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Colorimetric determination of acetazolamide in pharmaceutical preparation by simple diazotization method

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ABSTRACT

A colorimetric method for determination of acetazolamide is described. The method is based on the formation of red coloured azo product by diazotization of acetazolamide molecule followed by a coupling reaction with N-(1-naphthyl) ethylenediamine dihydrochlorhydrate in acid medium. Absorbance of the resulting red azo product is measured at 493nm and is conforms to Beer's law over the range 10-200μgml^{-1}. The optimum reaction conditions were evaluated and common excipients used as additives in pharmaceutical preparations do not interfere in the proposed method. The method is simple, sensitive and rapid also is successfully employed for the determination of acetazolamide in pharmaceutical preparations.

KEYWORDS

Acetazolamide;
Diazotization;
N-(1-naphthyl) ethylenediamine dihydrochlorhydrate;
Colorimetric.

INTRODUCTION

Acetazolamide (5-acetamido-1,3,4-thiazole-2-sulfonamide) is a carbonic anhydrase inhibitor which reduces the rate of aqueous humor formation and correspondingly decreases the intra ocular pressure in patients with glaucoma. It is also used, either alone or in association with other antiepileptics, for the treatment of various forms of epilepsy, and has been used clinically from 1954[1].

Historically, several different techniques have been used to measure acetazolamide in pharmaceutical preparation and biological fluids, including spectrophotometry[2,3], gas chromatography- mass spectrometry[4,5], polarography[6], RMN[7,8], amperometry[9], alkalimetry[10,11] and liquid chromatography[12-26].

N-(1-naphthyl) ethylenediamine dihydrochlorohydrate (NEDA) in acid medium or alkaline was used as coupling agent for determination spectrophotometry of different drugs in forms pharmaceuticals[27,32].

This report describes a new spectrophotometric method, simple, fast, and reliable for the determination of acetazolamide in either pure form or in its pharma-

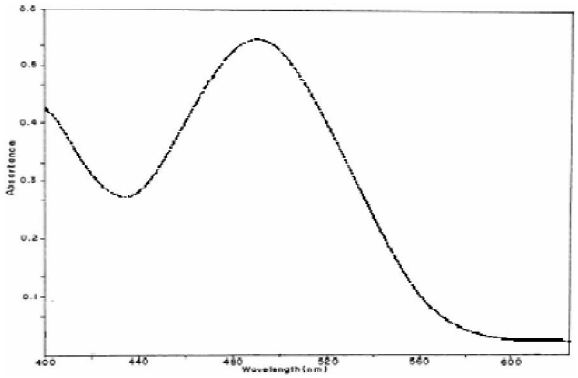
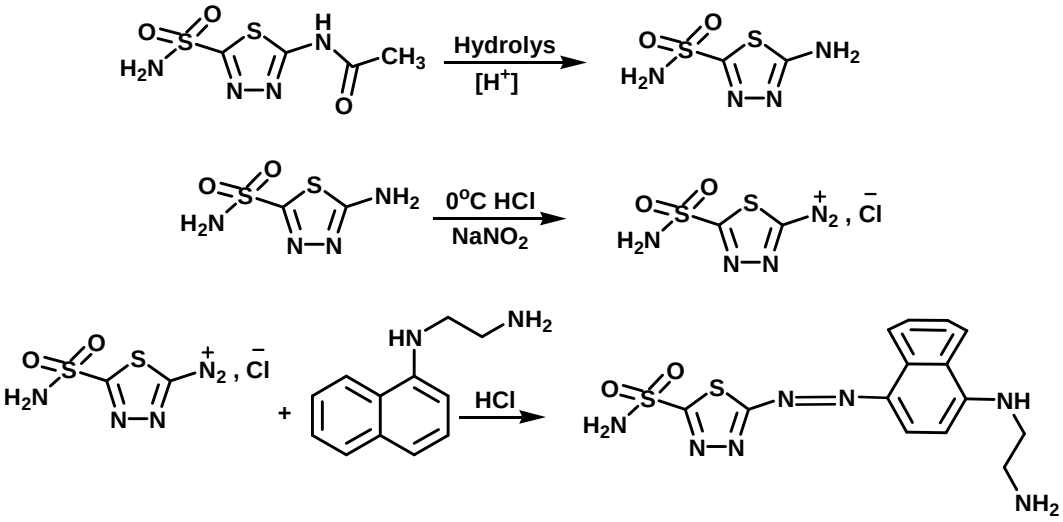


Figure 1 : Absorption spectra of acetazolamide product



Scheme 1 : Reaction sequence for the formation of red colored product

ceuticals formulations.

The scientific novelty of the present work is that the reagents used in both the methods are easily available and the chemistry of these reagents is already well established. The reactions involved with these reagents are simple, rapid and sensitive in their range of determination compared with other established methods.

