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QUANTITATION OF ACETAZOLAMIDE IN PHARMACEUTICAL DOSAGE FORMS USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Stability-indicating reverse phase high-pressure liquid chromatography methods to quantify acetazolamide in pharmaceutical dosage forms have been developed. The methods are accurate and precise with percent relative standard deviations based on six injections of 0.9 with a semipolar column and 0.4 with a nonpolar column. No preliminary extraction procedure is required to assay the acetazolamide sodium in vials and a very simple extraction is needed to extract acetazolamide from the tablets or contents of the capsules. Acetazolamide appears to be very unstable in the presence of 0.1N NaOH with a half-life of about 14 days. It underwent hydrolysis to acetic acid and 5-amino-1,3,4-thiadiazole-2 sulfonamide. The hydrolysis followed first-order law with a K value of 0.0495 day^{-1} at 25°.

BACKGROUND

Acetozolamide is one of the most commonly used diuretics and carbonic anhydrase inhibitor. It is available in a number of dosage forms such as acetazolamide sodium for injection in vials, sustained

release capsules, and tablets. The USP-NF method[1] for the quantitation of acetazolamide in injection is based on UV spectroscopy. The USP-NF method[2] for the determination of acetazolamide in tablets is based on polarography. The capsules are not official in the USP-NF.

The quantitation of acetazolamide in biological fluids has been recommended[3,4] using high-performance liquid chromatography. These methods were not tried for the quantitation of acetazolamide in dosage forms especially in the presence of its major product of decompositon, 5-amino-1,3,4-thiadiazole-2 sulfonamide.

The purpose of these studies was to develop a stability-indicating high-performance liquid chromatography method for the quantitation of acetazolamide in pharmaceutical dosage forms.

MATERIALS AND METHODS

Chemicals and Reagents: All the chemicals and reagents were either USP-NF or ACS grade and used as received. Acetazolamide powder[5] was used without further purification.

Apparatus: The high-pressure liquid chromatograph[6] was equipped with a multiple wavelength UV detector[7] and a recorder[8].

Columns: Two semipolar columns[9] (30 cm x 3.9 mm i.d.) and a nonpolar column[10] (25 cm x 4.6 mm i.d.) were used.

Chromatographic Conditions: The mobile phase for semipolar columns was 6% (V/V) of acetonitrile in 0.02 M ammonium acetate buffer in water. For nonpolar column, the mobile phase was 12% (V/V) methanol, 2% (V/V) acetonitrile in 0.02 M potassium phosphate monobasic in water. The flow rate for both columns was 2 ml/min. The sensitivity was 0.04 AUFS at 265 nm. The chart speed was 30.5 cm/hr and the temperature was ambient.

Peparation of Stock Solutions: The stock solution of acetazolamide (0.5 mg/ml) was prepared by dissolving 50.0 mg of the powder in 0.5 ml of ∿1 N NaOH solution and then adding enough water to make 100.0 ml in a volumetric flask. This solution was prepared fresh daily. The stock solution of hydrochlorothiazide (the internal standard for semipolar columns) was prepared by dissolving 100.0 mg of the powder in enough methanol to make 100.0 ml. The solution was stable for at least one month. The stock solution of sulfamerazine (1.2 mg/ml) was prepared according to procedure given above for actetazolamide. This solution was prepared fresh every week. Sulfamerazine solution was used as an internal standard with nonpolar column.

Preparation of Standard Solutions: The standard solutions containing the desired concentrations of acetazolamide and the internal standard were prepared by diluting the stock solutions with water. Before final dilution ∿5 ml of 0.1 M potassium phosphate monobasic solution was added to neutralize sodium hydroxide present in the stock solutions. The most commonly used standard solutions contained; 15 µg/ml of acetazolamide and 20 µg/ml of hydrochlorothiazide for semipolar columns; 15 µg/ml of acetazolamide and 18 µg/ml of sulfamerazine for nonpolar column.

Preparation of Assay Solution from Vials Containing Sodium Acetazolamide Powder: The contents of one vial were dissolved in enough water to make 100.0 ml. The solution was further diluted 10.0 to 100.0 ml with water. A 3.0 ml quantity of this solution was mixed with 2.0 ml of the stock solution of hydrochlorothiazide, 5 ml of ∿0.1 M KH_2PO_4 solution and brought to volume (100.0 ml) with water. For nonpolar column, 2.0 ml of hydrochlorothiazide solution was substituted with 1.5 ml of stock solution of sulfamerazine.

