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R E S E A E C H A R T I C L E

Method Development and Validation of Acetazolamide Single Drug Molecule by Using UV Double Beam Spectrophotometer

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A B S T R A C T

This UV-spectrophotometric technique is quite simple, accurate, precise, reproducible, and sensitive. The UV method has been developed for quantification of Acetazolamide in tablet formulation. The validation procedure confirms that this is an appropriate method for their quantification in the formulation. It is also used in routine quality control of the formulations containing this entire compound.

Keywords: Acetazolamide, UV-spectrophotometric technique

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C O N T E N T S

1. Introduction

Acetazolamide is chemically named as N-(5-sulfamoyl-1,3,4-thiadiazol-2-yl)acetamide[1]. The anticonvulsant activity of Acetazolamide may depend on a direct inhibition of carbonic anhydrase in the CNS, which decreases carbon dioxide tension in the pulmonary alveoli, thus increasing arterial oxygen tension. The diuretic effect depends on the inhibition of carbonic anhydrase, causing a reduction in the availability of hydrogen ions for active transport in the renal tubule lumen[2]. This leads to alkaline urine and an increase in the excretion of bicarbonate, sodium, potassium, and water[3].

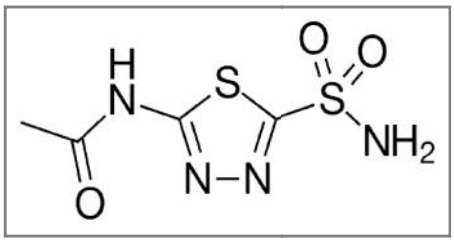

Fig 1: Structure of Acetazolamide

2. Materials and Methods

Material

Acetazolamide was a gift sample from Dr. Reddys Lab, Hyderabad. All chemicals(distilled water, methanol) and reagents used were of analytical grade and purchased from Qualigens Fine Chemicals, Mumbai, India[4].

Apparatus

A Labindia UV–visible spectrophotometer (UV-T60-India) was used for all absorbance measurements with matched quartz cells[5].

Methodology

Method Development

Preparation of standard stock solution: Accurately weighed 10 mg of Acetazolamide was transferred to a 100 ml volumetric flask, dissolved in 20 ml distilled water by shaking manually for 10 min[6]. The volume was adjusted with the same up to the mark to give the final strength, i.e. 100 µg/ml[7].

Selection of wavelength for analysis of acetazolamide: Appropriate volume 0.5 ml of standard stock solution of acetazolamide was transferred into a 10 ml volumetric flask, diluted to a mark with distilled water to give concentration of 5 µg/ml(and also 10, 15µg/ml)[8][9]. The resulting solution was scanned in the UV range (200–400 nm). In spectrum acetazolamide showed absorbance maximum at 288 nm[10][11].

Validation of the method

The method was validated in terms of linearity, accuracy, precision, and ruggedness[12].

Linearity study:

Different aliquots of Acetazolamide in the range 0.5–3 ml were transferred into series of 10 ml volumetric flasks, and the volume was made up to the mark with distilled water to get concentrations 5, 10, 15, 20, 25, and 30 µg/ml, respectively[13][14]. The solutions were scanned on a spectrophotometer in the UV range 200–400 nm. The spectrum was recorded at 288 nm[15]. The calibration plot was constructed as concentration vs. absorbance

Accuracy:

To the preanalysed sample solutions, a known amount of standard stock solution was added at different levels, i.e. 50%, 100%, and 150%. The solutions were reanalyzed by the proposed method[16][17].

Precision:

Precision of the method was studied as intraday and interday variations. Intraday precision was determined by analyzing the 10, 15 and 20 µg/ml of acetazolamide solutions for three times in the same day[18][19]. Interday precision was determined by analyzing the 10, 15, and 20 µg/ml of acetazolamide solutions daily for 3 days over the period of week**[20]**.

Sensitivity:

The sensitivity of measurements of acetazolamide by the use of the proposed method was estimated in terms of the limit of quantification (LOQ) and limit of detection (LOD)[21]. The LOQ and LOD were calculated using equation LOD = 3 × N/B and LOQ = 10 × N/B, where 'N' is standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve[22][23].

Repeatability:

Repeatability was determined by analyzing 20 µg/ml concentration of acetazolamide solution for six times[24].

Ruggedness:

Ruggedness of the proposed method is determined for 20 µg/ml concentration of acetazolamide by analysis of aliquots from a homogenous slot by two analysts using same operational and environmental conditions[25].

