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THIN-LAYER CHROMATOGRAPHY OF HYDRAZIDIC MONOAMINE OXIDASE INHIBITOR DRUGS

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SUMMARY

Seven solvent systems for the thin-layer chromatographic separation of hydrazidic monoamine oxidase inhibitor drugs have been developed. Detection of compounds at the microgram level was performed with the aid of thirteen spray reagents.

INTRODUCTION

Monoamine oxidase inhibitor (MAOI) drugs have frequently been used for the treatment of depressive states in psychotic patients. Several workers have described thin-layer chromatographic (TLC) procedures for the identification of isoniazid[1–4], iproniazid[1–7], isocarboxazid[1–8], nialamide[1–8] and iproclozide[2,4], using as the adsorbent silica gel G (Merck, Darmstadt, G.F.R.) and as the stationary phase water, 0.1 *N* sodium or potassium hydroxide solution or 0.1 *N* sodium hydrogen sulphate solution, with neutral or alkaline mobile phases based on methanol, chloroform and ammonia solution.

This paper reports TLC systems developed for the more unequivocal characterization of isoniazid, iproniazid, isocarboxazid, nialamide and iproclozide.

EXPERIMENTAL

TLC plates

Amounts of 12.5 g of cellulose MN 300 (Macherey, Nagel & Co., Düren, G.F.R.) and of 12.5 g of silica gel HF_{254} (Merck) were suspended in 95 ml of water and finely homogenized with the aid of a mixer. Using a Shandon Unoplan apparatus, five 20 × 20 cm glass plates were covered with the slurry to a thickness of 250 μm. The plates were dried in air and used without temperature activation.

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Solvent systems

Seven ternary mobile phase combinations were examined: (A) chloroform–methanol–13 *N* ammonia solution (90:10:1); (B) benzene–methanol–diethylamine (90:10:1); (C) chloroform–1-hexanol–13 *N* ammonia solution (90:10:0.2); (D) chloroform–ethyl acetate–13 *N* ammonia solution (50:50:1); (D) chloroform–ethyl acetate–13 *N* ammonia solution (50:50:1); (E) chloroform–acetone–acetic acid (90:10:1); (F) benzene–acetone–diethylamine (50:50:1); and (G) chloroform–acetone–acetic acid (50:50:1).

Detection

The plates were viewed under 254-nm UV light for presence of absorption on a fluorescent background. The thirteen spray reagents used are listed below.

(*a*) Molybdophosphoric acid reagent[1,8]: 5 g of molybdophosphoric acid (Merck) are dissolved in 100 ml of ethanol; after spraying, the TLC plates are exposed to ammonia vapour in a tank for 1 min.

(*b*) Iron(III) chloride–potassium hexacyanoferrate(III) reagent[9,10]: a freshly prepared 1:1 mixture of aqueous 1 % iron(III) chloride and 1 % potassium hexacyanoferrate(III).

(*c*) Folin–Ciocalteu reagent[1,4,7]: prior to use, this reagent (Merck) is diluted with an equal volume of distilled water; after spraying, the TLC plates are exposed to ammonia vapour in a tank for 1 min.

(*d*) Potassium permanganate reagent[3,5,8]: 0.1 g of potassium permanganate is dissolved in 100 ml of water.

(*e*) Ammoniacal silver nitrate reagent[11–13]: (*e'*)[12,13] just prior to use, 30 ml of 5 *N* ammonia solution and 30 ml of 50% silver nitrate solution are mixed, adding dropwise 5 *N* ammonia solution until the solution clears; after spraying, the TLC plates are heated for 1–2 min; (*e''*)[11] 5 g of silver nitrate are dissolved in 100 ml of water; after application of the former solution, the TLC plates are oversprayed with 1 % ammonia solution and then heated in a drying stove at 120°.

(*f*) Amminepentacyanoferrate(II) reagent[11]: 25 g of sodium nitroprussiate are treated with 75 ml of 13 *N* ammonia solution, left for 24 h in the refrigerator and diluted with absolute ethanol so as to precipitate yellow trisodium amminepentacyanoferrate(II), $Na_3[Fe(CN)_5NH_3]$; after washing with absolute ethanol, a solution of 1 g of trisodium amminepentacyanoferrate(II) is made in 100 ml of water, which serves as a spray reagent.

