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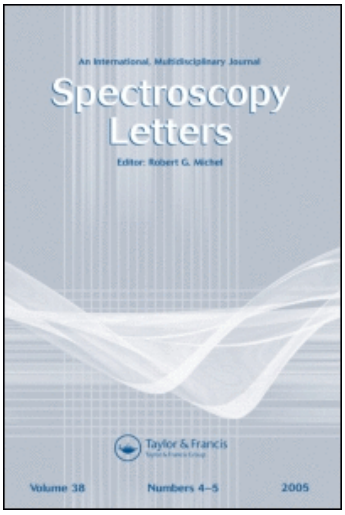

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Quantitative Analysis of Acetazolamide in Solid Dosage Forms with ^{1}H-Nmr Spectroscopy

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QUANTITATIVE ANALYSIS OF ACETAZOLAMIDE IN SOLID DOSAGE FORMS WITH ^{1}H-NMR SPECTROSCOPY

Keywords: *Acetazolamide, Quantitative Analysis, ^{1}H-NMR*

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ABSTRACT

A rapid and specific proton magnetic resonance spectroscopic method was developed for determining acetazolamide in tablets. Maleic acid was used as the internal standard and DMSO-d_6 served as the NMR solvent. The concentration of drug per unit dose was calculated from the integration values for the resonance signals of acetazolamide at 2.14 ppm and maleic acid at 6.20 ppm. The method using commercial products gave comparable results of those obtained by the method of USP XXIII.

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INTRODUCTION

Acetazolamide, N-[5-(aminosulfonyl)-1,3,4-thiadiazol-2-yl]acetamide, is a carbonic anhydrase inhibitor (CAI), and is used therapeutically for treatment of glaucoma, epilepsy and as a diuretic[1]. Methods used for the assay of acetazolamide in biological and pharmaceutical samples have included HPLC (2,3). In the USP XXIII, the assay of acetazolamide in dosage forms also relies on HPLC determination [4]. The aim of this study was to establish a simple and sensitive procedure for determination of acetazolamide in solid dosage forms. The applied NMR spectroscopic method is rapid, specific and very simple. One assay can be completed in less than 20 minutes and the method is selective enough to permit the assay in the presence of certain excipients of dosage forms.

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MATERIALS AND METHODS

Chemicals

Standard USP acetazolamide RS was obtained from Sanofi Inc., Turkey. Sulfadiazine (internal standard for HPLC experiment) was USP grade. Anhydrous sodium acetate, glacial acetic acid, sodium hydroxide, and acetonitrile of HPLC grade were purchased from Merck Chemical Industries (U.S.A.). DMSO-d_6 was of analytical grade, obtained from Aldrich (U.S.A.), and 0.45 μm nylon filters obtained from Waters (U.S.A.).

Apparatus

NMR, Bruker DPX-400, 400 MHz High Performance Digital FT NMR (Germany). HPLC, Hewlett Packard Series 1100 Liquid Chromatograph including 7725 rheodyne injector (20 µl loop), HP UV-Vis detector, vacuum degasser, gradient pump module and column compartment oven. A 5-µm packing L1 column (4.6mm x 25cm) from Phenomenex was used for the separations.

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Assay Preparation for NMR

Twenty tablets were weighed and finely powdered. A portion of well-mixed powder equivalent to 20-25 mg acetazolamide weighed accurately and transferred to a glass-stoppered tube. About 15-20 mg of accurately weighed maleic acid, 0.8-1 mL of DMSO-d_6 and 2 drops of D_2O were added. The solution was mixed by means of a vortex mixer and centrifuged. Using a capillary pipette, about 0.4-0.5 mL of the supernatant was transferred to an analytical NMR tube, the NMR spectrum was taken and from the spectrum the signals at 2.14 ppm and 6.20 ppm were integrated.

CALCULATIONS

The amount of acetazolamide ($C_4H_6N_4O_3S_2$) per unit dose was obtained from the equation as follows[4];

$$W_{acet}=W_{mal.Ac.} \text{ X } E_{acet.} / E_{mal.Ac.} \text{ X } A_{acet.} / A_{mal.Ac.}$$

Where, A_{acet} is the integral value for the methyl protons of acetazolamide absorbing at 2.14 δ ppm, $A_{mal.Ac.}$ is the integral value of olefinic protons absorbing at 6.20 δ ppm, $E_{acet.}$ is the formula weight of acetazolamide divided by the number of absorbing protons (222.25/3=74.08), $E_{mal.ac.}$ is the formula weight of maleic acid divided by the number of absorbing protons (116.07/2=58.04), $W_{mal.ac.}$ is the weight of maleic acid used in the assay, mg and W_{acet} is the weight of acetazolamide, in mg.

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RESULT AND DISCUSSION

Fig. 1 shows the 400 MHz ^{1}H-NMR spectrum of acetazolamide in DMSO-d_6. In the spectrum, the methyl protons of acetamido group give a sharp singlet at 2.14 ppm and the amine protons give a broad singlet at 8.20 ppm.

