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DETERMINATION OF BUSULFAN IN TABLETS BY NMR SPECTROSCOPY

Keywords: Busulfan, NMR Analysis; Busulfan Tablets, NMR Analysis.

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Abstract

A simple NMR spectroscopic method is described for the assay of busulfan in tablets. Methenamine is used as the internal standard and chloroform-d as the solvent. The mean recovery value for the drug from standard mixtures was $99.3 \pm 0.182\%$ w/w ($n = 5$) by the NMR method and 99.2% ($n = 2$) by the official method. The mean content of busulfan in commercial tablet samples was 99.4 ± 0.16 (range $99.1 - 99.6$)% w/w ($CV = 0.16\%$, $n = 10$) by the NMR method and 99.5 ± 0.17 (range $99.2 - 99.8$)% w/w ($CV = 0.17\%$, $n = 10$) by the official method.

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Introduction

Busulfan (1,4-butanediol dimethanesulfonate) is a potent anti-neoplastic drug used in the treatment of chronic myelogenous leukemia. It is an alkylating methane sulfonic acid ester which is unique in that it exerts virtually no pharmacological action other than myelosuppression⁽¹⁾. The only reported method for the assay of busulfan in dosage forms appears to be a nonspecific titration of its acid moiety liberated upon saponification⁽²⁾. Busulfan has also been determined in plasma samples by GC-MS with selected ion monitoring⁽³⁾. This paper describes the assay of busulfan in tablets by a simple, rapid, precise, and accurate proton NMR spectroscopic method, which in addition minimizes the analyst's physical contact with the analyte.

Experimental

Apparatus.- NMR spectra were recorded with a 200 MHz, wide bore, Fourier transform spectrometer¹ equipped with a 24K computer memory and using a 5 mm proton probe. The spectrometric conditions were: ambient temperature, 30°C; frequency ¹H observation, 200.06 MHz; observation frequency range, 1600 Hz; pulse width, 9.13 µsec; pulse repetition time, 14 sec; FTD data points, 8; observation time, 2.2 min.

Chemicals and Samples.- Standard: busulfan², stored in a refrigerator when not in use; internal standard: methenamine³; solvent: chloroform-d⁴. All chemical shifts are reported in ppm (δ) with reference to tetramethylsilane⁴ (TMS) at 0 ppm. Busulfan scored tablets, 2 mg, were obtained from commercial sources.

Preparation of Internal Standard Solution.- Transfer about 11.68 mg of methenamine to a 10 ml volumetric flask. Dissolve and dilute to volume with chloroform-d, and mix.

Preparation of Reference Standard Solution.- Transfer about 50 mg of tetramethylsilane to a 10 ml volumetric flask. Dissolve and dilute to volume with chloroform-d, and mix.

Preparation of Sample Solution.- Caution: busulfan is a highly toxic drug; do not touch or inhale its powder. Great care must be exercised in its handling, and procedural details of the method must be followed exactly. Weigh and finely powder not less than 20 busulfan tablets. Weigh accurately a quantity of powder, equivalent to about 6 mg of busulfan, and transfer to a 15 ml vial⁵. Add 1 ml of internal standard solution, close the vial with a septum⁶, and crimper-seal with an aluminum seal. Using a 3 ml syringe fitted with a 2 in needle, introduce about 2 ml of chloroform-d through the septum, and shake to dissolve (the exact volume of chloroform-d needed should be based on the signal-to-noise ratio of the NMR spectrometer used). Allow any insoluble matter to settle. Using a 2 ml syringe fitted with a 2 in needle withdraw about 0.5 to 1 ml of the solution, and transfer to an NMR tube. Add 1 drop of reference standard solution, cap the tube, and shake. Obtain the NMR spectra. Integrate the broad triplet at about 4.3 ppm due to the four methylene protons ($-\text{CH}_2-\text{O}-\text{SO}_2-$) of busulfan and the singlet at about 4.7 ppm due to the twelve methylene protons of the internal standard. The quantity of busulfan, in mg, is obtained from $(\text{Au}/\text{As}) \times (\text{Eu}/\text{Es}) \times \text{C}$, where Au = integral

value of the signal representing busulfan; A_s = integral value of the signal representing methenamine; E_u = formula weight of busulfan/4, 61.58; E_s = formula weight of methenamine/12, 11.68; and C = weight, in mg, of methenamine taken for the analysis.

