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Note

Separation of hydrazine, monomethylhydrazine, 1,1-dimethylhydrazine and 1,2-dimethylhydrazine by high-performance liquid chromatography with electrochemical detection

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Hydrazine (HY) is an important precursor for many industrial organic syntheses[1,2]. In addition, HY as well as monomethylhydrazine (MMH) and 1,1-dimethylhydrazine (UDMH) have seen extensive use as rocket propellants and fuels[3]. These compounds and, in particular, the closely related 1,2-dimethylhydrazine (SDMH) have been shown to be carcinogenic in laboratory animals[3–5]. Because of their widespread use and potent biological activity, considerable effort has been devoted to the development of methods for the detection and quantitation of these compounds in the environment and in biological fluids[6–11]. For the most part, such methods usually involve derivatization with either *p*-dimethylaminobenzaldehyde[7], pentafluorobenzaldehyde[9] or salicylaldehyde[11] followed by colorimetric estimation[7], gas chromatography with an electron-capture detector[9] or high-performance liquid chromatography (HPLC) with UV detection[11], respectively. Methods dependent on coupling with aldehydes are limited in application to hydrazines with an unsubstituted $-NH_2$ group; compounds such as SDMH which do not form hydrazones cannot be estimated in this way.

In this communication, we describe the separation of HY, MMH, UDMH and SDMH by ion-exchange HPLC using an electrochemical detector with glassy carbon working and auxiliary electrodes. The hydrazines do not require derivatization, and urine or blood plasma samples suspected of containing these compounds may be analyzed directly without previous work-up.

EXPERIMENTAL

HY, MMH, UDMH and SDMH–2HCl were purchased from Aldrich (Milwaukee, WI, U.S.A.). The hydrazines were dissolved in distilled deionized water (≥ 13.7 MΩ resistivity) which had been flushed with nitrogen to prevent air oxidation. In the case of SDMH–2HCl, all concentrations were calculated as the free base.

Stainless-steel columns of Aminex A-5 sulfonic acid type cation-exchange resin in the sodium form, particle size 13 ± 2 μm (Bio-Rad Labs., Richmond, CA, U.S.A.) were slurry packed using the same buffer and flow-rate as those ultimately used in the analyses. Various preliminary runs were made with short (5 to 13 cm long, 4 mm I.D.) columns and sodium borate buffers ranging from 0.01 to 0.05 *M* and from pH 8.6 to

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9.2 to establish optimal conditions. On the basis of these preliminary runs, a 30 cm × 4 mm I.D. column eluted with 0.05 *M* sodium borate buffer, pH 8.9, at 1 ml/min was selected for the separations described here.

A Waters Assoc. Model M6000A HPLC pump with a Model U6K injector were used in conjunction with a Metrohm Model EA1096 electrochemical detector. The latter used glassy carbon indicating and working electrodes and a Ag/AgCl reference electrode. Dead volume of the detector was approximately 3–4 μl. A Metrohm E506 Polarecord was used to supply the polarizing voltage (+1.0 V) and to detect cell current. Since this instrument was designed principally for application in differential pulse polarography where the movement of the chart recorder paper is discontinuous and is synchronized to the dropping of mercury, serrated HPLC recordings were obtained in our application. This circumstance was found not to interfere significantly with the determination of elution volumes or peak heights.

For the determination of hydrazines in rat blood plasma and urine, male F344 rats, approximately 300 g body weight, were injected subcutaneously with 50 mg/kg of UDMH or with 100 mg/kg SDMH, neutralized to pH 6.4 in 0.7% sodium EDTA. After injection, the rats were placed in metabolism cages within a fume hood[12] and blood samples were obtained from the orbital sinuses under light ether anesthesia. The blood, collected with heparinized capillaries, was centrifuged, and 100 μl of the resulting plasma was submitted directly to HPLC. Urine was collected for a period of 24 h after dosing in containers thermoelectrically cooled to 0–4°C. Because of the high levels of hydrazines excreted in the urine, it was necessary to dilute the samples with deionized water prior to HPLC in order to bring the concentrations to within the bounds of the standard curve.

RESULTS AND DISCUSSION

Typical HPLC profiles of the four hydrazines at three different concentrations are shown in Fig. 1. It is evident that the order of elution from the ion-exchange column is in order of increasing pK_a values. These are: UDMH, 7.21; SDMH, 7.52; MMH, 7.87; and HY, 8.07. While MMH, SDMH and UDMH show well defined peaks at levels down to 17, 10 and 8 ng, respectively, the HY peak is obscured by noise at levels below approximately 80 ng.

