BIOMEDICAL CHROMATOGRAPHY, VOL. 7, 134–135 (1993)

Determination of Acetazolamide in Dosage Forms by High Performance Liquid Chromatography

Z. S. Gomaa
Department of Chemistry, College of Science, King Saud University, Riyadh, Saudi Arabia

A high performance liquid chromatographic assay for the quantitation of acetazolamide in both tablet and injection form is described. Acetazolamide is extracted with 0.005 M NaOH solution containing 0.3 mg/mL sulphadiazine (internal standard). A commercially available μ-Bondapak C_{18} cartridge column was used for the separation together with a mobile phase made of acetonitrile, methanol and sodium acetate buffer mixture (10:2:88) (pH 4) at a flow-rate of 4 mL/min. Retention times of about 2.50 and 3.36 min were obtained for the drug and the internal standard, respectively.

INTRODUCTION

Acetazolamide (5-acetamido-1,3,4-thiadiazole-2-sulphonamide) is a carbonic anhydrase inhibitor which is used primarily to reduce intraocular pressure in the treatment of glaucoma.

Numerous methods are available for the determination of sulphonamide drugs in both pharmaceutical dosage forms and biological samples (Ebel, 1977; Berkarek and Kalova, 1976). Several high performance liquid chromatographic (HPLC) assays have been published for the determination of acetazolamide in biological fluids (Bayne *et al.*, 1975; Hossie *et al.*, 1980; Chambers *et al.*, 1981; Chapron and White, 1984), but no work seems to have been reported for its determination by HPLC in pharmaceutical dosage forms. The present work describes a modified HPLC method of analysis for acetazolamide in dosage forms.

EXPERIMENTAL

Apparatus. A Waters Associates (Milford, MA, USA) liquid chromatograph was used for analysis. The instrument was equipped with a pump (Model 45 Pump), an automated gradient controller (Model 680), an autosampler (Model 710B autosampler), a Lambda Max. detector (Model 481), a data module (Model 730 Data Module) and a radial compression separation system (Z. Module Radial Compression Separation System). A μ-Bondapak C_{18} cartridge column (8 mm × 10 cm; Waters Associates) was used for the separation. Standards and samples were filtered through a 0.45 μm Durapore membrane filter obtained from Millipore Corporation (Bedford, MA, USA) before injections.

Chemicals. Acetazolamide tablets (250 mg tablets) and sterile acetazolamide sodium parenteral (500 mg vials) were obtained locally. Acetazolamide (Pfaltz and Bauer Inc, Flushing, NY, USA), and sulphadiazine (BDH Chemicals Ltd., Poole, UK) were used as obtained without further purification. The chemicals used in the mobile phase and for drug extraction were acetonitrile, HPLC grade (BDH Chemicals Ltd.) methanol for chromatography (Merck, Darmstadt, Germany), sodium acetate, anhydrous analar (BDH Chemicals Ltd.), glacial acetic acid (Koch-Light, Colnbrook, UK), and sodium hydroxide (Merck). The mobile phase was prepared by mixing 50 mL acetonitrile with 10 mL methanol and 440 mL 0.05 M sodium acetate solution and adjusting the pH to 4 with glacial acetic acid.

Development of HPLC conditions. Preliminary runs based upon literature review (Chapron and White, 1984) using the μ-Bondapak C_{18} column and a acetonitrile:methanol:0.05 M sodium acetate (3:2:95) mixture as the mobile phase were unsatisfactory. Broad peaks were obtained and a need for adjustment in the mobile phase was indicated. By using the μ-Bondapak C_{18} column and a mobile phase made of acetonitrile, methanol and sodium acetate buffer mixture (10:2:88) with a pH of 4 at a flow-rate of 4 mL/min, sharp peaks and good separation of acetazolamide were obtained. A retention time for acetazolamide of about 2.50 min was observed. The

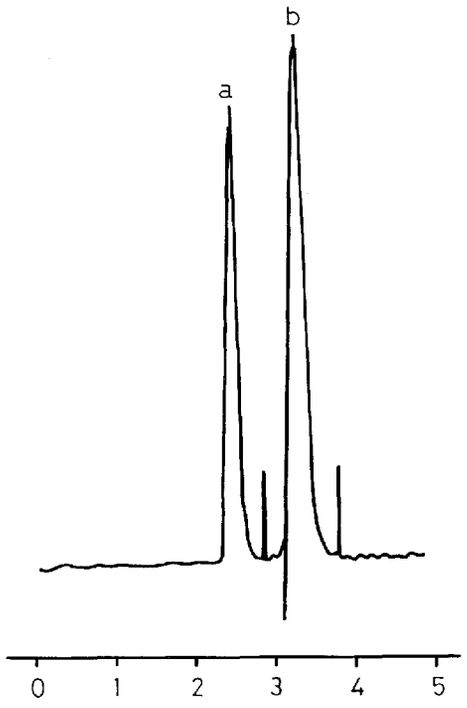

Figure 1. Typical chromatogram: (a) acetazolamide; (b) sulphadiazine.