Results and Discussion

Figure 1 shows the 200 MHz NMR spectra of busulfan and methenamine, the internal standard, in chloroform- d solution. Busulfan exhibited three resonance signals (labeled a, b, and c) whereas methenamine showed a strong singlet (labeled d). The respective structural assignments are given in Table 1. This table also lists the spin-lattice relaxation times (T_1 's) for a nondegassed mixture of busulfan and methenamine, which were obtained by the inversion recovery technique⁽⁴⁾ with an alternating phase of 90° pulse. Additional resonance signals are found at 0.0, 1.5, and 7.26 ppm and are ascribable to the reference standard, moisture, and the tablet excipient stearic acid, respectively.

TABLE 1

Structural Assignments and Spin-Lattice Relaxation Times for Busulfan and Methenamine

| Compound | Peak | Chemical shift, ppm (δ) | Substituent | T_1 , sec |
|-------------|------|-------------------------------------|---|-----------------|
| Busulfan | a | 1.914 | $-\text{CH}_2-\text{C}-\text{O}-\text{SO}_2-$ | 2.05 ± 0.01 |
| | b | 3.022 | $-\text{C}-\text{C}-\text{O}-\text{SO}_2-\text{CH}_3$ | 2.54 ± 0.01 |
| | c | 4.287 | $-\text{C}-\text{CH}_2-\text{O}-\text{SO}_2-$ | 2.37 ± 0.02 |
| Methenamine | d | 4.730 | $-\text{CH}_2-$ | 1.87 ± 0.02 |