Extraction Procedure from Tablets and Capsules: Ten tablets or contents of 10 capsules were weighed accurately and ground to a fine powder. A portion of the powder representing 50.0 mg of acetazolamide was mixed with 0.5 ml of ∿1 N NaOH solution and 0.5 ml of water. The mixture was stirred occasionally during 12-15 minutes and brought to volume (100.0 ml) with water. The mixture was filtered[11], first 15-20 ml of the filtrate was rejected and then collected for further dilution as described under preparation of assay solution from vials.

Samples of Decomposed Solution: Three stock solutions containing 0.25 mg/ml of acetazolamide were allowed to stand at 25° (± 1°) and assayed at 0 day and after appropriate intervals. Solution one was in water prepared from commercial powder of sodium acetazolamide; solution two was in 0.01 N NaOH solution; and solution three was in 0.1 N NaOH solution.

Assay Procedure: A 20 µl aliquot of the assay solution was injected into the chromatograph at the conditions described. For comparison, a 20 µl of the standard solution containing identical concentrations of acetazolamide and the internal standard based on the label claim was injected after the assay eluted. If the assay results were less than 50%, then the assay solution was prepared again using higher concentration (2 times of the first solution).

Calculations: Since preliminary investigations indicated that the ratio of peaks heights of acetazolamide/internal standard were directly related to the concentrations (range tested 0.15 to 0.36 µg), the results were calculated using the equation:

$$\frac{A}{S} \times 100 = \text{Percent of the label claim found}$$

TABLE 1 - Assay Results

Dosage Form	Acetazolamide mg/Dosage Form	Column Used	Percent of the Label Claim Found
Injection	500 mg as sodium salt	Semipolar	99.7
Injection	500 mg as sodium salt	Nonpolar	100.5
Capsules sustained release	500 mg	Semipolar	101.2
Capsules sustained release	500 mg	Nonpolar	99.5
Tablets	250 mg	Semipolar	100.3
Tablets	250 mg	Nonpolar	99.8
Tablets	250 mg	Nonpolar[a]	99.3
Synthetic mixture 1	390 mg and 500 mg of dextrose	Nonpolar	100.1
Synthetic mixture 2	400 mg and 450 mg of lactose	Nonpolar	100.8

[a]Using USP-NF procedure 2 for the extraction of acetazolamide i.e. with boiling water.

where A is the ratio of the peak heights of the drug/internal standard of the assay solution and S that of the standard solution.

RESULTS AND DISCUSSION

The assay results (Table 1) indicated that the developed method can be used for the quantitation of acetazolamide in pharmaceutical dosage forms, injections, capsules and tablets. The percent relative standard deviations based on 6 injections were 0.9 with semipolar column and 0.4 with nonpolar column. The results were reproducible

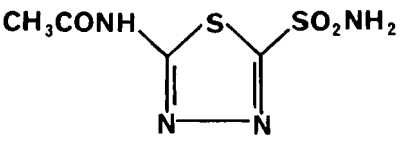

FIGURE 1

Structure of acetazolamide

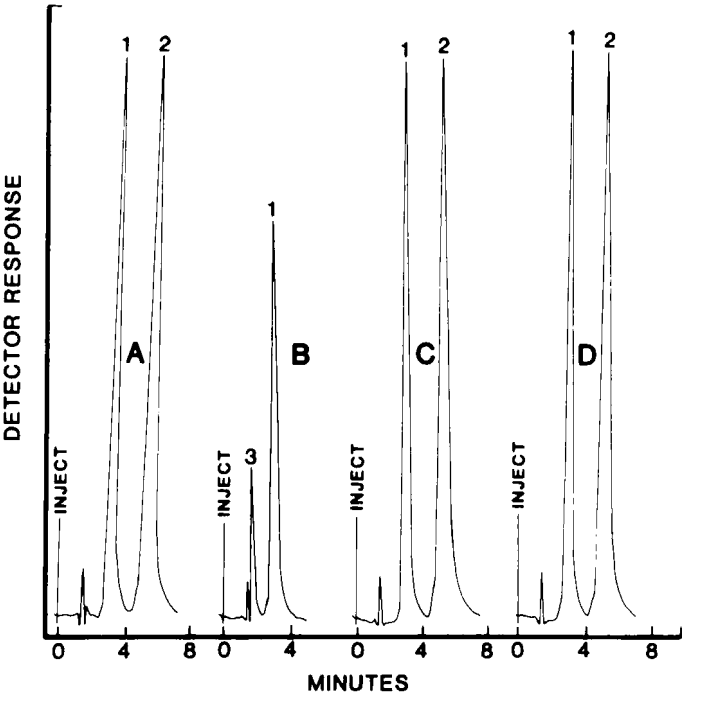

FIGURE 2

Sample chromatograms using semipolar column. Peaks 1-3 are from acetazolamide, hydrochlorothiazide and 5-amino-1,3,4-thiadiazole-2 sulfonamide, respectively. Chromatogram A is from a one day old standard solution (results about 97%); B from a stock solution which was heated for 10 minutes on a hot plate (results 70%); C from a vial containing sodium acetazolamide powder and D from the tablets. For chromatographic conditions, see text.

