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Note

Thin-layer chromatography of some methylated hydrazines and detection by a sensitive spray reagent

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During studies on the metabolic disposition of 1,2-dimethylhydrazine (1,2-DMH), a potent carcinogen for several rodent species[1,2], it became necessary to verify the radiochemical purity of the commercially synthesized ^{14}C-labeled compound. We report a simple thin-layer chromatographic (TLC) system which is highly effective in resolving the dihydrochlorides of hydrazine, methylhydrazine (MMH), 1,1-dimethylhydrazine (1,1-DMH) and 1,2-DMH. The system is applicable to both analytical (thin layer) and preparative (thick layer) scale separations. In addition, we found the Folin–Ciocalteau[3] reagent a very sensitive tool for the detection of hydrazines.

EXPERIMENTAL

MMH (free base) and 1,2-DMH·2HCl were obtained from Aldrich (Milwaukee, Wisc., U.S.A.), 1,1-DMH (free base) from K & K Labs. (Plainview, N.Y., U.S.A.), and hydrazine·2HCl from Eastman-Kodak (Rochester, N.Y., U.S.A.). Dihydrochlorides of MMH and 1,1-DMH were obtained by adding an excess of concentrated HCl to ethanolic solutions of the respective free bases in ice. After addition of 20 volumes of absolute ethanol and 10 volumes of diethyl ether, samples were allowed to stand overnight at −70°. The resulting white crystals (hygroscopic) were washed with cold ether–ethanol (10:1). Glass plates precoated with Avicel cellulose (250-μm thickness) were from Analtech (Newark, Del., U.S.A.). Folin–Ciocalteau reagent ("Phenol Reagent, 2N") was obtained from Fisher Scientific (Pittsburgh, Pa., U.S.A.).

Hydrazine salts were dissolved in 50% ethanol and applied as 1- to 2-cm streaks, representing up to 240 μg of sample, 2 cm from the bottom edge of the plate. Plates were developed with 2-propanol–water–conc. HCl (130:40:30) for a distance of 12.5 cm from the origin. For unlabeled compounds, the developed plates were partially dried by a current of air at 22° for 30 min and sprayed with the Folin–Ciocalteau reagent. After drying for 20 min at room temperature, the plates were exposed to NH_3 fumes whereupon the yellow background was bleached to white and prominent blue zones corresponding to separated hydrazines appeared immediately. For ^{14}C-labeled compounds, developed plates were dried overnight at room temperature and 1/8-in. zones were scraped sequentially. Material from the zones was suspended in Scintisol cocktail (Isolab, Akron, Ohio, U.S.A.) and radioactivity was determined in a liquid scintillation counter.

RESULTS AND DISCUSSION

A typical separation of the dihydrochlorides of hydrazine, MMH, 1,1-DMH and 1,2-DMH is represented in Fig. 1. The R_F values of these compounds are, 0.24, 0.47, 0.65, and 0.72, respectively. Some tailing of hydrazine and MMH at higher levels (> 20 µg) was evident but the zones for 1,1-DMH and 1,2-DMH are sharp and clearly defined even up to approximately 240 µg. The separation of all four hydrazines, including that of 1,1-DMH from 1,2-DMH, which is not feasible using other published systems[4], is complete.

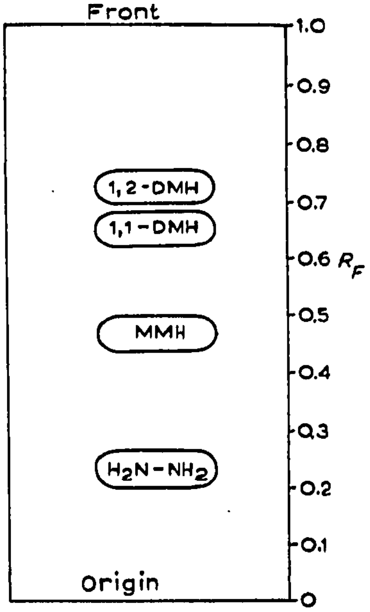

Fig. 1. R_F values of hydrazine, MMH, 1,1-DMH and 1,2-DMH on Avicel precoated plates. Solvent system, 2-propanol–HCl–water (130:30:40).

It should be noted that plates coated with Avicel F (with fluorescent indicator), also obtained from Analtech, cannot be used in this application since phase separation of the system occurs.