Fig.2 shows the 400 MHz. ^{1}H-NMR spectrum of acetazolamide + maleic acid in DMSO-d_6 after deuterium exchange with D_2O. The singlets at 2.14 and 6.20 ppm were used in the quantitative analysis.

The method is also applied to the medicament involving acetazolamide. Since the excipients in the medicament give multiplets between 3.00 and 4.00 ppm and do not interfere with the signals used in quantitative analysis, the quantification of acetazolamide in pharmaceutical dosage

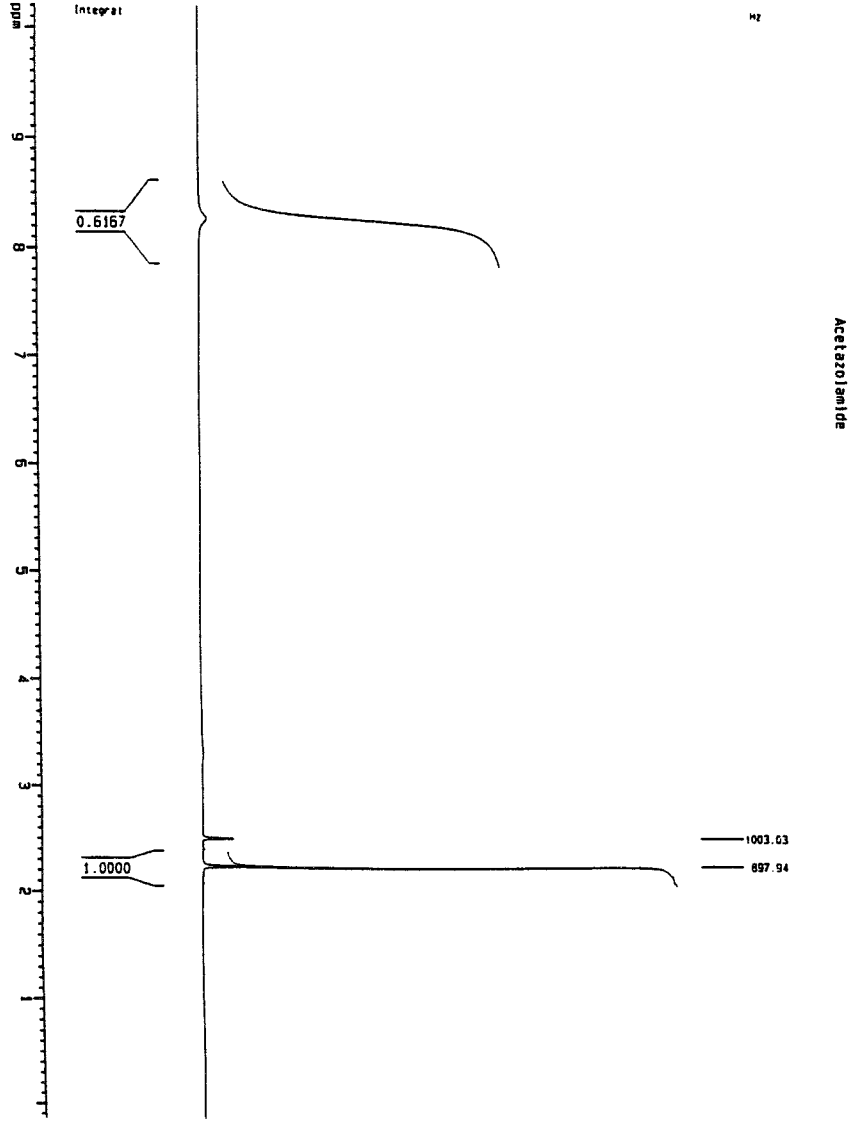

Fig. 1 ^{1}H-NMR Spectrum of Acetazolamide in DMSO-d_6

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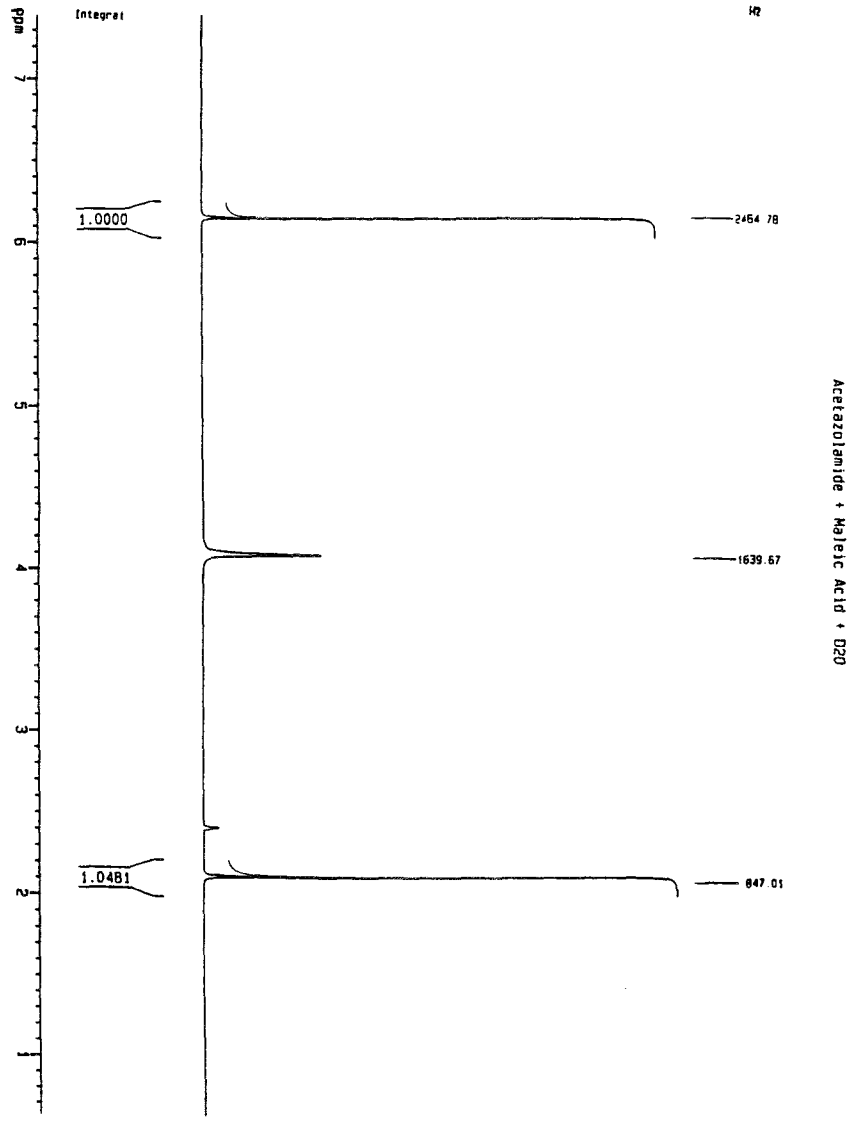

Fig. 2. ^{1}H-NMR Spectrum of Acetazolamide + Maleic Acid in DMSO-d_6 after D_2O exchange

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TABLE 1
Determination and the Statistical Results of the Proposed ^{1}H- NMR and USP XXIII methods

Acetazolamide Tablet, 250 mg.				
NMR Method			USP XXIII Method	
Sample No	Amount Found mg	Amount Found %	Amount Found mg	Amount Found %
1	249.75	99.90	249.95	99.98
2	248.80	99.52	249.45	99.78
3	249.00	99.60	249.45	99.78
4	249.75	99.90	249.25	99.70
5	249.25	99.70	248.80	99.52
6	249.10	99.64	248.70	99.48
7	249.25	99.70	249.00	99.60
8	249.75	99.90	249.05	99.62
9	249.70	99.88	248.75	99.50
10	249.70	99.88	249.50	99.80
Mean : 249.41 % : 99.76 SD : 0.3654 CV% RSD : 0.1465			Mean : 249.19 % : 99.68 SD : 0.4019 CV% RSD: 0.1612	

forms was realised easily. The method given for acetazolamide tablets in USP XXIII was also applied and the results of the proposed and USP XXIII method are given in Table 1.

