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Original Article

Spectrophotometric estimation of Acetazolamide in pharmaceutical formulations

Garikapati Devala Rao, Madhusudhanareddy Induri

Department of Pharmaceutical Analysis, K.V.S.R Siddhartha College of Pharmaceutical Sciences, Pinnamaneni Polyclinic Road, Siddhartha Nagar, Vijayawada-520 010, Andhra Pradesh, India

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ABSTRACT

A precise and sensitive UV spectrophotometric method for the quantification of acetazolamide in pharmaceutical formulations has been described. The acetazolamide shows absorption maxima at 265 nm and obeyed Beer's law in the range of 6-16 µg/mL using methanol as solvent. The results were treated statistically and were found highly accurate, precise and reproducible. This statistical approach gives optimum results for the eliminating fluctuations coming from instrumental or experimental conditions. It was concluded that the proposed method is simple, easy to apply, economical and could be used as an alternative to the existing spectrophotometric and non-spectrophotometric methods for the routine analysis of acetazolamide in pharmaceutical formulations.

Keywords: Acetazolamide, Pharmaceutical Formulations, UV-Spectrophotometry

INTRODUCTION

Glaucoma is becoming an increasingly important cause of blindness, as the world's population ages. It is group of eye disorders that have few symptoms in their early stages, but eventually leads to damage of the optic nerve (the bundle of nerve fibers that carries information from the eye to the brain), which can then lead to vision loss or complete blindness. Glaucoma is a leading cause of irreversible blindness with 60 million cases worldwide and 2.2 million in the United States. Up to 50 percent of those with glaucoma are not aware they have it. Early diagnosis and treatment is critical to managing glaucoma [1-3].

Glaucoma, however, presents perhaps an even greater public health challenge than cataracts: because the blindness it causes is irreversible. In primary open angle glaucoma, the channels that drain fluid within the eye become blocked, causing the pressure within the eye to rise. It causes gradual loss of vision. There are few symptoms so that people may not notice for a long time that they are losing their sight. In angle closure glaucoma, there is a similar build-up of fluid within the eye, but the onset is much more sudden. Symptoms include headaches, blurred vision and pain in the eye [4, 5]. People of Asian descent are much more likely to suffer from angle closure glaucoma, while those of African or European origin are more likely to develop primary open angle glaucoma. In Southern India, studies have shown a prevalence of glaucoma of 2.6% and 90% of these cases have never been diagnosed before, compared to about 50% previously undiagnosed when similar studies are done in Europe. In African populations, the prevalence is 1–2%, but can rise to about 10% in the Caribbean. There are several reasons why glaucoma has now become the second leading cause of blindness, experts say. One is age. As a population grows older, the prevalence of glaucoma rises [6, 7].

Carbonic anhydrase inhibitors work in glaucoma by reducing the production of fluid in the eye. Carbonic anhydrase inhibitors were initially developed as agents to treat high blood pressure (hypertension) but then were found to also decrease intraocular pressure. They reduce the action of an enzyme called carbonic anhydrase, which is necessary for the production of aqueous fluid. The carbonic anhydrase inhibitors include oral agent's acetazolamide and methazolamide, and eye drops brinzolamide and dorzolamide. Acetazolamide works by blocking the action of an enzyme called carbonic anhydrase. Blocking this enzyme reduces the amount of fluid that you make in the front part of your eye (called aqueous humour), and this helps to lower the pressure within your eye. Accurate and

***Corresponding Author Address:** Madhusudhanareddy Induri, Department of Pharmaceutical Analysis, K.V.S.R Siddhartha College of Pharmaceutical Sciences, Pinnamaneni Polyclinic Road, Siddhartha Nagar, Vijayawada-520 010, Andhra Pradesh, India*

very precise analytical methods are required to quantify these drugs in pharmaceutical formulations to help medical community for providing effective dose during treatment of glaucoma and to have better pharmacokinetic applications [8, 9].

