out by standard addition method to check the accuracy of the developed methods and to study the interference of formulation additives, at 20, 40 and 60 % level. The % recovery was found to be in the range of 98.81-101.03(Table 2). The validated method was successfully applied for the determination of clonazepam in tablets and the results are given in Table 3 indicate that the amount of drug in tablet samples met with requirements (95%-105% of the label claim).

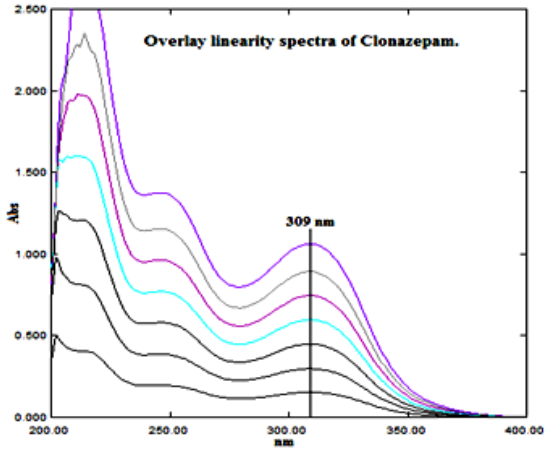


Figure 4: Overlay linearity spectra of Clonazepam. (4-28 µg/ml)

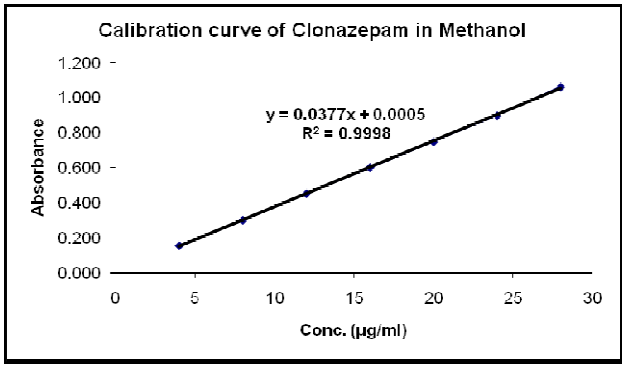


Figure 5: Calibration curve of Clonazepam at 309 nm.

**Table 1.**

**Regression analysis data and Summary of Validation Parameter for the proposed method.**

| Sr no. | Parameter | Clonazepam |
|---|---|---|
| 1 | Wavelength of measurement(nm) | 309 nm |
| 2 | Beer's law limit (µg/ml) | 4-28 µg/ml |
| 3 | Regression Equation(y = mx + c) | y= 0.0377 x + 0.0005 |
| 4 | Slope | 0.0377 |
| 5 | Intercept | 0.0005 |
| 6 | Correlation coefficient ($R^2$) | 0.9998 |
| 7 | Precision (% R.S.D) | |
| | Intraday (n=6) | 0.43 |
| | Interday (n=6) | 0.87 |
| 8 | Accuracy (% recovery), n=3 | 98.81-101.03 % |
| 9 | Limit of Detection | 0.22 µg/ml |
| 10 | Limit of Quantitation | 0.66 µg/ml |

**Table 2.**

**Recovery Data of Proposed Method**

| % Std. Added | Amount in test (µg/ml) | Amount of Std. Added (µg/ml) | Total Absorbance (n=3) | Total Amount Found (µg/ml) | % Recovery |
|---|---|---|---|---|---|
| 0 | 10 | 0 | 0.383 | 10.15 | - |
| 20 | 10 | 2 | 0.459 | 12.17 | 101.03 |
| 40 | 10 | 4 | 0.533 | 14.13 | 99.59 |
| 60 | 10 | 6 | 0.607 | 16.08 | 98.81 |

**Table 3.**

**Analysis of marketed formulation of Clonazepam tablet**

| Formulation | Label claim (mg/tablet) | % Assay(Mean±S.D, n=6) |
|---|---|---|
| CLONOTRIL-1 | 1 | 104.91 ± 0.92 |

## *CONCLUSION*

Identification test is proposed based on distinctive UV spectra of clonazepam under acidic and alkaline condition. Clonazepam underwent Hyperchromic and Blue shift in 0.01 N HCl and in 0.01 N NaOH underwent Hypo chromic and Red shift. The developed UV spectrophotometric method was found to be rapid, simple, inexpensive, reproducible and applicable over a wide concentration range with high precision and accuracy. The method was validated as per the guidelines laid by ICH. The results of the validated tests were found to be satisfactory and therefore this method can be applied successfully for routine quality control analysis of clonazepam in bulk and pharmaceutical formulation

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