0269–3879/93/030134–02 $06.00

Received 30 October 1992
Accepted (revised) 25 January 1993

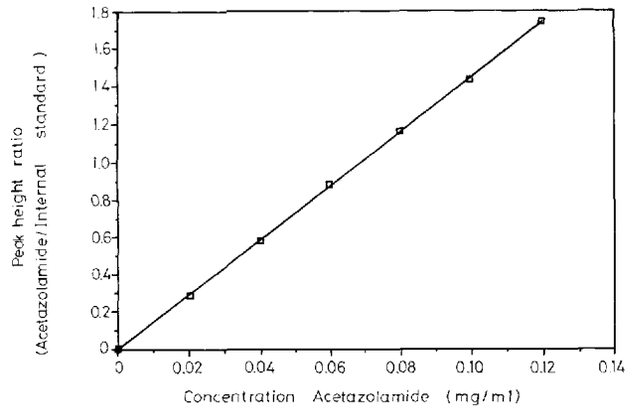

Figure 2. Relation between peak-height ratio and concentration of acetazolamide ($r = 0.9999$).

Table 1. Acetazolamide recovery from spiked tablets

Amount labelled (mg/mL)	Amount added (mg/mL)	Total found (mg/mL)	%Recovery of added
	0.0200	0.217[a]	108.50
0.041	0.0000	0.0478[a]	—
0.041	0.0205	0.0700	108.29
0.041	0.0400	0.0860	95.50
0.041	0.0800	0.1310	104.00

[a]Average of two determinations.

internal standard sulphadiazine was found to have a well-defined peak, separate from that of the drug, with a retention time of about 3.36 min. Ultraviolet absorption at a wavelength of 254 nm was used for detection.

Preparation of the standard curve. Stock solutions were prepared by dissolving 0.1 g of acetazolamide in 0.005 M NaOH solution and 0.1 g sulphadiazine in 0.01 M NaOH. Concentrations of acetazolamide in the range of 0.02–0.12 mg/mL were prepared by diluting the stock standard in the mobile phase. Injections of 5 µL of each solution were made onto the column. The peak-height ratio (acetazolamide:sulphadiazine) was plotted against the concentration of acetazolamide.

Table 2. Analysis of acetazolamide in tablets

Sample	Labelled amount	%Labelled amount
A	250 mg/tablet	116.7
B	250 mg/tablet	105.4
C	250 mg/tablet	100.6

Table 3. Analysis of acetazolamide in sterile sodium acetazolamides

Sample	Labelled amount	%Labelled amount
1	650 mg/vial	94.2
2	650 mg/vial	107.9

Determination of acetazolamide in commercial tablets. Acetazolamide was determined in commercial tablets containing 250 mg of the active ingredient. Five tablets were accurately weighed and finely powdered in a mortar. After thorough mixing, a fraction of the powder was weighed and extracted with 50 mL of 0.005 M NaOH containing 0.3 mg/mL internal standard. The mixture was warmed and stirred for about 30 min, then allowed to cool to room temperature. The volume was adjusted to 100 mL with a solution containing 0.3 mg/mL sulphadiazine in 0.005 M NaOH and the resulting solution was filtered. A 5 mL aliquot from the filtrate was diluted to 25 mL and 5 µL samples were injected onto the column. In order to evaluate the efficiency of the extraction process, powdered tablets were pooled, then divided into four fractions. Three of these fractions were spiked with different amounts of pure drug. After thorough mixing, the total acetazolamide was determined in each fraction as before.

Determination of acetazolamide in sterile sodium acetazolamide injections. Acetazolamide was determined in commercial sterile sodium acetazolamide injections containing 650 mg of the active ingredient. The contents of one vial were dissolved in 0.005 M NaOH solution containing 0.3 mg/mL internal standard and the volume was adjusted to 100 mL. A 5 mL aliquot from the solution was diluted to 25 mL and 5 µL samples were injected onto the column.

RESULTS AND DISCUSSION

Figure 1 shows a typical chromatograph obtained by HPLC and indicates the retention times for acetazolamide and sulphadiazine. The standard curves were obtained by plotting the peak-height ratio of acetazolamide and the internal standard against concentration (mg/mL) of acetazolamide. Figure 2 shows the straight-line relationships between the concentration of acetazolamide and the peak-height ratio (acetazolamide/internal standard).

Table 1 shows the percentage recovery of acetazolamide from spiked tablets. Recovery was calculated by subtracting the experimentally found acetazolamide content of the tablet from the experimental total acetazolamide for the spiked samples and the percentage of recovered amount to added amount was then determined.

The results of applying the HPLC method to the determination of acetazolamide in three commercial tablets is shown in Table 2, and the results for the determination of two commercial sterile sodium acetazolamide injections are shown in Table 3.

Acknowledgements

The author would like to acknowledge the support of the Research Center of the Center for University Women Students (Medical Studies and Sciences sections), King Saud University and Ms. Connie Lobrin for typing the manuscript.

REFERENCES

Bayne, W. F., Rogers, G., and Crisologo, N. (1975). *J. Pharm. Sci.* **64**, 402.

Berkarek, V. and Kalova, H. (1976). *Chem. Listy* **70**, 17.

Chambers, D. M., White, M. H. and Hostenbauder, H. B. (1981). *J. Chromatogr.* **225**, 231.

Chapron, D. J. and White, L. B. (1984). *J. Pharm. Sci.* **71**, 985.

Ebel, S. (1977). *Handbuch der Arzneimittel-Analytik.* Verlag Chemie, Weinheim.

Hossie, R. D., Mouseau, N., Sved, S. and Brien, R. (1980). *J. Pharm. Sci.* **69**, 384.