CHROM. 4394

The separation of parasympatholytics by thin-layer chromatography*

Clinical pharmacological investigations of parasympatholytics have previously been made and their presence and quantity have been determined by bioassay in different biological fluids (urine, saliva, bile, serum) of patients[1-4]. This method was found to be very sensitive but not specific for different parasympatholytic drugs.

In order to study the drug cross-tolerance developed in patients treated with parasympatholytics, we needed a method suitable for the identification and separation of several parasympatholytics simultaneously. A simple thin-layer chromatographic method for the separation and identification of various parasympatholytics simultaneously is presented in this paper.

The following solutions were used as developing solvents: chloroform–ethanol (5:5) and chloroform–ethanol (7:5). Potassium iodoplatinate reagent (3 ml of 10% H_2PtCl_6 solution mixed with 97 ml water, added to 100 ml of 6% aqueous KI) was used as the chromogenic reagent[5]. Silica Gel G (Merck; 25 g in 50 ml of 1.0 *N* NaOH solution) and Aluminium Oxide G (Merck; 25 g in 50 ml of water) were used as coating materials. The slurry was spread over glass plates (20 × 20 cm) to a thickness of 250 μ with a Desaga equipment. The coated plates were air dried at room temperature. The plates were activated before use by heating them at 110° for 1 h.

After activation the plates were allowed to cool on a large glass plate for 5 min, spots (usually 5 to 10 μl) of the parasympatholytic solutions under investigation were applied, and the treated plates were put into a tank equilibrated with the solvent to be used for chromatography. The atmosphere in the tank was kept saturated with the developing solvent vapour by lining the tank walls with strips of filter-paper which dip into the solvent.

Development was by the ascending method for 15–16 cm. The plates were dried at room temperature for 30 min and sprayed with the chromogenic reagent.

The following parasympatholytics were examined: atropine, Novatropine (methylhomatropinum bromatum), isopropamide (2,2-diphenyl-4-diisopropyl-amino-methyliodide) and Gastrixone (8-methyl-tropiniumbromide-xanthene-9-carboxylate). These were all obtained from the United Drug and Foodstuffs Factory, Budapest. Atropine is a tertiary amine, whereas Novatropine, isopropamide and Gastrixone are quaternary ones.

The results are presented in Table I. The spots obtained after treatment with the chromogenic reagent were of different shades of violet. The sensitivity of the method is 1 to 30 μg.

According to our results the Aluminium Oxide G was found to be suitable for the separation of each parasympatholytic drug tested when both chloroform–ethanol solvents were used, while the Silica Gel G thin-layer was found to be suitable only for the separation of atropine, isopropamide and Gastrixone. Densitometry following the chromatography was found to give a quantitative evaluation of the parasympatholytics.

Other solvent solutions[5-9] (benzene–methanol; benzene–ethanol; ethanol–am-

* This work was supported by a grant from OMFB (National Committee for Technical Development).

TABLE I

R_F VALUES × 100 FOR PARASYMPATHOLYTICS

Developed in chloroform–ethanol (5:5), chloroform–ethanol (7:3) and sprayed with potassium iodoplatinate.

Parasympatholytics	*Solvent solutions*	
	Chloroform–ethanol (5:5)	*Chloroform–ethanol (7:3)*
Silica Gel G impregnated with 1.0 N NaOH		
Atropine	53	63
Novatropine	00	00
Isopropamide	48	30
Gastrixone	16	07
Aluminium Oxide G		
Atropine	77	87
Novatropine	12	05
Isopropamide	70	70
Gastrixone	36	16

monia; methanol–acetone–triethylamine; chloroform–diethylamine; cyclohexane–chloroform–diethylamine; cyclohexane–diethylamine; benzene–ethyl acetate–diethylamine; dimethylformamide–diethylamine–ethanol–ethyl acetate used in different ratios) and thin-layers[9] (Silica Gel G with water and impregnated with 0.05 *N*, 0.10 *N*, 0.25 *N*, or 0.50 *N* NaOH, and Silica Gel G mixed with Aluminium Oxide G) did not give a satisfactory simultaneous separation of the parasympatholytics mentioned above.

First Department of Medicine, University Medical School, Pécs (Hungary) GY. MÓZSIK

Department of Hygiene, University Medical School, Debrecen (Hungary) ELISABETH TÓTH*

1 GY. MÓZSIK AND T. JÁVOR, *Med, Pharmacol. Exp.*, 14 (1966) 183.
2 GY. MÓZSIK, Á. GYÓRFFY, S. DOBI AND T. JÁVOR, *III. Conferentia Hungarica pro Therapia et Investigatione in Pharmacologia*, Academic Press, Budapest, 1965, p. 497.
3 GY. MÓZSIK, I. VIZI AND T. JÁVOR, *Recent Advances in Gastroenterology*, Vol. I, Kenkyusha Printing Co., Ltd., Tokyo, 1967, p. 681.
4 GY. MÓZSIK AND T. JÁVOR, *European J. Pharmacol.*, 6 (1969) 169.
5 D. WALDI, K. SCHNACKERZ AND F. MUNTER, *J. Chromatog.*, 6 (1961) 61.
6 J. BAUMLER AND S. RIPPSTEIN, *Pharm. Acta Helv.*, 36 (1961) 382.
7 S. HERMANEK, V. SCHWARTZ AND Z. CEKAN, *Pharmazie*, 16 (1961) 566.
8 K. TEICHERT, E. MUTSCHLER AND H. ROCHELMEYER, *Deut. Apotheker-Ztg.*, 100 (1960) 477.
9 B. KISS, J. KOVÁCS AND L. GYARMATI, *Élelmiszervizsgdlati Közlemen.*, 12 (1966) 55.

Received September 15th, 1969

* Present address: Department of Radiology, University Medical School, Debrecen, Hungary.