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Area Under Curve UV Spectrophotometric Method for the Determination of Clonazepam in Tablets



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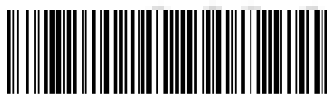
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ABSTRACT

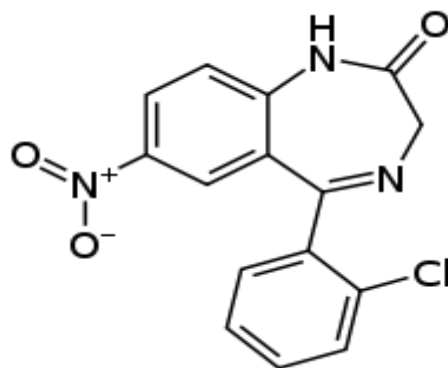
A simple, precise and economical procedure has been developed for the estimation of Clonazepam in bulk drug and pharmaceutical dosage form using UV- spectrophotometer Shimadzu model UV 1800. Area under curve method was employed for estimation of Clonazepam using analytical grade methanol as solvent. Clonazepam obeys Beer's law in concentration range 20-32 μ g/ml for the area between 240nm to 260nm. The recovery studies ascertained accuracy of purposed method and result validated according to ICH guideline. The result of analysis has been validated statistically by recovery studies. This method was successfully carried out for the estimation of Celecoxib in capsule and clonazepam in tablet dosage form without the interference of common excipients.



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INTRODUCTION



Clonazepam

Clonazepam belongs to category of benzodiazepine drug. Chemically it is 5-(2-Chlorophenyl)-7-nitro-2, 3-dihydro-1, 4-benzodiazepin-2-one used for epilepsy or anxiety disorders¹. Only very few analytical methods are reported so far for the determination of Clonazepam in pharmaceutical formulations and bulk drugs. Four HPLC methods^{2,3,4,5} and only one UV spectrophotometric method⁶ have been reported which are useless for routine analysis. Therefore; the aim of the present study was to develop new method which is simple, rapid, economical and suitable for the routine determination of Clonazepam in its bulk drug and dosage forms.

MATERIALS AND METHODS

Materials

Shimadzu 1800 spectronic model UV Spectrophotometer with 1cm matched quartz cells was used as the instrument for data collection and analysis. Methanol 95% was used as the solvent obtained from Qualigens. Tablet brands were obtained from the local market for assay and recovery studies.

Methodology

Preparation of standard stock solution:

Standard stock solution of clonazepam was prepared by dissolving accurately weighed quantity of clonazepam (10mg) in 100ml of methanol with sonication and transferred it to 100ml of

volumetric flask. Volume was made upto the mark with methanol for obtaining standard stock solution of 100 μ g/ml concentration.

Determination of Area under curve:

The standard solution of clonazepam (22 μ g/ml) was scanned in the wavelength range of 230nm to 270nm and the absorption maximum was found to be 245nm. Therefore, area between 240nm to 250nm was selected as analytical range for the determination and the area was found to be 9.4909. (Figure 1)