3. Results and Discussions

Linearity:

Table 1: Results of Precision for linearity

Concentration (ug/ml)	absorbance(nm)
0	0
5	0.142
10	0.273
15	0.404
20	0.532
25	0.659
30	0.791

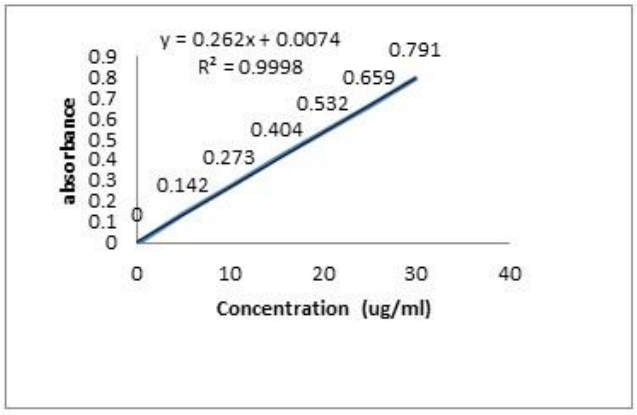

Fig 2: Results of Calibration graph

Accuracy:

The accuracy study was performed for 50%, 100% and 150 % for Acetazolamide [26][27]. Each level was injected in triplicate into chromatographic system[28]. The area of each level was used for calculation of % recovery [29].

