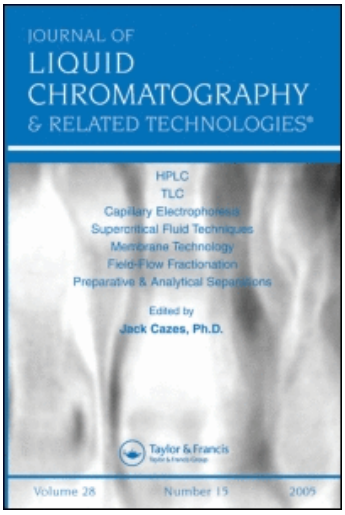

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Thin-Layer Chromatographic Separation of Antipyrine and Its Derivatives

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THIN-LAYER CHROMATOGRAPHIC SEPARATION OF ANTIPYRINE AND ITS DERIVATIVES

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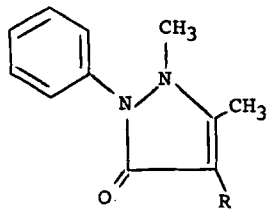
ABSTRACT

A convenient thin-layer chromatographic procedure that utilizes neutral solvent systems for the separation of antipyrine, aminopyrine, 4-aminoantipyrine, and 4-nitrosoantipyrine on silica gel adsorbent is reported.

INTRODUCTION

In continuation of our studies on thin-layer chromatographic (TLC) analysis of drugs, oral hygiene products and their metabolites (1-3), we investigated the TLC separation of antipyrine and its 4-substituted derivatives on silica gel adsorbent. Antipyrine (I) and its 4-dimethylamino derivative, aminopyrine (II) have been

used as antipyretic and analgesic agents (4). 4-Aminoantipyrine (III) is a N-demethylated metabolite of aminopyrine. Antipyrine readily yields the 4-nitroso derivative, IV in the presence of nitrite under physiological conditions (5).



(I) R = H

(II) R = $N<^{CH_3}_{CH_3}$

(III) R = NH_2

(IV) R = NO

A literature survey suggested that the TLC analysis of antipyrine and its 4-substituted derivatives, II-IV has not been reported earlier. The present paper describes a simple and rapid TLC procedure that utilizes neutral solvent systems for the separation of compounds I - IV on silica gel adsorbent.

EXPERIMENTAL

Commercially available silica gel 60F-254 pre-coated TLC plates (E. Merck, Darmstadt, G.F.R.), 5x20 cm or 20x20 cm, layer thickness, 0.25 mm, were used after activation at 105°C for 5

min. Compounds I - III were obtained from Aldrich Chemical Co. Inc., Milwaukee, Wisconsin. The 4-nitroso derivative of antipyrine (IV) was synthesized as described earlier(5). Requisite amounts of samples in chloroform were spotted on TLC plates and developed in solvent systems A-E (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 for 5 min.

RESULTS AND DISCUSSION

The TLC data on the separation and detection limits of antipyrine (I) and its three 4-substituted derivatives, II - IV are given in Table 1. The chromatographic development time for all five systems (A-E) employed was about 1 hr. All the solvent systems used gave satisfactory separation of the four compounds. 4-Aminoantipyrine (III) and 4-nitrosoantipyrine (IV) were visible on the chromatograms as yellow and green spots, respectively. All test compounds were detectable as blue spots under the UV light and when the chromatograms were exposed to iodine vapors, all compounds gave brown colored spots.

The R_f value of 4-nitrosoantipyrine (IV) relative to those of compounds I - III changed dramatically in various solvent systems employed for chromatographic development. Thus, in solvent systems B, C and E, IV exhibited the lowest R_f value, while it was

TABLE 1

TLC Separation of Antipyrine and its Derivatives

Compound (Number)	R_f [a] solvent system [b]					Color observed (detection limit, μg)		
	A	B	C	D	E	Visible	UV	Iodine
Aminopyrine (II)	0.48	0.50	0.42	0.34	0.50	--	Blue (0.5)	Light Brown (1.0)
Antipyrine (I)	0.40	0.39	0.34	0.20	0.45	--	Blue (0.5)	Dark Brown (0.5)
4-Aminoantipyrine (III)	0.32	0.30	0.29	0.11	0.37	Yellow (1.0)	Blue (0.5)	Brown (0.5)
4-Nitrosoantipyrine (IV)	0.36	0.20	0.22	0.24	0.33	Green (1.0)	Blue (1.0)	Dark Brown (2.0)

[a] Mean of six determinations. Standard errors of all mean values were ± 0.01 or less.

[b] Solvent systems: A, chloroform-acetone-methanol (5:2:1); B, chloroform-diethyl ether-methanol (2:2:1): C, chloroform-ethyl acetate-methanol (4:2:1); D, acetone-chloroform-diethyl ether (10:8:1); E, chloroform-benzene-methanol (4:1:1).

higher than III in solvent system A and higher than both compounds I and III in solvent system D. The TLC separation of the C-nitroso derivative, IV appeared to be influenced by both the polarity and nature of individual components of the solvent mixtures used.

The development of chromatograms in any of the five solvent systems (A - E) and detection of 4-nitrosoantipyrine (IV) under the UV light could be used as a rapid procedure for monitoring the formation of this C-nitroso derivative from antipyrine-nitrite interactions (5). The solvent system B appeared to be best suited for the drug-nitrite interaction studies with antipyrine.

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