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Letter to the Editor

Determination of hexamethylene bisacetamide by capillary gas chromatography

Sir,

Hexamethylene bisacetamide (HMBA) is one of several polar–planar compounds [1] capable of inducing cancer cells to differentiate in vitro [2] towards a mature phenotype. Because it is the most potent and effective of the known differentiating agents and animal toxicologic studies suggested acceptable side-effects [3], HMBA has recently entered Phase I clinical trial in cancer patients. Pharmacokinetic studies during the clinical trial are important because in vitro work indicates that HMBA-induced differentiation is dependent on both drug concentration and duration of exposure [2]. Optimal concentrations for differentiation range from 1 to 5 m*M* whereas early clinical data suggest that toxic effects are encountered at concentrations >2 m*M* [4]. Currently available analytic methods employ gas chromatography with packed columns [5]. In our hands deterioration of the packing of these columns occurs rapidly with loss of sensitivity and resolution; accordingly, we decided to evaluate a capillary system in an effort to improve the long-term stability of the analytic method. Use of a capillary system has resulted in improved stability and better sensitivity.

EXPERIMENTAL

Materials

HMBA was of pharmaceutical purity; both HMBA and the internal standard, pentamethylene bisacetamide (PMBA), were supplied by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute.

Apparatus

A Perkin-Elmer (Norwalk, CT, U.S.A.) Sigma 2B gas chromatograph equipped with a nitrogen–phosphorus detector was used. The glass capillary column (30 m $\times$ 0.75 mm) contained 35–65% methylphenyl polysiloxane (Supelco SPB-35; Bellefonte, PA, U.S.A.). The column was placed in a Sigma 2B oven using a Supelco capillary column conversion kit (2-3727). The oven temperature was set

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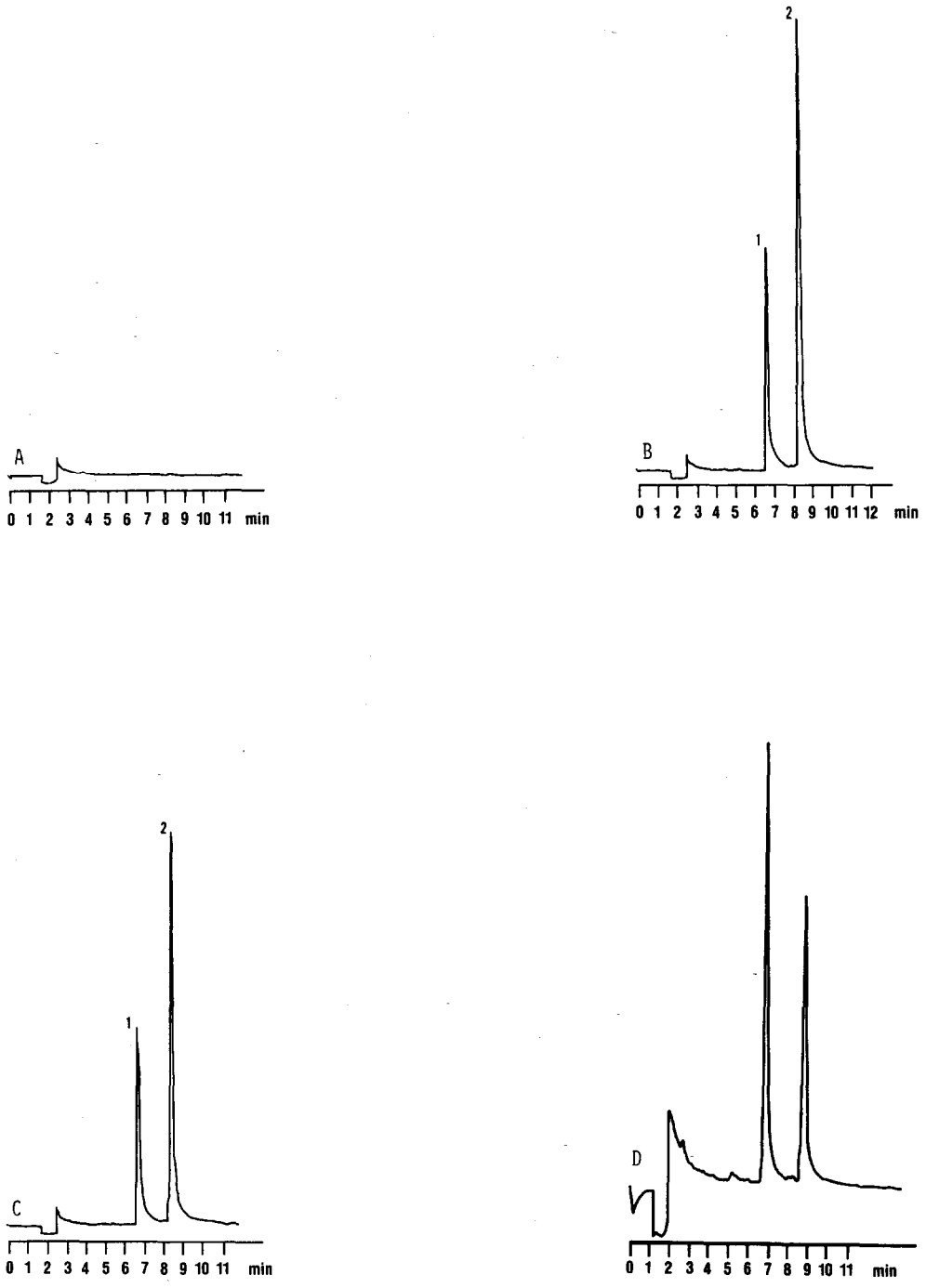