In contrast to the more commonly used Ehrlich's reagent (*p*-dimethylaminobenzaldehyde) which reacts preferentially with hydrazines containing an unsubstituted hydrazo group and thus does not give as strong a color reaction with 1,2-DMH as with the other hydrazines used here, the Folin–Ciocalteau reagent reacts with all four hydrazines apparently equally well. In a study of limits of detectability, 0.36 µg/cm^2 of separated hydrazines was easily visible and 0.12 µg/cm^2 was still detectable. This

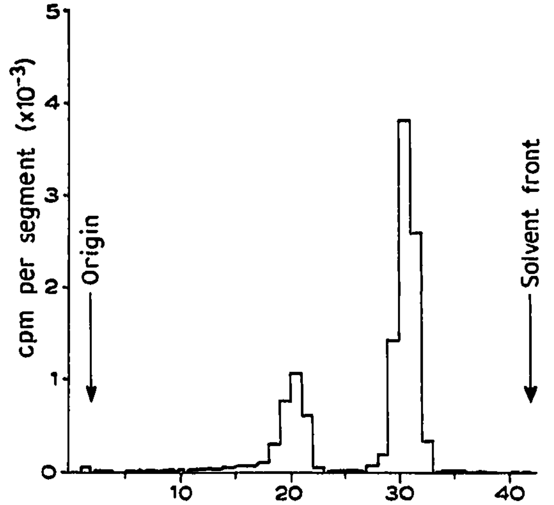

Fig. 2. TLC of a commercial preparation of ^{14}C-labeled 1,2-dimethylhydrazine. The sample was run as in Fig. 1. After drying overnight, consecutive 1/8-in. segments were scraped from the plate and radioactivity was determined by liquid scintillometry. The first peak (around segment 21) corresponds to ^{14}C-labeled MMH, the second peak (around segment 31) to ^{14}C-labeled 1,2-DMH.

is several orders of magnitude better than the sensitivity obtained with Ehrlich's reagent or with ammoniacal silver nitrate[4] and compares favorably even with gas chromatographic separations of hydrazines using the thermal conductivity detector[5]. The color developed is stable for at least a week.

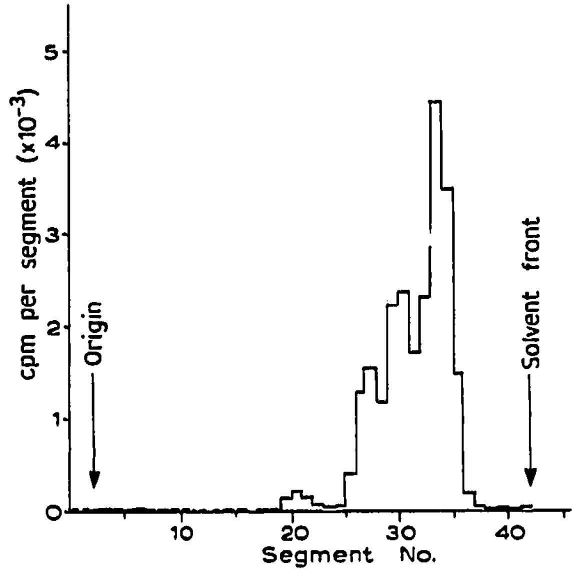

Fig. 3. Thin-layer chromatography of a commercial preparation of ^{14}C-labeled 1,2-DMH. The first peak (segment 21) corresponds to ^{14}C-labeled MMH, the second peak (segment 28) to ^{14}C-labeled 1,1-DMH, the third peak (segment 31) to ^{14}C-labeled 1,2-DMH, and the fourth peak is presumed to be ^{14}C-labeled trimethylhydrazine. Conditions, as in Fig. 1.

Detection of ^{14}C-labeled impurities in two commercial samples of ^{14}C-labeled 1,2-DMH is shown in Figs. 2 and 3. Interestingly, assay sheets furnished by the manufacturer for both samples claimed a purity of 99%, based on TLC on silica gel GF using *n*-butanol–acetic acid–water (4:1:5, upper phase) and acetone–chloroform (2:1), without however taking into consideration that these systems cannot resolve 1,2-DMH from MMH or 1,1-DMH. The sample in Fig. 2, showing a 21% contamination with ^{14}C-labeled MMH, was easily purified by scraping the zone corresponding to ^{14}C-labeled 1,2-DMH and eluting with a small volume of water containing 0.01% Na_2EDTA[1]. The sample in Fig. 3 was found to be grossly contaminated with ^{14}C-labeled MMH, ^{14}C-labeled 1,1-DMH and a substance of undetermined nature, probably ^{14}C-labeled trimethylhydrazine. In this case, purification by TLC was not attempted because of the close mobilities of the interfering compounds. However, pure ^{14}C-labeled 1,2-DMH was nevertheless successfully obtained by liquid chromatography[6].

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