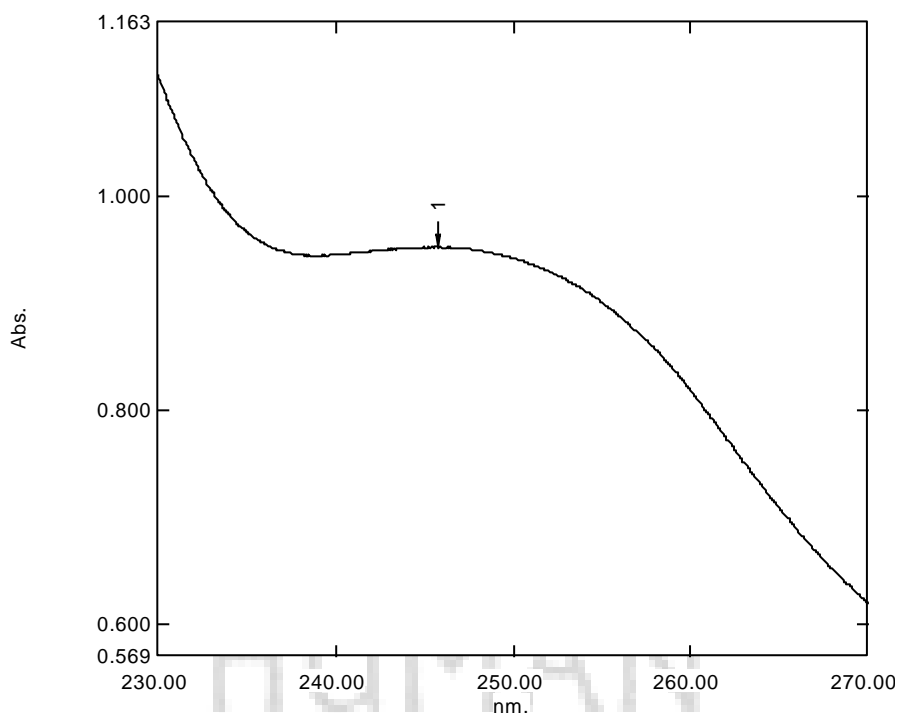


Figure 1: Area under curve of clonazepam in solution of methanol (22 μ g/ml).

Stability of Drug in Selected Solvent:

The stability of drug in selected solvent was determined by measuring the absorbance of the drug solution (20 μ g/ml) at different time intervals. After every 5min. of interval the abs. was measured the solution was found to be stable. (Table 1)

Table 1: Stability Data for Clonazepam

Sr. No.	Time (min.)	AUC
1	0	8.8838
2	05	8.8945
3	10	8.8967
4	15	8.9054

Linearity:

From the standard stock solution of Clonazepam, appropriate aliquots were pipetted out into 25ml of volumetric flask and dilutions were made with methanol to produce working standard solution of Clonazepam 20, 24, 28, 32 μ g/ml. The difference in AUC of Clonazepam was measured in the area from 250 to 240. The calibration curve of the drug Clonazepam was plotted.

The concentration range over which the drug followed linearity was chosen as an analytical concentration range i.e. 20 to 32 μ g/ml for Clonazepam. (Table 2 and Figures 2 to 6)