(*g*) Iodoplatinate reagent[5,13]: 5 ml of 5 % platinum chloride and 45 ml of 10 % potassium iodide solution are mixed and water is added to 100 ml.

(*h*) Iodine reagent[6]: 1 g of iodine is dissolved in 100 ml of methanol.

(*i*) Dragendorff's reagent[2,5,6,8,13]: 1.7 g of bismuth nitrate are dissolved in 100 ml of 20 % acetic acid (solution 1) and 40 g of potassium iodide are dissolved in 100 ml of water (solution 2); the reagent is prepared by mixing 20 ml of solution 1 with 5 ml of solution 2 and adding 70 ml of water.

(*j*) Cinnamaldehyde reagent[7]: 5 ml of cinnamic aldehyde are dissolved in 95 ml of ethanol to which 5 ml of concentrated hydrochloric acid are added.

(*k*) Alkaline triphenyltetrazolium reagent[2]: 2 g of triphenyltetrazolium chloride are dissolved in 100 ml of ethanol to which, just prior to use, is added an equal volume of a 1 *N* solution of sodium hydroxide in ethanol.

(*l*) Dithiocarbamate reagent[14]: 10 ml of carbon disulphide are poured into a 150-ml beaker containing 10 ml of 13 *N* ammonia solution; the beaker is placed inside the developing chamber, which fills with dense white vapour in 10–15 min; the TLC plates are exposed to this vapour for 10 min and then sprayed with 5% copper(II) chloride solution.

(*m*) Saturated ammonium molybdate and oxalic acid solutions[4]: the TLC plates are first sprayed with a saturated aqueous solution of ammonium molybdate and then with a saturated aqueous solution of oxalic acid.

Chromatographic procedure

A series of parallel strips, 3-cm wide, were marked out on each chromatographic plate using a scraper. Volumes of 10 μl of a solution containing 10 mg of active substance in 10 ml of chloroform or methanol were applied with a 10-μl micropipette on to 3 cm of the lower edge of a TLC plate in a 2-cm line. The plates were placed in a chromatographic tank lined with Whatman chromatography paper, saturated with the solvent system for at least 30 min, and developed over a distance of 10 cm measured from the lines of application. In order to determine the detection limits with spray reagents (*a*), (*b*) and (*c*), volumes of 1, 2, 4 and 10 μl of a solution containing 5 mg of active substance in 10 ml of chloroform or methanol were applied on TLC plates and developed in mobile phases A and G.

RESULTS AND DISCUSSION

Results on chromatographic behaviour in terms of R_{Ic} values (calculated with reference to iproclozide) are given in Table I. Taking as criteria the highest separation possible from structurally related compounds and an intermediate absolute R_F value (0.22–0.72), the following mobile phases seem to be the most suitable: isoniazid, E and F; iproniazid, A, B and F; isocarboxazid, D, E and G; nialamide, A and B; iproclozide, D, E and G.

TABLE I

RELATIVE R_F (R_{Ic}) VALUES OF HYDRAZIDIC MAOI DRUGS EXAMINED IN SEVEN MOBILE PHASE SYSTEMS

Drug	*R_{Ic} value**						
	*A***	*B*	*C*	*D*	*E*	*F*	*G*
Isoniazid	0.22	0.16	0.04	0.10	0.61	0.34	0.41
Iproniazid	0.75	0.48	0.20	0.35	0.22	0.62	0.48
Isocarboxazid	1.01	1.02	1.01	1.20	1.49	1.08	1.18
Nialamide	0.56	0.33	0.04	0.06	0.02	0.17	0.16
Iproclozide	1.00(0.81)****	1.00(0.63)	1.00(0.81)	1.00(0.51)	1.00(0.41)	1.00(0.65)	1.00(0.61)

* $R_{Ic} = \dfrac{R_F \text{ of compound}}{R_F \text{ of iproclozide}}$.

** For mobile phase systems A to G, see text.

*** Values in parentheses are absolute R_F values of iproclozide as measured on the particular chromatogram (for each mobile phase, all five compounds studied were investigated on the same plate).

