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Short communication

Quantitative determination of azathioprine in tablets by ^{1}H NMR spectroscopy☆

Nilgün Günden Göğer [a,*], H. Kürşat Parlatan [a], Hasan Basan [a], Aysel Berkkan [a], Tuncel Özden [b]

[a] *Department of Analytical Chemistry, Faculty of Pharmacy, Gazi University, 06330 Ankara, Turkey*
[b] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gazi University, 06330 Ankara, Turkey*

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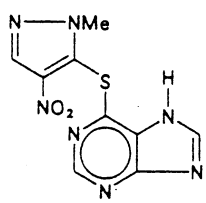
1. Introduction

Azathioprine, 6-(1-methyl-4-nitroimidazole-5-ylthio)-purine, is a potentially useful antileukaemic and immunosuppressive drug.



$C_9H_7N_7O_2S$ 277.3

According to the literature, the electrochemical behaviour of azathioprine has been studied by differential pulse polarography [1]. Separation and fluorodensitometric determination of azathioprine was realised [2]. A colorimetric quantitative assay was applied to tablet forms [3]. In addition to these, some studies based on the determination of azathioprine and 6-mercaptopurine, a metabolite of azathioprine in plasma, were performed by HPLC [4–12].

This work describes a rapid, accurate, specific and simple method for the assay of azathioprine, involving the application of ^{1}H NMR spectroscopy. The method involves the addition of an internal standard to the sample and subsequent dilution with DMSO-d_6. The appropriate analytical peaks are integrated after the ^{1}H NMR spectrum has been recorded and the amount of azathioprine is then calculated. The assay can be completed in less than 20 min and is selective enough to permit the assay of azathioprine in the presence of tablet excipients.

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* Corresponding author. Fax: +90-312-433-3419.
E-mail address: artcamera@artcamera.com.tr (N.G. Göğer)

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2. Experimental

2.1. Apparatus

For the ^{1}H NMR method, A Brucker DPX 400 spectrometer was used. All chemical shifts are reported in parts per million (ppm) relative to tetramethyl silane (TMS) at 0 ppm.

For the UV method, a Shimadzu UV 160 A spectrophotometer was used.

For the titration method, a Jenway 3040 ion analyzer, a digital ionmeter and an Ingold combined pH electrode were used.

2.2. Chemicals and reagents

Azathioprine powder (working standard), fumaric acid, dimethyl sulphoxide-d_6 (DMSO-d_6), tetra butyl ammonium hydroxide (TBAH), dimethyl formamide (DMF), hydrochloric acid and azathioprine tablets were obtained from various commercial sources.

2.3. Assay of tablets

For ^{1}H NMR, 20 tablets were weighed and homogenised. A portion of tablet equivalent to 60.0 mg azathioprine was weighed and transferred into a glass-stoppered tube. Twenty five milligrams of fumaric acid were added. The mixture was dissolved in 1.5 ml DMSO-d_6. After centrifugation, 0.4 ml of the supernatant were transferred into an analytical PMR tube and the spectrum was recorded. The average height of the integral steps was obtained and the amount of azathioprine (as $C_9H_7N_7O_2S$) per unit dose calculated using the equation given below.

$$Ws = Wis(Nis \times Ms \times Hs)/Ns \times Mis \times His$$

Where Ms and Mis are the molecular weights of azathioprine and fumaric acid, respectively; Ws and Wis are the weights of azathioprine and fumaric acid used in the assay. Hs is the integral value of the signal that belongs to azathioprine at 8.24 ppm, and His is the integral value of the signal that belongs to fumaric acid at 6.63 ppm. Ns, the proton number of azathioprine signal, is 1, and Nis, the proton number of fumaric acid signal, is 2. The azathioprine content of one tablet was calculated using the obtained Ws value.

