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Thin-Layer Chromatographic Separation of Piperazine and Its Carcinogenic *N*-Nitroso Derivatives

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NOTE

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Abstract

A simple and rapid thin-layer chromatographic procedure that utilizes neutral solvent systems for the separation of piperazine and its *N*-nitroso and *N,N'*-dinitroso derivatives on silica gel adsorbent is reported.

INTRODUCTION

A number of orally administered drugs were recently found to react with dietary nitrite and form carcinogenic *N*-nitroso derivatives in experimental animals (*1*). Piperazine, a widely used anthelmintic agent, readily undergoes such nitrosation reaction to yield *N*-nitroso and *N,N'*-dinitroso derivatives under physiological conditions (*1, 2*). In mice and rats, both nitroso derivatives of piperazine produce multiorgan tumors (*3–6*). Coadministration of piperazine and sodium nitrite to mice by the oral route induces lung adenomas attributable to *in vivo* nitrosation of the drug (*7*).

Recently, during the development of a high-pressure liquid chromatographic (HPLC) assay for the toxic *N*-nitroso derivatives of piperazine (*8*), we had an occasion to study the thin-layer chromatographic (TLC) separation of these compounds on silica gel adsorbent. Only one acidic

solvent system, *n*-butanol–acetic acid–water (4:1:1), appeared to be reported earlier (*9*) for the TLC analysis of the two nitroso compounds. A major objective of our study was to achieve their TLC resolution employing neutral solvent systems which could then be used in the projected HPLC assay. The present paper describes a convenient TLC procedure that utilizes neutral solvent systems for the rapid separation of piperazine and its *N*-nitroso derivatives.

EXPERIMENTAL

Silica gel 60 F-254 pre-coated TLC plates (E. Merck, Darmstadt, G.F.R.), 20 × 20 cm, layer thickness 0.25 mm, were used after activation at 105°C for 5 min. The mono and dinitroso derivatives of piperazine were synthesized according to the literature procedure (*3*, *10*). Appropriate amounts of samples in chloroform or methanol were spotted on TLC plates and developed in solvent system I, II, or III (Table 1) by the ascending technique. The resolved compounds on chromatograms were visualized by either viewing under short-wavelength UV light (254 nm) or exposing to iodine vapors.

RESULTS AND DISCUSSION

The chromatographic development time for all three solvent systems (I–III) used was about 50 min. The data on the TLC resolution and detection limits of piperazine and its *N*-nitroso derivatives are given in Table 1. Satisfactory separation of the three compounds was achieved in

TABLE 1
TLC Separation of Piperazine and its *N*-Nitroso Derivatives

Compound	$R_F \times 100$, solvent system[a]			Color observed (detection limit, μg)	
	I	II	III	UV	Iodine
Piperazine	3.0	2.0	3.0	—	Dark brown (0.5)
N-Nitroso-piperazine	25.0	15.0	24.0	Blue (1.0)	Brown (2.0)
N, *N'*-Dinitroso-piperazine	62.0	66.0	67.0	Blue (0.5)	Light Brown (5.0)

[a] Solvent systems: I, ethyl acetate–methanol (1:1); II, chloroform–methanol (1:9); III, diethyl ether–methanol (1:4).

all the three solvent systems employed. When exposed to iodine vapors, all test compounds gave brown colored spots. Under the UV light, both *N*-nitroso derivatives of piperazine were detectable as blue spots on the chromatograms. However, piperazine could not be visualized with the UV light.

Thus development of chromatograms in any of the three solvent systems (I–III) and detection of the *N*-nitroso derivatives under the UV light could be used as a rapid procedure for monitoring the formation of these carcinogenic compounds from drug-nitrite interactions (*2*). The solvent system I has been successfully adopted in the HPLC assay of the two *N*-nitroso derivatives of piperazine (*8*).

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