Accuracy (recovery) data for acetazolamide

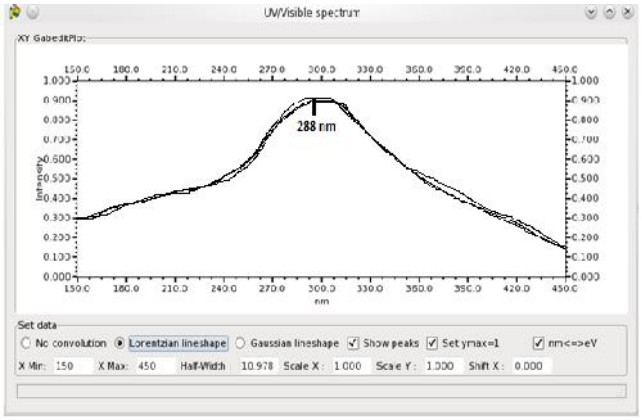

Fig 3: Accuracy 50%

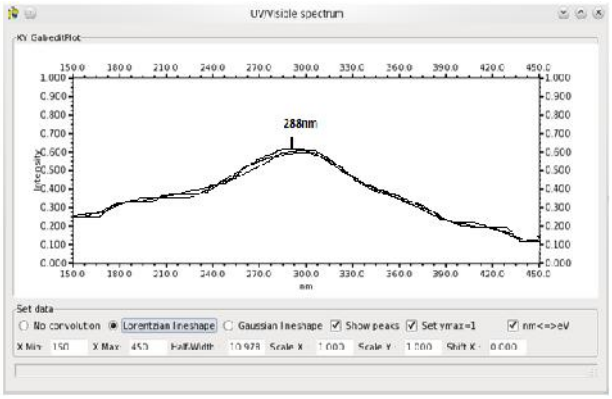

Fig 4: Accuracy 100%

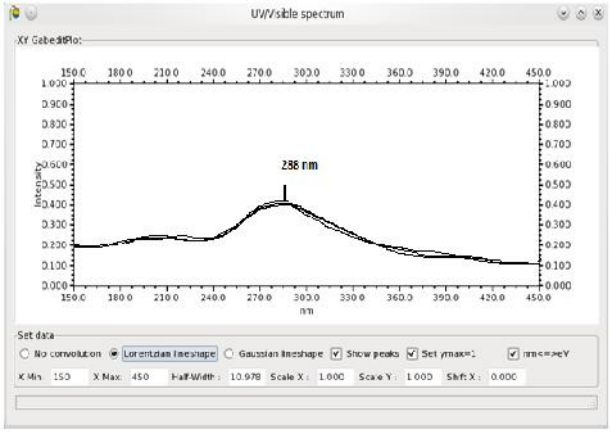

Fig 5: Accuracy 150%

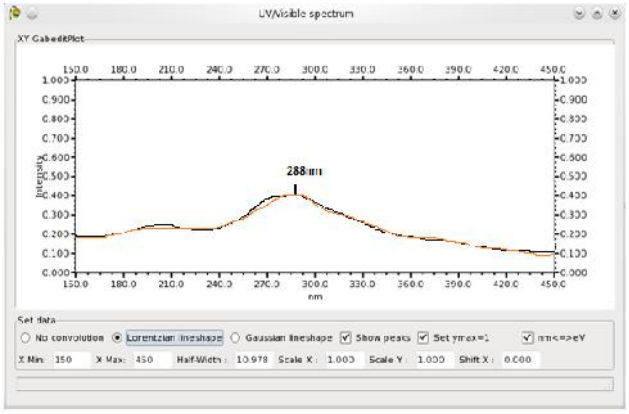

Fig 6: Intrday precision

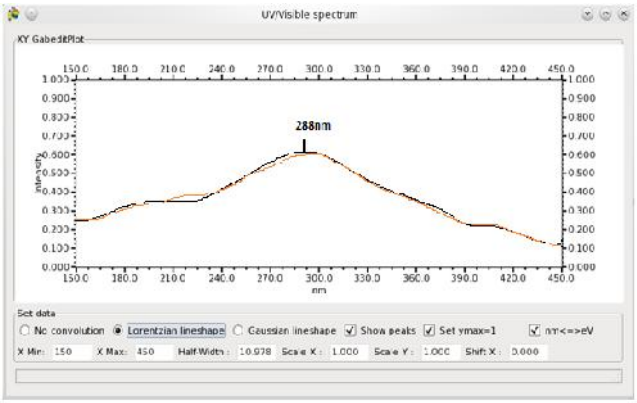

Fig 7: Intermediate precision

Sensitivity:

The linearity equation was found to be Y = 0.262X + 0.0074. The LOQ and LOD for acetazolamide were found to be 0.51 µg and 2.99 µg, respectively[30][31].

Repeatability:

Repeatability was determined by analyzing 20 µg/ml concentration of acetazolamide solution for six times and the % amount found was 99.69 % RSD < 2.

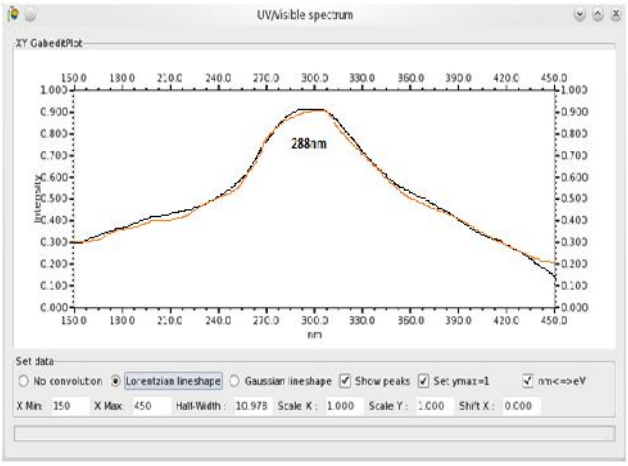

Fig 8: Results for Repeatability

Ruggedness:

The peak area was measured for same concentration solutions, six times. The results are in the acceptable range for both the drugs[32]. The result showed that the % RSD was less than 2%.

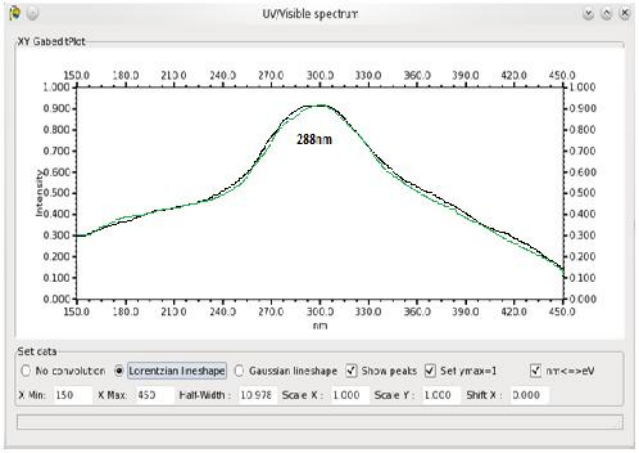

Fig 9: Results of Ruggedness

4. Conclusion

This UV-Spectrophotometric technique is quite simple, accurate, precise, reproducible, and sensitive. The UV method has been developed for quantification of Acetazolamide in tablet formulation. The validation procedure confirms that this is an appropriate method for their quantification in the formulation. It is also used in routine quality control of the formulations containing this entire compound.

Table 2: Results of Accuracy

%Concentration (at specification Level)N=3	absorbance	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	0.4213	2.5	2.498	99.92	99.65
100%	0.6213	5.0	4.990	99.08	
150%	0.9199	10	9.995	99.95	

Table 3: Intra-day and inter-day precision determined for three different concentrations of acetazolamide (*n*=3)

Concentration (µg/mL)	Intra-day precision			Inter-day precision		
	Absorbance measured	RSD (%)	Average (%)	Absorbance measured	RSD (%)	Average (%)
10	0.4113	0.140	98.96	0.4110	0.240	98.96
15	0.6147	0.094	98.60	0.6153	0.094	98.70
20	0.9210	0.122	98.77	0.8213	0.070	98.81

Table 4: Results of Repeatability

Concentration (µg/mL)	Absorbance measured (Mean ± SD)	Amount Found (%)	RSD (%)
20	0.8310±0.0324	99.69	0.02

Table 5: Results of Ruggedness

Analyst	Concentration (µg/mL)	Absorbance measured (Mean ± SD)	Amount Found (%)	RSD (%)
I	20	0.8116±0.0015	98.98	0.02
II	20	0.8214±0.0010	99.12	0.01

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