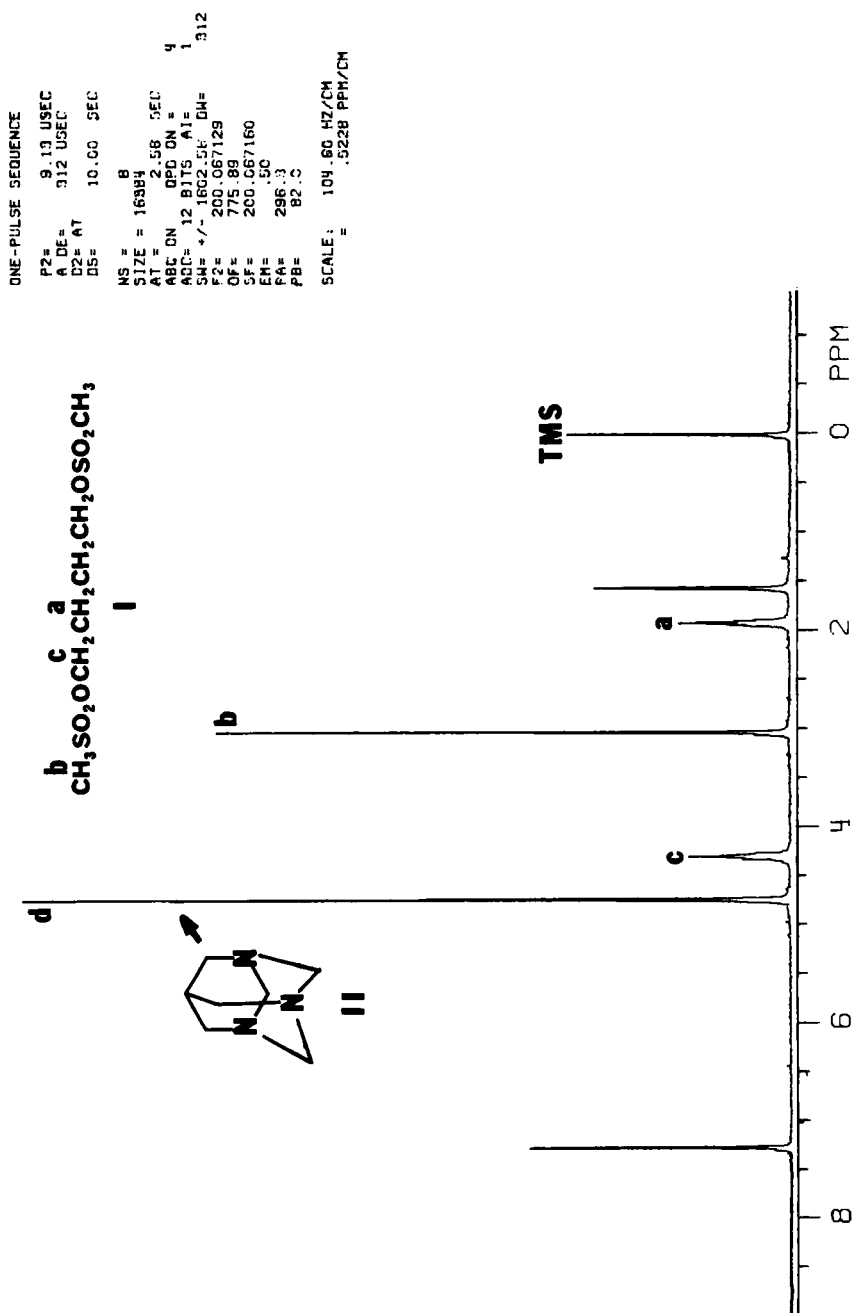


FIG. 1. 200 MHz ^1H -NMR spectra of (I) busulfan, (II) mthenamine, and TMS in chloroform- d .

The accuracy and precision of the proposed method were tested on five standard mixtures of busulfan and methenamine with the results shown in Table 2. The mean recovery value was $99.3 \pm 0.182\%$ w/w, which was in good agreement with that obtained by the official titrimetric method⁽²⁾, which gave a mean recovery of 99.2% for two standard mixtures. These results also indicate that the ratio of busulfan to internal standard has no significant bearing on the accuracy of the proposed method for the range of concentrations studied.

Ten equal portions of a powdered tablet composite sample were analyzed by the NMR and official methods. The NMR method gave a mean assay value of 99.4 ± 0.16 (range 99.1 - 99.6)% w/w and a CV of 0.16%; for the official method these values were 99.5 ± 0.17 (range 99.2 - 99.8)% w/w, CV of 0.17%. Again, the NMR

TABLE 2

Determination of Busulfan in Standard Mixtures by NMR

| Mixture No. | Methenamine, mg/3 ml | Busulfan added, mg | Busulfan found, mg | Recovery, % w/w |
|-------------|-------------------------|-----------------------|-----------------------|--------------------|
| 1 | 1.175 | 6.155 | 6.106 | 99.2 |
| 2 | 1.170 | 6.155 | 6.112 | 99.3 |
| 3 | 1.173 | 6.155 | 6.118 | 99.4 |
| 4 | 1.570 | 6.155 | 6.112 | 99.1 |
| 5 | 2.015 | 6.155 | 6.112 | 99.3 |
| Mean | | | | 99.3 |
| SD | | | | 0.182 |
| CV, % | | | | 0.182 |

method showed good reproducibility as well as a close agreement with the official method. Furthermore, it has the added advantage of being able to provide a positive means of identification of the drug in tablets.

Since busulfan is a highly toxic drug and very few reports have dealt with its proper handling during chemical analysis, we recommend the analyst to wear plastic gloves throughout the procedure and to avoid inhaling the powders. In addition, all glassware that has come in contact with the drug or its solutions should be immersed in sufficient 5% aqueous sodium hydroxide, the solution heated to boiling for about 2 hours, cooled, and then thoroughly rinsed with running water. As an added precaution the analyst is strongly urged to become acquainted with the proper handling of antineoplastic substances⁽⁵⁾.

FOOTNOTES

- 1- Model NTC 200, Nicolet Magnetic Corporation, Fremont, CA 94539.
- 2- Burroughs Wellcome Co., Research Triangle Park, NC 27709.
- 3- Laboratory working standard.
- 4- Merck and Co., Inc., Rahway, NJ 07065.
- 5- Hypo-vials, clear, Pierce Chemical Co., Rockford, IL 61105.
- 6- Hycar septa, Pierce Chemical Co., Rockford, IL 61105.

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