TABLE II

DETECTION CHARACTERISTICS OF HYDRAZIDIC MAOI DRUGS

Drug	*UV light (254 nm)*	*Spray reagent*													
		(a)	*(b)*	*(c)*	*(d)*	*(e')*	*(e'')*	*(f)*	*(g)*	*(h)*	*(i)*	*(j)*	*(k)*	*(l)*	*(m)*
Isoniazid	+	Blue	Blue	Blue	Yellow	Brown	Brown	Yellow-brown	White	Yellow-brown	–	(Yellow)***	(Red)***	Green-brown	(Brown)***
Iproniazid	+	Blue	Blue	Blue	Yellow	Brown	Brown	Yellow	White	Yellow-brown	–	(Yellow)***	Red	(Green-brown)***	Brown
Isocarboxazid	+	Blue	Blue	Blue	Yellow	Brown	Yellow-brown	–	White	Yellow-brown	–	(Yellow)***	Red	Green-brown	(Brown)***
Nialamide	+	Blue	Blue	Blue	Yellow	Brown	Brown	Yellow	White	Yellow-brown	–	(Yellow)***	Red	(Green-brown)***	Brown
Iproclozide	–	Blue	Blue	Blue	Yellow	(Brown)*	(Brown)*	–	(White)**	Yellow-brown	–	–	Red	–	–

* Colour is seen only after the heating step.

** Intensified by exposure to ammonia vapour (see text).

*** Less intense colours are obtained.

Results on the detection of compounds are given in Table II. All compounds, except iproclozide, showed absorption on a fluorescent background when inspected under 254-nm UV light. The molybdophosphoric acid reagent (*a*), the iron(III) chloride–potassium hexacyanoferrate(III) reagent (*b*) and Folin–Ciocalteu reagent (*c*) yield good results; although no colour differentiation is produced, universal detection involving the hydrazidic functional group is possible. The potassium permanganate spray reagent (*d*) gives yellow stains on a pink background but the colours obtained are less intense and disappear shortly after spraying. The ammoniacal silver nitrate reagents (*e'*) and (*e''*) allow good detection of all products examined; for iproclozide, however, a heating step is necessary. As shown in Table II, the amminepentacyanoferrate(II) reagent (*f*) is more specific for isoniazid, iproniazid and nialamide, but is inapplicable to isocarboxazid and iproclozide. The universal iodoplatinate reagent (*g*) gives clear white spots on a brown background, except for iproclozide, which is only just visible. Intensification of the latter occurs after exposure of the plate to ammonia vapour, respraying with reagent (*g*) and final treatment with ammonia vapour. The unspecific iodine reagent (*h*) produces intense yellow-brown stains for all products. Dragendorff's reagent (*i*) is useless because it does not permit any colour development of the compounds studied. Application of the cinnamaldehyde reagent (*i*) results in slightly yellow spots that are better detected by viewing the plate under 366-nm light. Heating the plate at 110° intensifies the isocarboxazid stain. In general, the cinnamaldehyde reagent (*j*) results in a less sensitive method of detection. Alkaline triphenyltetrazolium reagent (*k*) develops clearly all compounds as red stains, except isoniazid, which gives a less intense spot even when heated in a drying stove at 90°. Isocarboxazid is well located by the dithiocarbamate reagent (*l*) as a green-brown spot, but the other compounds are only just visible and iproclozide remains invisible. Application of saturated ammonium molybdate solution (*m*) reveals nialamide well, and subsequent spraying with saturated oxalic acid solution (*n*) intensifies the nialamide spot and also reveals isoniazid and iproniazid.

In our experience, sensitive detection is achieved with one of the three reagents (*a*), (*b*) and (*c*): an amount of 0.5–1 μg of active substance is just visible for all compounds chromatographed in mobile phase A or G. The clarity of the stains observed decreases in the order (*b*) > (*a*) > (*c*). Other reagents (*d–h, j–m*) might be useful for particular purposes, but are not universally applicable. Even when a good colour differentiation between similar products is not possible, combination of the solvent systems permits clear identification of the MAOI drugs studied to be achieved.

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