For the UV method, 20 tablets were weighed and homogenised. A portion of tablet powder equivalent to 150 mg azathioprine was dissolved in 20.0 ml DMSO and was diluted to 500.0 ml with 0.1 M HCl. The solution was filtered and 25.0 ml of the filtrate was diluted to 1000.0 ml with 0.1 M HCl. Using the absorbance value of the resulting solution at the maximum wavelength at 280 nm and taking 628 as the value of A (1%, 1 cm) at the same wavelength, the content of azathioprine was calculated.

For the non-aqueous titrimetric method, 20 tablets were weighed and homogenised. A portion of tablet powder equivalent to 250 mg of azathioprine was dissolved in 25 ml of DMF and the titration was performed using 0.1 M TBAH as titrant, according to the Pharmacopoeia method and the end point potentiometrically determined.

3. Results and discussion

Since DMSO-d_6 promptly dissolves both azathioprine and fumaric acid, this solvent was used for the assay. Fumaric acid was chosen as an internal standard because of its solubility in DMSO-d_6 and importantly it does not overlap with any of the azathioprine peaks in the spectrum.

In the ^{1}H NMR spectrum of azathioprine, the methyl protons are observed at 3.70 ppm (3h, s) and aromatic protons are observed at 8.24, 8.54 and 8.58 ppm (3h,3 s). In the spectrum of fumaric acid, there is a singlet at 6.63 ppm belonging to the ethilenic protons (2h, s) and a singlet at 12.28 ppm belonging to hydroxyl protons (2h, s). The azathioprine signal at 8.24 ppm (s) and the fumaric acid signal at 6.63 ppm (s) were chosen for quantitative determination. Fig. 1 shows the spectrum of the mixture of azathioprine and fumaric acid in DMSO-d_6.

In addition to this, the UV method for azathioprine tablets from BP 93 was performed. As a third study, the non-aqueous titration method for standard azathioprine from BP 93 was applied to tablet forms.