Fig. 1. Gas chromatograms obtained from the analysis of (A) blank plasma, (B) methanolic recovery standard of HMBA (2.5 m*M*), (C) spiked plasma extract of HMBA (2.5 m*M*) and (D) patient plasma. Peaks: 1 = pentamethylene bisacetamide; 2 = hexamethylene bisacetamide.

at 240°C and the injector and detector temperatures both at 300°C. Helium (5 ml/min) was used as a carrier gas; it was also used as the make-up gas to give a total flow-rate (both measured at the detector) of 30 ml/min. A Perkin-Elmer AS100 automatic sampler was used for sample injection. A Spectra-Physics (San Jose, CA, U.S.A.) SP-4290 computing integrator was used to record chromatograms.

Methods

Determination of HMBA in plasma was carried out as follows. Pooled plasma was first centrifuged at 1300 *g* for 10 min. To 0.8 ml of plasma (or methanol for recovery standards) were added 0.2 ml of methanolic stock solutions of HMBA (0.01–10.0 m*M*) and 50 μl of PMBA methanolic solution (50 m*M*). Tubes were then vortex-mixed for 15 s. Plasma samples were applied to prepared C_{18} sorbent extraction columns (Sep-Pak, Waters Assoc., Milford, MA, U.S.A.) and the drugs were eluted with 2 ml of HPLC-grade methanol. Samples (2 μl) of the methanol extract were then injected into the column using direct (heated) on-column injection.

RESULTS AND DISCUSSION

We used the extraction procedure of Egorin et al. [4] and our results agree with theirs. The mean recovery of HMBA after addition of pure drug to human plasma was 99% over the range of the standard curve (0.1–10.0 m*M*) when compared to methanolic recovery solutions. PMBA was selected as internal standard because of its structural similarity to HMBA. Typical gas chromatograms of a blank plasma, spiked plasma and recovery standard are shown in Fig. 1.

For the determination of drug concentrations in plasma, 2-μl direct injections were made into the chromatograph. A linear standard curve between peak-area ratios and HMBA concentration was obtained ($r=0.998$) for plasma concentrations of 0.01–10.0 m*M*. Injection of methanolic recovery standards ($n=10$) of HMBA (1 m*M*) showed a coefficient of variation of 6.3%. The precision of the method was tested at 1.0 and 2.5 m*M* spiked plasma extracts of HMBA ($n=10$ each) revealing coefficients of variation of 7.5 and 5%, respectively. The accuracy of the method was assessed by analyzing quality-control samples of spiked plasma ($n=10$). The quality-control sample variability had a coefficient of variation of 2.8% and a mean concentration of 1.009 m*M* for a theoretical concentration of 1.0 m*M* HMBA. Replicate injections ($n=10$) of spiked plasma extracts of HMBA (2.5 m*M*) showed coefficients of variation of 3 and 4% for between-day injections. The minimum detectable concentration of HMBA extracted from plasma was 0.005 m*M*. Use of this system for three months did not produce deterioration in the chromatography.

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