Journal of Chromatography, 177 (1979) 174–176

CHROM. 12,069

Note

Separation of isomeric monocyanopyridine and pyridinemonoamidoxime hydrochlorides by thin-layer chromatography

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(Received April 9th, 1979)

Pyridineamidoximes and their hydrochloride salts have been investigated as potential acetylcholinesterase activators[1,2], however, a search of the literature did not reveal any micro method for the detection of these compounds. We have investigated the separation parameters and present a simple thin-layer chromatographic (TLC) method which will differentiate the three pyridinemonoamidoximes from each other as well as from their cyanopyridine starting materials. The procedure given will work equally well for either the hydrochloride salts or their parent compounds.

EXPERIMENTAL

Chemicals and reagents

2-, 3- and 4-Cyanopyridine (-CNPy) were purchased from Aldrich-Europe (Beerse, Belgium). Pyridine-2-amidoxime (Py-2-AO) was synthesized as previously described[3]. Pyridine-3-amidoxime (Py-3-AO) was prepared by adding 75 ml of an aqueous solution containing 14.6 g of hydroxylamine hydrochloride and 8.4 g of sodium hydroxide to 20.8 g of 3-CNPy dissolved in 75 ml of 95% ethanol. The solution was heated overnight on a steam-bath, evaporated to dryness under a stream of air, the solid dissolved in a minimum volume of absolute ethanol, the solution filtered and crystallized by slow evaporation of the ethanol to give a 72.3% yield (19.8 g) of Py-3-AO. A sample recrystallized from ethanol and dried *in vacuo* melted at 128° (Lit. 127.5–128°)[4]. The sealed tube reaction described in the literature was found to be unnecessary[4,5]. Pyridine-4-amidoxime (Py-4-AO) was prepared by the method of Bernasek[4]. The hydrochloride salts of the cyanopyridines and amidoximes were prepared using the following two procedures. In the first procedure, the compound was dissolved in excess concentrated hydrochloric acid, evaporated to dryness, and the solid recrystallized from hot absolute ethanol. The second procedure consisted of dissolving the compound in a minimum of glacial acetic acid and saturating the solution with hydrogen chloride gas. The second procedure gives a purer product

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TABLE I

CHLORIDE ANALYSIS OF HYDROCHLORIDE SALTS

Compound	*Chloride (%, calc.)*	*Chloride (%, found)**
2-CNPy · HCl	25.22	25.09
3-CNPy · HCl	25.22	25.13
4-CNPy · HCl	25.22	25.18
Py-2-AO · 2HCl	33.76	33.63
Py-3-AO · 2HCl	33.76	33.39
Py-4-AO · 2HCl	33.76	33.53

* Average of 5 or more determinations.

but a lesser yield than the first. The results of the gravimetric analyses of the hydrochloride salts by chloride precipitation with silver nitrate are summarized in Table I.

Chromatography

Three types of commercially available pre-coated plates (Merck, Darmstadt, G.F.R.) were used. These plates were: silica gel 60 GF TLC (20 × 20 cm, 0.25 mm thickness); silica gel 60 HPTLC (10 × 10 cm, 0.25 mm thickness) and cellulose F TLC, aluminum sheets (20 × 20 cm, 0.10 mm thickness). Two solvent systems were investigated: solvent system A, consisting of isoamyl alcohol–acetone–acetic acid–water (55:25:10:10) and solvent system B, consisting of isoamyl alcohol–acetone–ammonia–water (70:5:10:15). The spots were visualized using both UV radiation (254 nm) and reaction with 5% ferric chloride solution.

Method

The plates developed with solvent system A were spotted with 1.0 μl of a 1% aqueous solution of each compound and eluted using a sandwich chamber at 20°. The silica gel 60 TLC plates were similarly spotted and eluted using solvent system B. The high-performance TLC (HPTLC) plates were spotted with 200 nl of the compounds prior to elution with solvent system B. In all instances the plates were dried after sample application and development.

RESULTS AND DISCUSSION

Using solvent system A and the cellulose F plate gave only partial separation of the compounds. The 2-isomers were separated from the 3- and 4-isomers, however both of the 2-isomers had approximately the same R_F value (Table II). Substitution of the silica gel 60 GF TLC plate using the same solvent system gave marginally better separation, but showed a significant decrease in tailing. Based upon this observation, only silica gel plates were used with solvent system B utilizing multiple development. Solvent system B separated into two phases when prepared and after thorough mixing the upper, water–ammonia-saturated alcoholic phase was used for development. For samples developed twice over a 10-cm path on silica gel TLC plates, the development took 7 h. By using HPTLC plates on a 5-cm path with 2 developments, the spots became more concentrated with an optimum separation. The time for plate development was reduced to 2 h and 40 min. Solvent system B was more effective than

TABLE II

R_F VALUES FOR CHROMATOGRAPHY OF NITRILE AND AMIDOXIME HYDROCHLORIDES

R_F valuez are the average of three or more riens.

	2-CNPy · HCl	*3-CNPy · HCl*	*4-CNPy · HCl*	*Py-2-AO · HCl*	*Py-3-AO · 2HCl*	*Py-4-AO · 2HCl*
Solvent system A						
Cellulose F	0.30	0.50	0.50	0.35	0.50	0.50
Silica gel 60 (TLC)	0.13	0.50	0.40	0.15	0.48	0.50
Solvent system B						
Silica gel 60 TLC	0.07	0.17	0.18	0.05	0.55	0.61
Silica gel HPTLC	0.06	0.20	0.24	0.04	0.72	0.78

A because the ammonia neutralized the hydrochloride salt leaving the free organic moiety to elute.

The spots in all cases were first marked under UV radiation. Subsequently, a drop of 5% ferric chloride solution was placed on each spot. Cyanopyridines did not undergo a color reaction under these conditions. Both 2- and 3-PyAO reacted with ferric ion to produce a red coloration thus allowing a confirmatory chemical identification in addition to UV visualization.

Because solvent system B does neutralize the hydrochlorides with the formation of ammonium chloride, the development actually involved only the organic molecules. This procedure can therefore be used starting with cyanopyridines and pyridine-amidoximes with the same results as those obtained with the hydrochlorides.

ACKNOWLEDGEMENTS

This investigation was supported by the Department of Analytical Chemistry, University of Stockholm.

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