Table 2: Calibration data Table for Clonazepam

Sr. No.	Conc. (μ g/ml)	AUC
1.	20	8.8838
2.	24	10.669
3.	28	12.670
4.	32	14.505

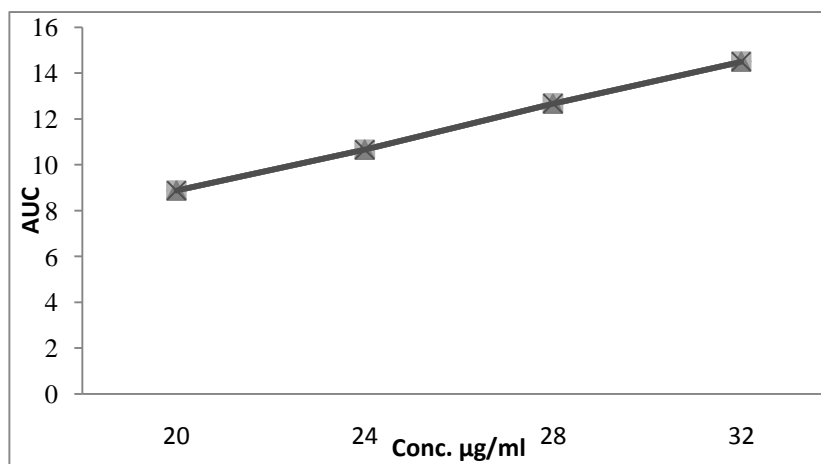


Figure 2: Calibration plot of Clonazepam

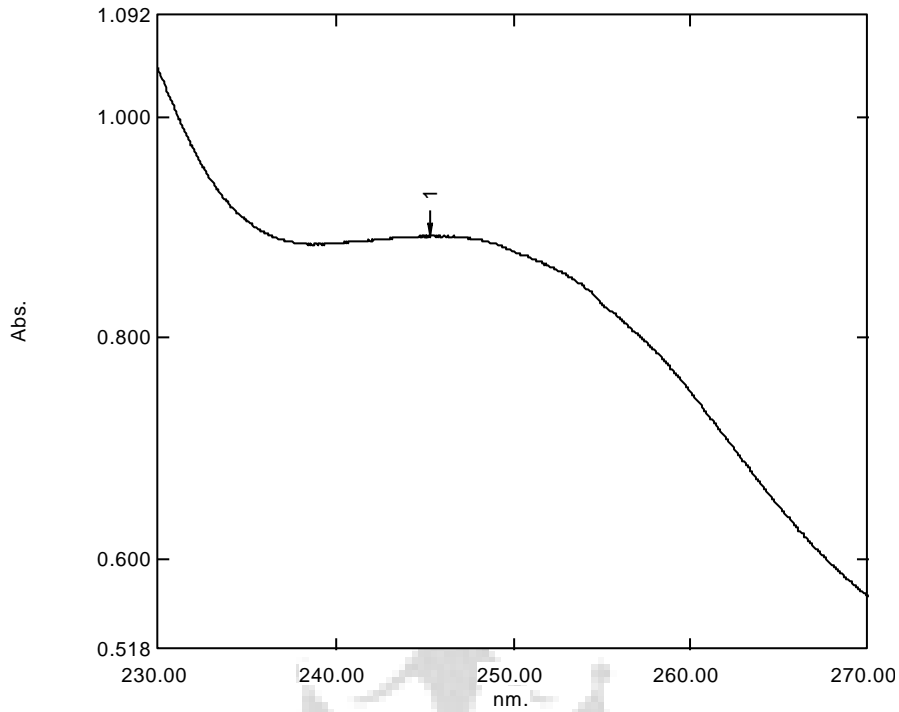


Figure 3: Area Under curve of Clonazepam 20µg/ml

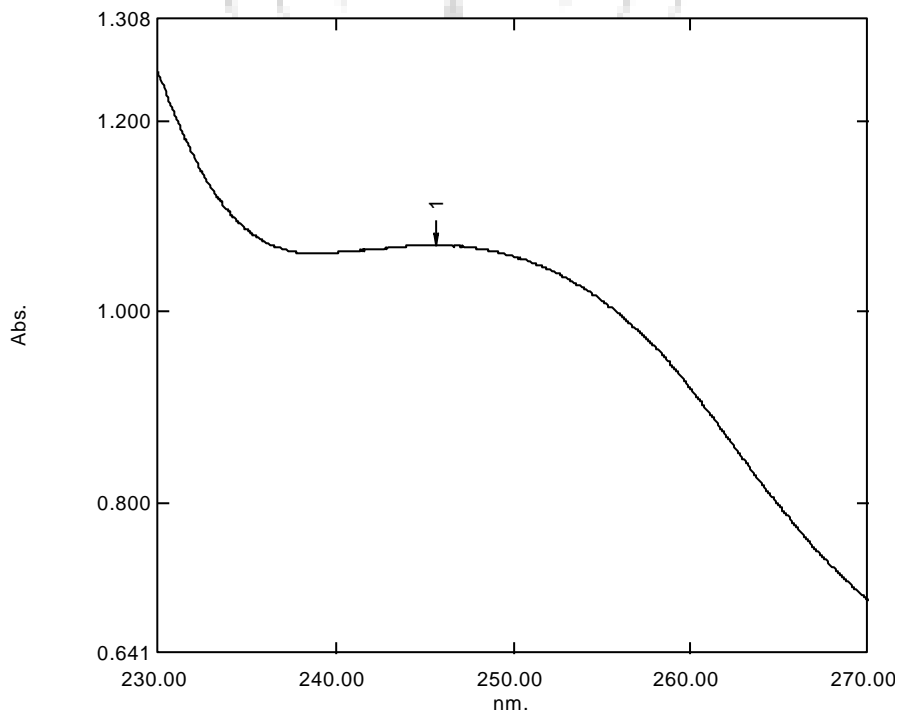


Figure 4: Area Under curve of Clonazepam 24µg/ml

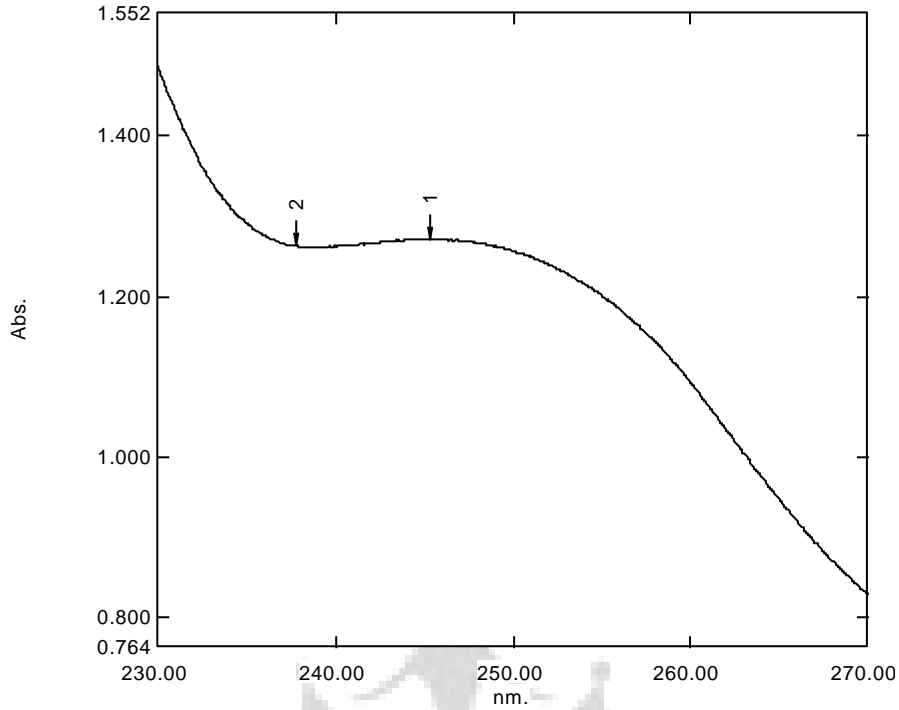


Figure 5: Area Under curve of Clonazepam 28µg/ml

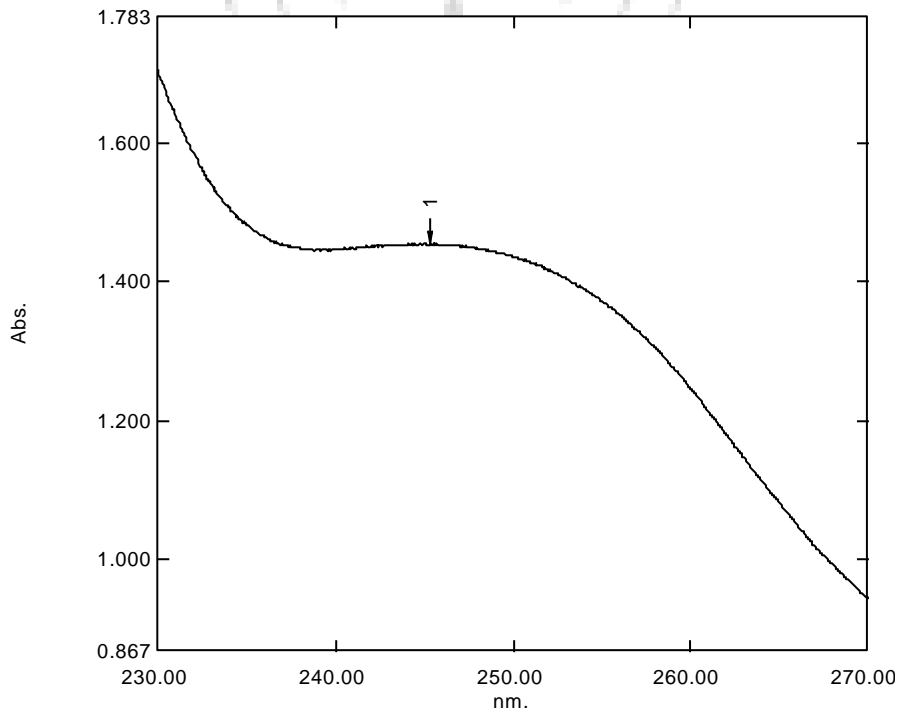


Figure 6: Area Under curve of Clonazepam 32µg/ml

Validation of purposed method:

Estimation of Drug from Dosage Form: (Tablet Assay Study)

Brand name- Revotril

Standard:

From the standard stock solution of Clonazepam, appropriate aliquots were pipetted out into 25ml volumetric flask and dilutions were made with methanol to obtain working standard solution of Clonazepam 20µg/ml. This concentration was scanned at area of 250 to 240m.

Sample:

Twenty tablets of brand Lonazep and containing 0.5mg of Clonazepam weighed, and finally powered. A quantity of powder sample of Lonazep equivalent to 100mg of Clonazepam was taken into volumetric flask. And dilution was made to get concentration of 20µg/ml. respectively. These concentrations were scanned at area between 250 to240.