The results were also compared statistically in each series by paired t-

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TABLE 2
Recovery Data Obtained for Acetazolamide by Using ^{1}H-NMR Method

Sample No	Acetazolamide Added (mg)	Found (mg)	Recovery %
1	25	24.98	99.92
2	25	24.98	99.92
3	25	24.96	99.84
4	25	25.02	100.08
5	25	24.98	99.92
6	25	24.99	99.96
7	20	19.96	99.80
8	21	21.02	100.10
9	22	21.98	99.91
10	23	22.96	99.83
11	24	23.98	99.92
12	25	25.00	100.00
13	26	25.96	99.85
14	27	26.96	99.85
	8: 99.92	RSD : 0.0809	

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test and no significant difference was found. As it can be seen from Table 1, the relative standard deviation values are very low showing the repeatibality of the proposed NMR method is high enough.

Mean recovery and relative standard deviation of the method were obtained as 99.12 and 0.0809 for acetazolamide in the synthetic preparations by adding known amounts of acetazolamide (TABLE 2)

CONCLUSION

Acetazolamide content of a pharmaceutical dosage form can be

determined with the use of maleic acid as the internal standard by ^{1}H-NMR spectroscopy. The statistical results show that the method can be easily applied since it is simple, rapid, and specific. It is sufficiently sensitive and rapid to be utilised in assaying individual tablets and also can serve as an identification test for acetazolamide.

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