A standard curve relating the peak current obtained with the amount of hydrazines injected is shown in Fig. 2. For UDMH, SDMH and MMH the peak current is a linear function of quantity up to approximately 250 ng, 600 ng and 1100 ng, respectively. For HY a hyperbolic rather than linear relationship between peak current and quantity was obtained in the range examined.

Profiles obtained from the direct HPLC of blood plasma and urine from rats given UDMH or SDMH subcutaneously are shown in Figs. 3 and 4, respectively. Interestingly, the profile of urine from the rat given SDMH shows a small peak, corresponding in elution volume to MMH, which may represent a metabolite. However, SDMH normally contains a small amount of MMH as an impurity and an alternative possibility is that the MMH impurity in the dose may be preferentially concentrated by the animal and excreted in the urine.

Blood plasma levels of UDMH and SDMH following subcutaneous administrations of 50 and 100 mg/kg, respectively, are shown in Fig. 5. Although large in-

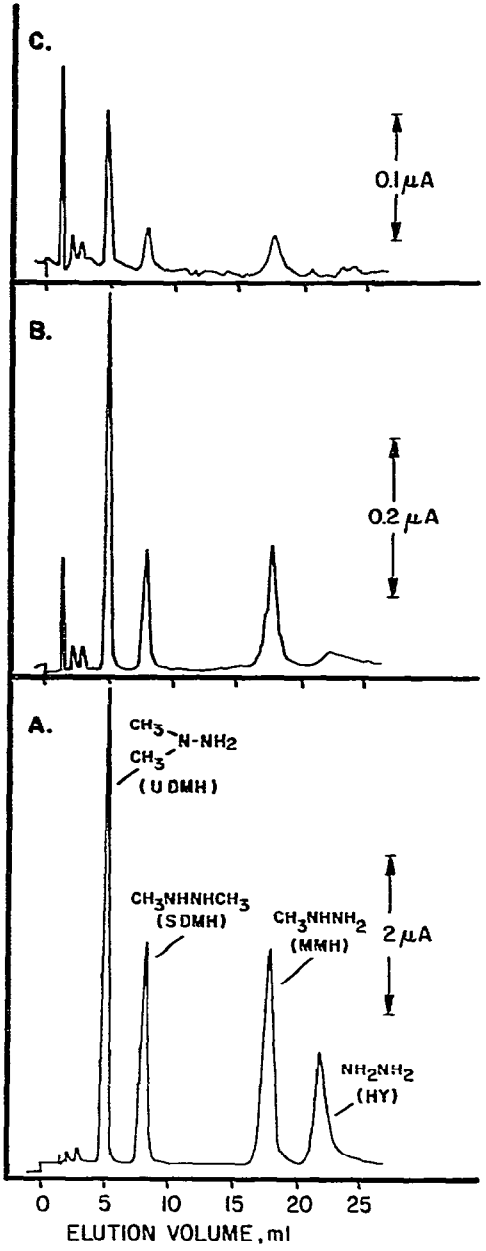
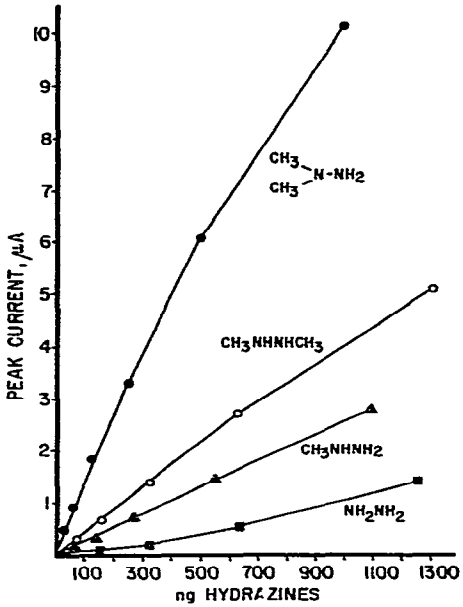


Fig. 1. HPLC of hydrazines on a 30 × 0.4 cm column of Aminex A-5 cation-exchange resin eluted with 0.05 *M* sodium borate, pH 8.90, at 1 ml/min. The amounts (in micrograms) of UDMH, SDMH, MMH and HY were, respectively, A: 0.5, 0.66, 1.09 and 1.26; B: 0.03, 0.04, 0.07 and 0.08; and C: 0.008, 0.01, 0.017 and 0.02.