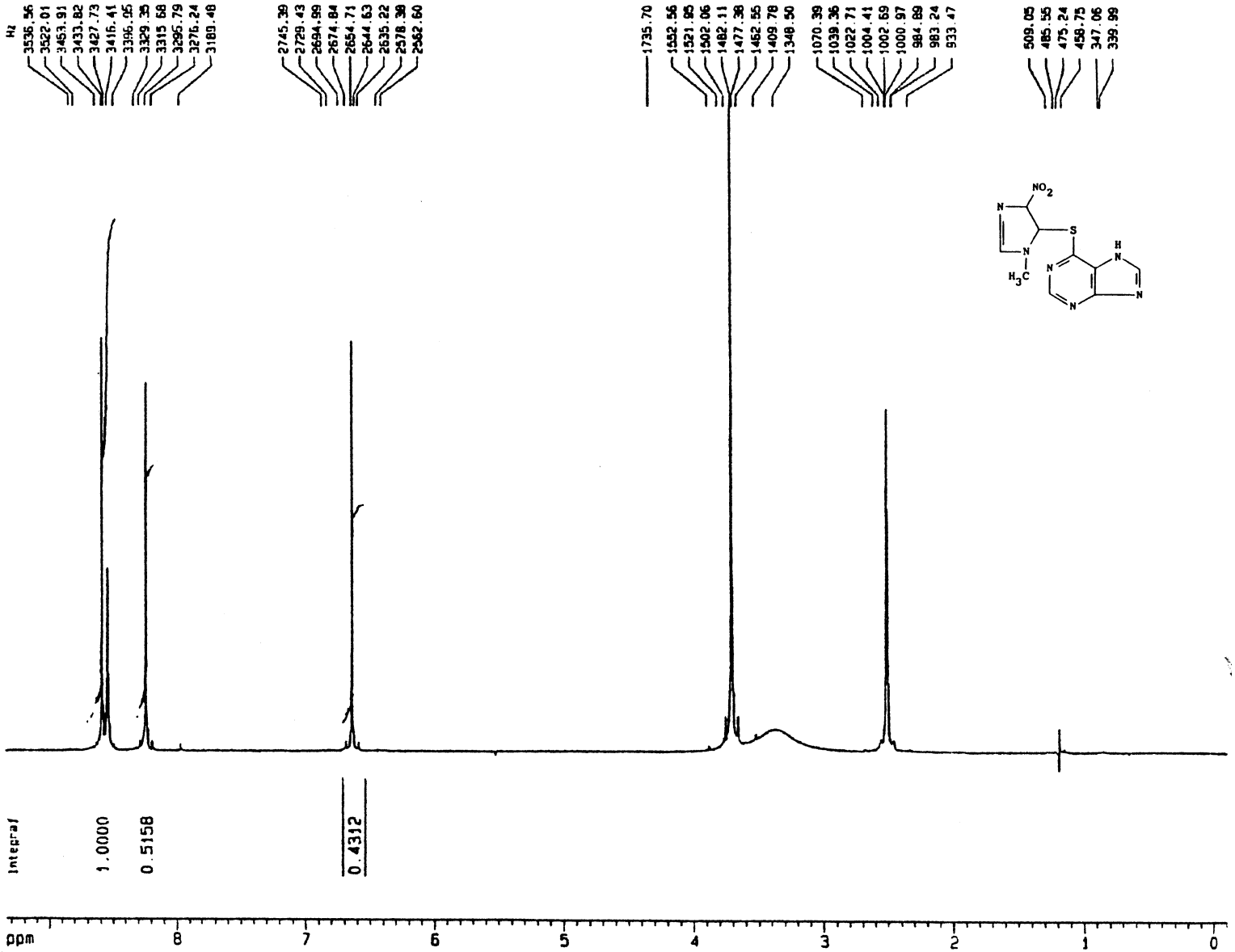

Fig. 1. ^{1}H NMR spectrum of azathioprine and fumaric acid in DMSO-d_6.

Table 1
Comparative study for azathioprine tablets

Azathioprine tablet (declared amount: [1] 50 mg per tablet)	^{1}H NMR method	UV method [14]	Non-aqueous titration method [14]
Amount found, mg[a]	49.73	49.92	49.33
Rsd%[b]	0.276	1.26	1.24
Confidence limits[c]	49.56–49.90	49.14–50.70	48.57–50.09
$t_{calculated} = 0.34$ (^{1}H NMR–Titration) $t_{calculated} = 0.65$ (^{1}H NMR–UV)	$t_{theorical} = 2.31$[c]		

[a] Mean of five measurements.
[b] Rsd: relative standard deviation.
[c] ($p = 0.05$).

Table 1 compares the three methods. With regard to Student's t test, there is no significant difference between ^{1}H NMR and the other methods as regards accuracy and precision. In addition the result of the recovery study, $99.86\% \pm 0.03\%$ (SD), shows the reliance of the proposed method.

A unique aspect of NMR spectra is the direct proportionality between peak areas and the number of protons responsible for the peak. A quantitative determination of a specific compound does not require pure samples for calibration. Thus, if an identifiable peak for one of the constituents of a sample doesn't overlap the peaks of the other constituents, the area of this peak can be employed to establish the concentration of the species directly, provided that only the signal area per proton is known. This later parameter can be obtained conveniently from a known concentration of an internal standard, if the peak of the internal standard does not overlap with any of the sample peaks.

In comparison with HPLC, the NMR spectrum can provide information on the quantity of an impurity in a sample so that the isolation of the impurity is not necessary in an NMR analysis. It is known that a HPLC procedure includes time-consuming steps, but the NMR method does not require these steps. Therefore, this method would be superior to the HPLC method with respect to selectivity, rapidity and simplicity.

In comparison with the UV method, in an NMR spectrum, the signals are a measure of the molar ratios of molecules, independent of the molecular weight. Therefore, there would be no response factors as in UV detection caused by varying extinctions dependent on molecular structures. There would also be no non-linear calibration curves as found with light scattering detectors in UV [13].

On the other hand, NMR spectroscopy is quicker and easier to perform and more specific because there is no probability of interferences as in UV and HPLC procedures. In addition to that, in most cases, such as in this study, the integration of signals used for quantitative analysis is more precise and accurate than the other two methods.

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