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Letter to the Editor

Determination of busulfan in plasma by high-performance liquid chromatography

Sir,

Gas chromatographic methods have been described for the determination of busulfan in plasma of patients receiving preparative regimens of busulfan for bone marrow transplantation [1,2]. Recently a method using high-performance liquid chromatography (HPLC) with ultraviolet detection was reported by Henner et al. [3], which involved derivatization of busulfan with sodium diethyldithiocarbamate to form the derivative 1,4-bis(diethyldithiocarbamoyl)butane (DDCB). Derivatization was necessary because busulfan has no ultraviolet absorption.

We have not had success with this method because of an interfering peak and incomplete derivatization. The pure derivative DDCB was synthesized as described by Henner et al. [3], with a yield similar to that reported (16%). According to the method of Henner et al. [3], which uses a Waters μ Bondapak C₁₈ column (10- μ m particles, 30 \times 0.39 cm), a mobile phase of methanol-water (80:20) at 1.0 ml/min and detection wavelength 251 nm, the retention time of DDCB is 12 min. When we used the same column and mobile phase, we obtained a chromatogram for the derivatized plasma extract similar to that reported by Henner et al. [3], but even in the absence of busulfan in plasma the sample profile was obtained (Fig. 1). That is, an impurity co-chromatographed with DDCB. We also found that the presence and size of several other peaks depended on the length of the interval between derivatization and chromatography.

The other discrepancy we observed was in the derivatization of plasma busulfan. Henner et al. [3] precipitated plasma proteins, mixed the plasma supernatant with aqueous diethyldithiocarbamate solution and pH 5.5 buffer and immediately extracted the formed DDCB with ethyl acetate. Derivatization

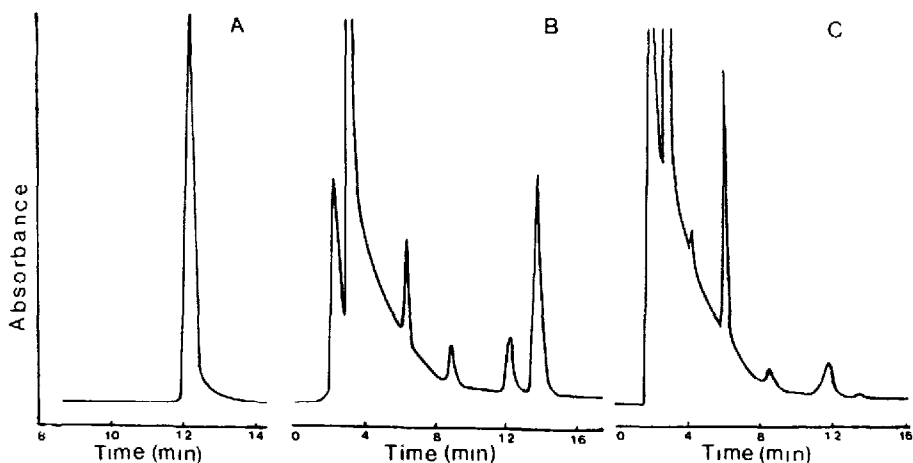


Fig. 1. Chromatograms of derivatized busulfan using methanol-water (80:20) as mobile phase. (A) Authentic DDCB; (B) extract of derivatized plasma spiked with busulfan (7.4 mg/l); (C) extract of derivatized blank plasma.

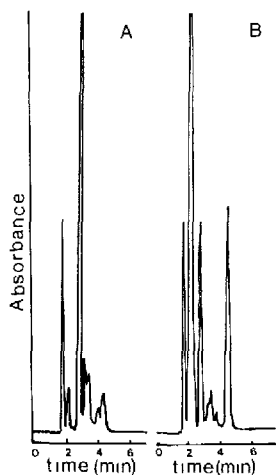


Fig. 2. Chromatograms of derivatized plasma extracts using acetonitrile-water (80:20) as mobile phase. (A) Blank plasma; (B) plasma containing busulfan (10 mg/l).

was reported as nearly complete (97.5%), in contrast to the low yield for the synthesis at 50°C of pure DDCB (16%). When we used this procedure derivatization was very slow, incomplete and yielded another major unidentified product, in addition to DDCB. Using a mobile phase of acetonitrile-water (80:20) at 2.0 ml/min, the retention time of this unidentified product was 2.1 min and that for DDCB was 4.5 min (Fig. 2). The yields of DDCB and the unidentified product both increased with time, but the yield of DDCB was only

approximately 20% after 80 h. The yields of both derivatives were not linearly related to the starting busulfan concentration in the range 5–20 mg/l. Conducting the derivatization at temperatures up to 50°C and changing the pH (5.5–7) did not improve the yield.

Our observations indicate that in the plasma assay described by Henner et al. [3], the derivatization reaction is incomplete and not quantitative and an impurity co-elutes with the derivative under the conditions described. We have also found that these limitations are apparent in the assay of busulfan in pharmaceutical dose forms. Therefore this assay is not useful for measuring busulfan concentration.

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