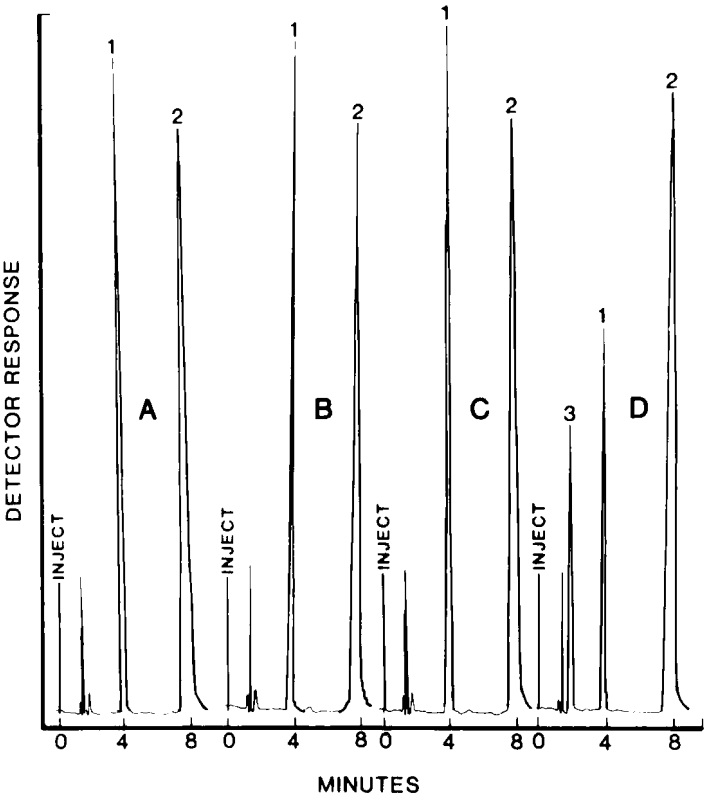

FIGURE 3

Sample chromatograms using nonpolar column. Peaks 1-3 are from acetazolamide, sulfamerazine and 5-amino-1,3,4-thiadiazole-2 sulfonamide, respectively. Chromatogram A is from a freshly prepared standard solution; B from capsules; C from tablets usig the USP-NF[2] extraction procedure and D from 19 days old solution 2 (Table 1) when stored at 25° (results 53.3%). For chromatographic conditions, see text.

from day to day within 1%. The ratio of peaks heights (drug/internal standard) were directly related to the concentration of acetazolamide between 0.15-0.36 μg. The assay method is stability-indicating since the hydrolyzed products (acetic acid and 5-amino-1,3,4-thiadiazole-2 sulfonamide) of acetazolamide (Figure 1) eluted with the solvent (peak 3 in Figures 2 and 3). This was confirmed by injecting sample of the hydrolyzed product. Since 5-amino-1,3,4-thiadiazole-2 sulfonamide

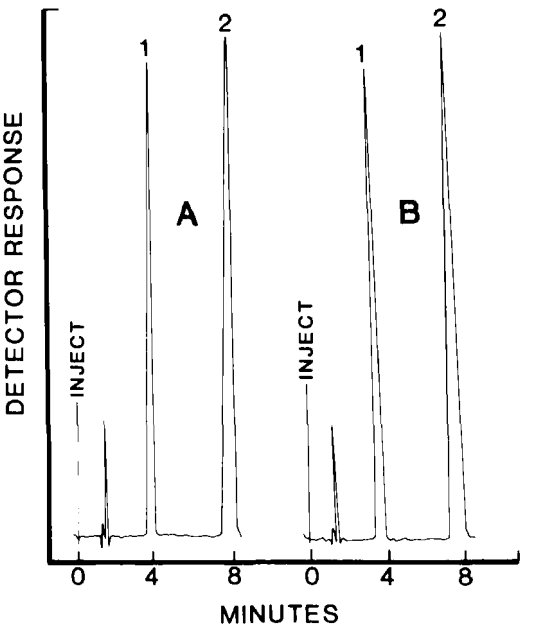

FIGURE 4

Sample chromatograms using a new semipolar column. Peaks 1 and 2 are acetazolamide and hydrochlorothiazide, respectively. Chromatogram A is from a standard solution and B from capsules. For chromatographic conditions, see text.

also absorbs light at 265 nm (peak 3 in Figures 2 and 3), the USP-NF method[1] for the quantitation of acetazolamide sodium in vials cannot be stability-indicating.