In line to these expectations, over a decade there were many methods developed on acetazolamide like HPLC [10-12], Spectrophotometric [13], LC-MS [14] and HPTLC [15] instrumental techniques. However, due to various limiting factors like low sensitivity, high plasma requirement for establishing methods, expensiveness, longer run times, thermal stability of molecules and difficulty in processing large samples using above said analytical techniques, it has have paved the way for researchers to shift their focus to develop more sensitive and accurate spectrophotometric methods.

MATERIALS AND METHODS

Chemicals: The Acetazolamide reference standard (assigned purity 99.63%) was kindly supplied by Hetero Drugs Limited (Hyderabad). The commercial pharmaceutical formulations were obtained from local Pharmacies. Methanol and dimethyl formamide was procured from SDF Chemicals, India. Distilled water was prepared by Aquatron deionizing water system.

Instrumentation: The analysis was performed in 10mm quartz cells using T60U UV-Visible spectrophotometer (PG Instruments Ltd., England) with a fixed 2nm spectral bandwidth and UV-Win5 software v5.1.1 was used for all absorbance measurements.

Preparation of Standard Solutions: The standard solution (1000 µg/mL) was prepared by accurately weighing 100 mg of Acetazolamide in 100 mL volumetric flask containing 50 mL of methanol and sonicated for about 20 min, and then the volume was made up to the mark with methanol. From this 10 mL was pipette out into a 100 mL volumetric flask and volume was made up to the mark with methanol to get final concentration of 100 µg/mL.

Preparation of sample solution: For analysis of marketing formulations, twenty tablets were weighed accurately and powdered. The powder equivalent to 100 mg of the drug weighed accurately and transferred to 100mL volumetric flask containing 50mL of methanol. The mixture was sonicated to dissolve, make up the volume with methanol. The above solutions were filtered through Whatmann filter paper and the solution was transferred into volumetric flask, and was made up to the mark with methanol to obtain a final concentration of 20 µg/mL. All determinations were conducted with six replicates.

Method Validation:
The method was validated according to International Conference on Harmonization (ICH) 2QB guidelines for validation of analytical procedure in order to determine the linearity, limit of detection, limit of quantitation, accuracy and precisions [16].

RESULTS AND DISCUSSION

Acetazolamide was analyzed using proposed UV spectrophotometric method in pharmaceutical formulation. It was completely soluble in methanol and hence methanol was selected as the solvent for acetazolamide to obtained UV spectrum in the range of 200-400 nm. After the evaluation of the spectrum, acetazolamide showed maximum absorption at 265 nm (Figure 1).

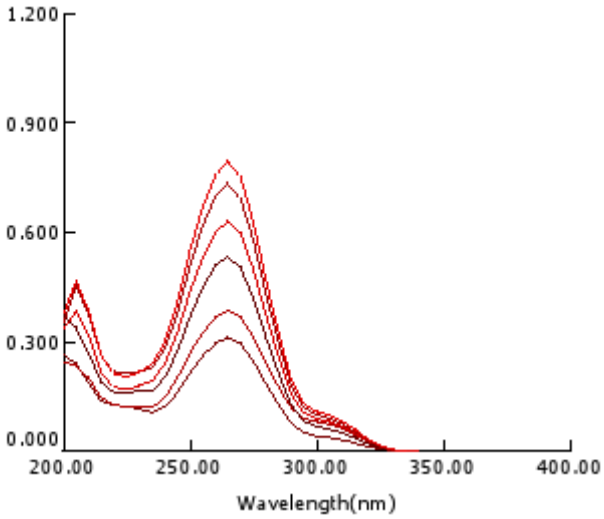

Figure 1: Absorbance spectrum of Acetazolamide

Table: Calibration data of Acetazolamide

Parameters	**Results**
λ_{max} (nm)	265
Beer's law range (µg/mL)	6-16
Molar extinction coefficient (1/mol/cm)	0.05336
Sandell's sensitivity (µg/cm^2)	0.018741
Limit of detection (µg/mL)	0.05
Limit of quantitation (µg/mL)	0.14
Regression equation	
Intercept (a)	0.0108
Slope (b)	0.0521
Correlation coefficient (r^2)	0.9993

A linearity set consisting of six solutions in working range of 6-16 µg/mL were freshly prepared and scanned in the UV region. The absorbance was recorded and calibration curve against concentration was plotted, which followed the Beer's law and gave a straight line (Table 1 and Figure 2).