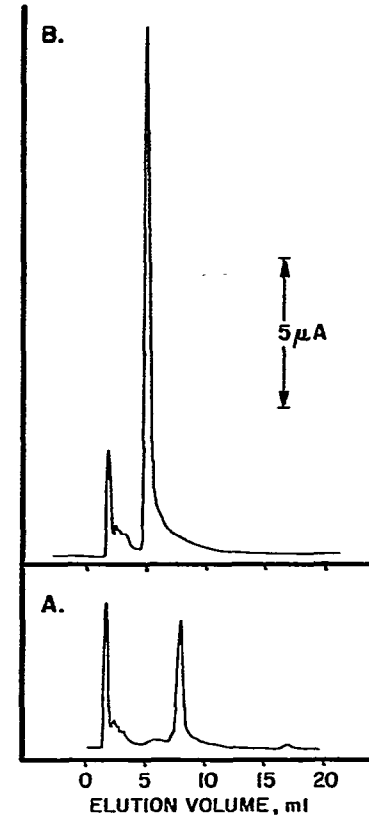
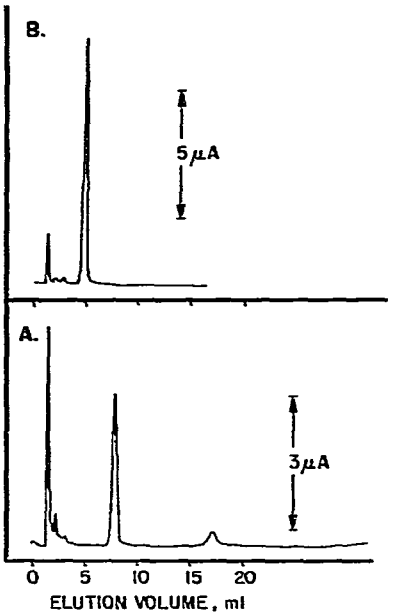


Fig. 2. Standard curve relating amounts of hydrazines injected and peak current. HPLC conditions same as for Fig. 1.

Fig. 3. HPLC of 100 μl of blood plasma obtained from a rat injected s.c. with 100 mg/kg SDMH (A) and with 50 mg/kg UDMH (B).

dividual variations in plasma levels of UDMH are apparent, nevertheless it is clearly evident that the plasma levels of UDMH are higher than those of SDMH at all but the earliest time points even though twice as much SDMH as UDMH was administered. The faster clearance of SDMH may be related to the metabolic conversion of the compound to azomethane gas[5,12] which is rapidly excreted via the exhaled air. Beyond 3 h, SDMH could not be detected in the blood, although UDMH was still detectable 6 hours after administration.

Previously, Rucki *et al.*[13] have reported the determination of procarbazine, a pharmacologically active hydrazine, by HPLC on amino-cyano columns with an amperometric detector with a carbon paste working electrode. The results of Rucki *et al.* together with ours demonstrate the utility of HPLC with electrochemical detection to the separation and estimation of various important hydrazine derivatives.



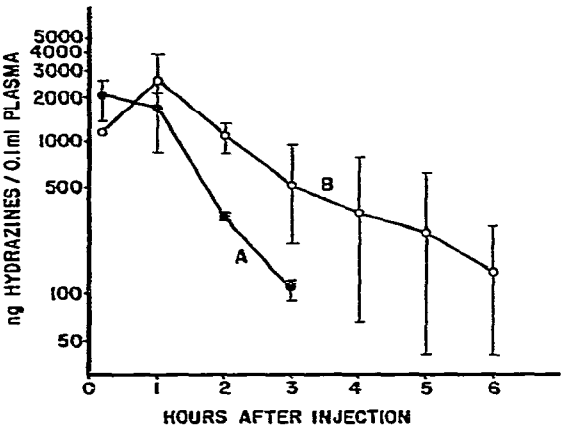


Fig. 4. HPLC of diluted urine samples obtained from a rat injected with 100 mg/kg SDMH (A) and with 50 mg/kg UDMH (B).

Fig. 5. Blood plasma levels of SDMH (A) and UDMH (B) in rats given these compounds as in Figs. 3 and 4. Data from three animals were averaged for each point. Brackets denote standard deviation.

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