MATERIALS AND METHODS

Instrumentation

A Perkin Elmer UV-visible spectrophotometer type lambda 20 version (1.01) with 1.0cm matched cells was used.

Reagents

All chemicals used were of analytical-reagent grade.

N-(1-naphthyl)-ethylenediamine dihydrochloride (NEDA) was purchased from Merck, Sodium nitrite and sulfamic acid was purchased from Fluka. Deionized water was used to prepare all solution and in all experiments.

Solutions

Accurately weighed (100mg) acetazolamide was transferred to a 100ml beaker containing 30 ml of 40% sulfuric acid and refluxed for 60 min. The solution was cooled and diluted to volume with water. The working standard solution of the hydrolysis acetazolamide containing 100µg ml^{-1} was prepared by further dilution.

0.1% of (NEDA) solution, 1% sodium nitrite solution, 2% sulfamic acid solution and HCl 1M solution were prepared separately in Deionized water in amber-glass volumetric flasks.

Procedure

A liquots of standard solution of acetazolamide were transferred into a 20ml calibrated flask.

2ml of HCl 1N was added, cool in an ice bath and add 2ml of 1% $NaNO_2$, stir the solution during 5min. add 2ml of 2% sulfamic acid stir again for 5min. Add 2ml of 0.1% (NEDA). After 15min made up to the mark with HCl 1M solution.

Procedure for assay of pharmaceutical tablets

Twenty tablets were powdered and mixed thoroughly. An amount equivalent to 100mg of Acetazolamide was taken and subjected to hydrolysis using 30ml of 40% sulfuric acid. The filtrate was made up to 100ml and an aliquots of this solution was treated as described above for pure sample in both the method.

RESULTS AND DISCUSSION

The spectrophotometric method for the determination of acetazolamide is based on the hydrolysis of the amide to amino group with sulfuric acid flowed by diazotization and coupling with (NEDA) to form the red colored product.

Spectral characteristics

The absorption spectra of the red colored product with λ_{max} = 493nm is presented below (Figure 1).

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TABLE 1 : Parameters for the spectrophotometric determination of acetazolamide parameters/characteristics acetazolamide

Parameters/Characteristics	Acetazolamide
Colors	Red
λ_{max}(nm)	493
Stability (in days)	2
Beer's law range (μg ml^{-1})	10-200
Molar absorptivity (l mol^{-1} cm^{-1})	1.17×10^3
Regression equation	(y)[a]
Slope (a)	0.0057
Intercept (b)	0.0169
Correlation coefficient	0.9916
R.S.D. (%)[b]	1.01

[a]y = ax + b where x is the concentration of acetazolamide in μg ml^{-1}, [b]relative standard deviation (n=5)

The reagent blank has practically negligible absorption at this wavelength.

Reaction mechanism

The stochiometric equation derived is illustrated in Schema 1.

Optimization of reactions conditions

The factors affecting color development, reproducibility, sensitivity, and conformity with Beer's law investigated.

It was found that (0.5-4)ml of HCl 1M, (1-4)ml of 1% Na NO_2 solution,(1-4)ml of 0,1% (NEDA) solution was necessary to achieve maximum color intensity.

The exec of nitrite sodium could be removed by the addition of 2% sulfamic acid solution. An excess of sulfamic acid has no effect on the color intensity of the product formed.

Quantification

Beer's law is obeyed over the acetazolamide concentration range of 10-200μg/ml.

The proposed procedure is validated by determination optical parameters, which are listed in TABLE 1.

Interference

The effects of various substances that often accompany acetazolamide in pharmaceutical preparations were studied. Majority of the common excipients do not interfere in the present method. The results are given in TABLE 2.

TABLE 2 : Determination of acetazolamide in presence of excipients

Excipients	Amount (mg)	Recovery of Acetazolamide, (±RSD)[a]
Calcium carbonate	100	98.1±1.1
Gallatin	40	97.5±0.9
Magnesium stearate	20	97.9±0.7
Starch	100	98.2±1.2

[a]average of five determination, R.S.D., relative standard deviation

TABLE 3 : Assay of acetazolamide in pharmaceutical preparation

Product	Label claim (mg)	Recovery of Acetazolamide, (± RSD)[a]
Diamox	250/tablet	99.8 (±0.9)

[a]Average of five determinations, R.S.D., relative standard deviation

Application

The application of the method to assay pharmaceutical preparation was examined. The assay of Acetazolamide, singly and in various combinations is shown in TABLE3.

The excellent recoveries obtained indicated the absence of any interference from the excipients.

CONCLUSION

The method is found to be simple, economical, selective and more sensitive than most of the spectrophotometric methods reported. The statistical parameters and recovery study data clearly indicate the reproducibility and accuracy of the method. Analysis of the authentic samples containing acetazolamide showed no interference from the common excipients. Thus the method can be adopted for routine analyse in quality control laboratories.

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