Table 3: Assay for Revotril in Tablet Form for Area Under Curve method

Brand Name	Label Claim (mg/capsule)	Amount Found (mg/capsule)	% Of Label claim	Mean	SD	CV
Revotril	0.5	0.491	98.2	100.68	4.1703	17.39
	0.5	0.54	108			
	0.5	0.50	100			
	0.5	0.496	99.2			
	0.5	0.49	98			

Accuracy (Recovery Study):

Recovery experiments are used for the study of accuracy method. This study was carried out by adding known amount bulk sample to the capsule and recovery was performed at three levels, 80, 100 and 120% of Clonazepam standard concentration. Samples for recovery studies were prepared according to aforementioned procedure. 3 samples were prepared for each recovery

level. The solutions of sample were analyzed and % recoveries were calculated by using following formula.

$$\% \text{ Recovery} = \frac{\text{observed amount of compound in sample}}{\text{Amount of all compound present in sample}} \times 100$$

The recovery values are summarized in following table 4.

Table 4: Result for accuracy parameters of Clonazepam (Brand Revotril)

Label % recovery	*Amount present (mg/tablet)	Amount of Standard added (mg/tablet)	Total Amount Recovered (mg/tablet)	% recovery	%mean recovery	SD	CV
80	0.5	0.4	0.404	101.23	100.8	0.52	0.272
80	0.5	0.4	0.401	100.25			
80	0.5	0.4	0.403	100.93			
100	0.5	0.5	0.4997	99.95	99.76	0.243	0.0594
100	0.5	0.5	0.4993	99.86			
100	0.5	0.5	0.497	99.49			
120	0.5	0.6	0.599	99.98	99.98	0.0602	0.0036
120	0.5	0.6	0.6003	100.05			
120	0.5	0.6	0.5995	99.93			

Precision:

The precision (inter-day) was carried out by using four independent sample of Clonazepam. The intermediate precision (inter-day precision) of the method was evaluated using four different analysts in the same laboratory. (Table 5)

Table 5: Determination of Precision of Clonazepam

Sample Number	Assay of Clonazepam as % of Labeled amount (inter – day precision)			
	Analyst 1	Analyst 2	Analyst 3	Analyst 4
1	100.15	99.97	99.98	100.11
2	100.13	100.04	99.85	99.97
3	100.20	100.15	99.95	99.96
4	100.01	99.92	100.06	100.08
Mean	100.12	100.02	99.96	100.03
SD	0.08057	0.0996	0.08679	0.07615
CV	0.00649	0.00993	0.007533	0.00580

RESULTS AND DISCUSSION

The standard solutions of Clonazepam in Methanol (22µg/ml each) subjected to scanning under area between 260nm to 240nm and the area was found to be 9.4909 for area under curve method using Shimadzu 1800 spectronic UV-Visible spectrophotometer. The calibration curve of Celecoxib was found to be linear at conc. range 20 to 32µg/ml at area between 250 to 240nm. Therefore, it was clear that Clonazepam can be determined in the presence of methanol with no intervention of any irrelevant substance in pharmaceutical products.

With the intention of determining the practicability of the developed technique for the assessment of commercially available brands (Revotril) of medicinal formulations, the technique was initially attempted on bulk drugs in their synthetic mixture sample as well as concentrations were estimated. Then the technique was subjected to the assay of in marketed dosage forms and satisfactory results were attained within the appropriate limits as per the content of the label claim for Clonazepam.

The newly developed method was validated as per the international guidelines and parameters. The novel method for the quantitative investigation of Clonazepam was subjected to different validation parameters like specificity and selectivity in presence of formulation additives and excipients, studied for Linearity and range at different levels of concentrations and calibration

standards where the determination range was optimized, accuracy was proved by recovery studies at different concentration levels, precision was established through inter-day precision studies, where the samples were subjected to changed conditions other than optimized parameters.

CONCLUSION

From the above experimental studies, it can be concluded that Area under curve method by UV spectrophotometry instrument developed for estimation of Clonazepam. The proposed methods for the selected drugs were found to be precise and accurate. The most important features of spectrophotometric methods are their rapidity & simplicity. Results of validation parameters demonstrate that these performed analytical procedures are suitable for its intended purpose and meet the criteria defined in ICHQ2A/B guidelines. The method is an excellent alternative to HPLC methods for routine analysis and accurate and better than the zero order UV spectrophotometric method.

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