The accuracy of the method was evaluated through the recovery studies. Recovery studies were carried out by addition of known quantity of pure drug solution to pre-analyzed sample solution at three different concentration levels (50, 100 and 150%). The percentage recovery values were found to be 100.93-101.63 with %RSD of <2% (Table 2), which indicates that the proposed method was accurate. Precision was determined as intra-day and intermediate precision, in accordance with ICH guidelines. The intra-day and intermediate precision were determined by analyzing the samples of acetazolamide at a concentration of 8, 10 and 12 µg/mL. The results of intra-day and intermediate precision studies were shown in Table 3. The low %RSD values obtained from the analysis of tablets indicated that the method was highly precise.

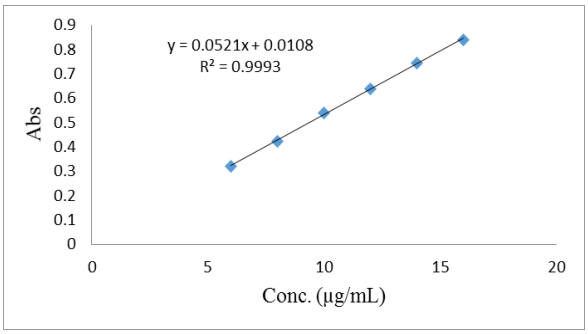

Figure 2: Linearity graph of Acetazolamide

Table 2: Accuracy results of Acetazolamide (standard addition technique)

Amount (%) of drug added to analyte	Theoretical content (µg/mL)	Conc. found (Mean± SD)*	%RSD	% Recovery
50	2.5	2.54±0.04	1.573	101.63
100	5	5.03±0.04	0.794	100.59
150	7.5	7.57±0.0966	1.276	100.93

Table 3: Precision data of Acetazolamide

Analyte Conc. (µg/mL)	Intra-day precision		Intermediate precision			
			Analyst 1		Analyst 2	
	*Mean ±SD	%RSD	*Mean ±SD	%RSD	*Mean ±SD	%RSD
8	0.354±0.0067	1.879	0.338±0.0031	0.903	0.335±0.0035	1.047
10	0.463±0.0085	1.838	0.421±0.0032	0.764	0.437±0.0036	0.825
12	0.553±0.0059	1.060	0.537±0.0025	0.467	0.528±0.0026	0.501

*Triplicate results

The UV spectrophotometric method was applied to the quantification of acetazolamide in tablet dosage forms available in local market. The results were tabulated in Table 4. It can be seen that, the results obtained by proposed method was very much similar to that of established methods.

Conclusion

The proposed method is rapid, accurate, precise and sensitive for the quantification of acetazolamide from its pharmaceutical dosage forms by the UV spectrophotometric method. The method relies on the use of simple working procedure comparable to that achieved by sophisticated and expensive technique like HPLC, and hence this method can be routinely employed in quality control for analysis of acetazolamide in pharmaceutical formulations.

Table 4: Assay results of acetazolamide in tablet dosage forms

Brand Name	Label Claim (mg)	% Label Claim	SD	%RSD
Aceact	250	99.43	0.948	0.953
Acetamide	250	99.94	1.011	1.011
Aceta-SR	250	99.83	0.732	0.733
ACMox	250	99.62	0.877	0.88

* Average of six determinations

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