The sample chromatograms of the dosage forms (Figure 2 using semipolar column and Figure 3 using nonpolar column) indicate excellent separation of the drug and the internal standard. The sample chromatograms in Figure 4 are produced to emphasise the fact that a new column (apparently more efficient) can make a difference in separation. In Figure 4, the retention time of the internal standard (hydrochlorothiazide) is higher than in Figure 2. These two semipolar

TABLE 2 - Assay Results of Decomposed Stock Solutions (0.25 mg/ml) When Stored at 25° (± 1°)

Number of Days Stored	Percent of the Label Claim Found[a] Solution 1 Initial pH[b] 8.8	Solution 2 Initial pH 10.8	Solution 3 Initial pH 12.7
3	95.8	-[c]	-[c]
4	-[c]	87.9	83.3
7	-[c]	80.7	72.0
9	76.8	-[c]	63.7
14	-[c]	59.7	49.7
18	67.9	56.7	-[c]
19	-[c]	53.3	39.5
	Final pH 8.0	Final pH 10.2	Final pH 12.3

[a]Results with both semipolar and nonpolar columns were similar.
[b]Determined using Beckman Model 4500 digital pHmeter.
[c]Not determined on this day.

columns were of the same manufacturer with same particle size, length and the internal diameter.

The recovery from the synthetic mixtures (Table 1) were quantitative. The tablets were also assayed (Table 1) using the extraction procedure given in the USP-NF[2] which requires the use of boiling water and heating for 15 minutes. The results with the USP-NF procedure were similar to the results with our procedure which did not require the use of heat.

Finally, acetazolamide appears to be very unstable and can get hydrolyzed very fast. The stock solutions were stable when used on the same day. On overnight storage, loss in potency was approximately

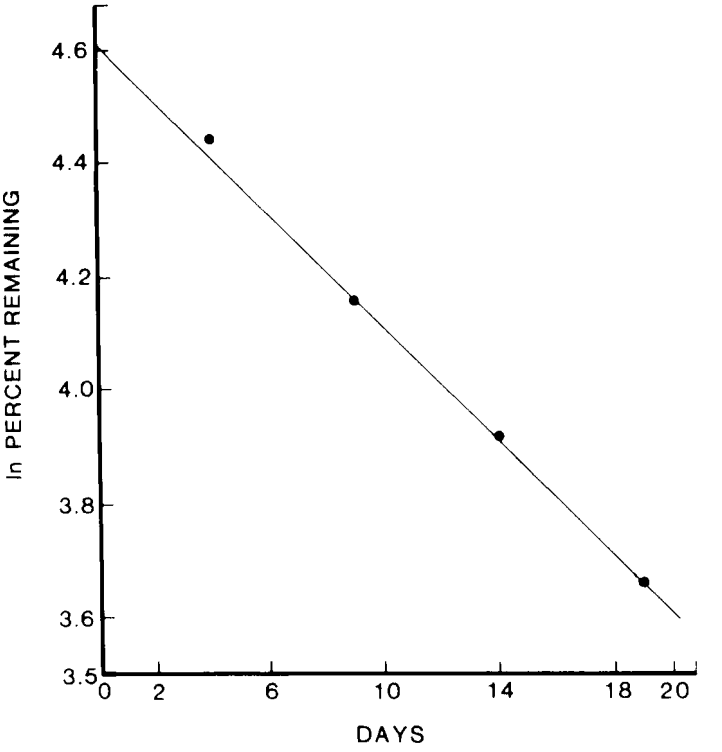

FIGURE 5

First-order plot of solution 3 (Table 2) when stored at room temperature (25° ± 1°).

3%. The data of 3 solutions (Table 2) of different pH values indicated decrease in pH values on storage. This was expected due to the production of acetic acid on hydrolysis. Because of decrease in pH values, the first-order law was not followed in solutions 1 and 2. In these solutions, the decomposition constant decreased as the pH decreased. However, in solution 3 which contained 0.1 N NaOH, the first-order law was followed (Figure 5) although there was slight decrease in the pH value of this solution (from 12.7 to 12.3). Apparently, in this pH range, the K value did not change significantly. The half-life of the

solution 3 was approximately 14 days and K value 0.0495 day^{-1} at 25° (± 1°).

Further investigations are in progress to determine the best pH value and vehicle for the formation of a stable liquid dosage form.

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