Journal of Chromatography, 114 (1975) 329–333

CHROM. 8432

SEPARATION OF THIAZIDE DIURETICS AND ANTIHYPERTENSIVE DRUGS BY THIN-LAYER CHROMATOGRAPHY

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(First received February 24th, 1975; revised manuscript received May 12th, 1975)

SUMMARY

A procedure is reported for the thin-layer chromatographic (TLC) separation and identification of thiazide diuretics and other antihypertensive drugs. Several new solvent systems and a variety of possible detection reagents were examined. Twenty thiazides used routinely in therapy were successfully separated and identified. Use of the TLC systems and combinations of the detecting methods described should be useful for the identification of these drugs in biological fluids and in various dosage forms.

INTRODUCTION

The thiazide diuretics are most useful in the management of edema of chronic cardiac decompensation. They are also useful in treating edema due to chronic hepatic or renal disease as well as hypertensive diseases with or without edema[1]. Ethacrynic acid and furosemide are effective in all types of edema[1]. No comprehensive methods have been reported for the thin-layer chromatographic (TLC) separation and identification of thiazide diuretics and other antihypertensive drugs used routinely in therapy. Adams and Lapière[2] achieved poor separation of twelve thiazides on alumina plates developed with ethyl acetate and on silica gel plates developed with ethyl acetate or ethyl acetate–benzene (8:2). Duchene and Lapière[3] subsequently examined the TLC separation of several additional diuretics on Alumina GF_{254} plates (Merck) and silica gel DSF5 plates (Camag) developed with ethyl acetate or ethyl acetate containing up to 3% water. Kiger and Kiger[4] reported on the TLC separation of a large number of sulfonamides which included acetazolamide, buthiazide and methyclothiazide. Flinn and Smith[5] concentrated polythiazide extracted from tablets on SilicAR 7GF plates developed with methylene chloride–methanol (90:2), but reported no R_F values. Sohn *et al.*[6] reported the column extraction and subsequent TLC of bendroflumethiazide, cyclothiazide, furosemide, hydrochlorothiazide and hydroflumethiazide; ethyl acetate was used to extract the thiazides which were developed on silica gel G plates with ethyl acetate–benzene (8:2). Recently Agarwal *et al.*[7] resolved seven thiazides into essentially two groups using ethyl acetate as the developing reagent.

The most common detection methods employed to date have been ultraviolet light[2,3], iodine[4], Bratton–Marshall reagent[6], *p*-dimethylaminobenzaldehyde reagent[6], and several metal ion containing reagents[7].

We have separated twenty thiazide diuretics and antihypertensive drugs by TLC, using several new solvent systems and examining a variety of possible detection reagents.

EXPERIMENTAL

Reagents

The following is a list of the drugs that were investigated with the source from which they were obtained: cyclothiazide (Eli Lilly), methyclothiazide (Abbott Labs.), hydrochlorothiazide (Abbott Labs.), acetazolamide (Lederle Labs.), quinethazone (Lederle Labs.), benzthiazide (A. H. Robins Research Labs.), trichloromethiazide (Schering), hydroflumethiazide (Bristol Labs.), polythiazide (Pfizer), bendroflumethiazide (E. R. Squibb & Sons), chlorothiazide (Merck, Sharp & Dohme), chlorthalidone (USV Pharmaceutical) ethacrynic acid (Merck, Sharp & Dohme), triamterene (Smith Kline & French Labs.), furosemide (Hoechst Pharmaceuticals), diazoxide (Schering), propranolol (Ayerst Labs.), hydralazine (CIBA Pharmaceutical), reserpine (CIBA Pharmaceutical), and metolazone (Pennwalt).

All chemicals were analytical grade. The coating material for the TLC plates was silica gel G (EM Reagents, Art. No. 7731). The developing solvents used were: methyl ethyl ketone (MEK)–*n*-hexane in the ratios 1:1, 2:1 and 3:2, and chloroform–acetone–triethanolamine (50:50:1.5). The R_F values for the various drugs in each of these systems are given in Table I.

The following spray reagents were employed: Dragendorff's reagent[8], 50% sulfuric acid with heat, alkaline *p*-dimethylaminobenzaldehyde (DMAB) with heat[6], anisaldehyde reagent with heat[9], 1% potassium permanganate in water, chloroplatinic acid solution, 0.1% diphenylcarbazone in chloroform, mercurous nitrate solution, and Bratton–Marshall reagent (BMR) with and without hydrolysis[6]. Dragendorff's reagent was prepared from two solutions: solution A consisted of 1.7 g bismuth subnitrate in 100 ml water–acetic acid (80:20); solution B contained 40 g potassium iodide in 100 ml water. The spray reagent was a freshly prepared mixture of 5 ml solution A, 5 ml solution B, 20 ml acetic acid and 70 ml water[8]. Alkaline DMAB consisted of 2 g *p*-dimethylaminobenzaldehyde in 75 ml 80% acetone and 25 ml conc. ammonium hydroxide; it should be freshly prepared[6]. The anisaldehyde reagent was prepared with 0.5 ml anisaldehyde, 1.0 ml sulfuric acid and 50 ml acetic acid[9]. The chloroplatinic acid solution consisted of 2 g potassium iodide in 100 ml water with 4 ml 5% platinum chloride solution added. The mercurous nitrate solution contained 0.50 ml 0.6 *N* HNO_3 with 1.0 g $HgNO_3$ in 100 ml water. The Bratton–Marshall reagents are sprayed in the following order: (a) 1% (w/v) sodium nitrite in 1% sulfuric acid, freshly prepared; (b) 5% (w/v) ammonium sulfamate, stored in a refrigerator; (c) 2% (w/v) N-(1-naphthyl)-ethylene diamine dihydrochloride in 95% ethanol stored in a brown bottle in a refrigerator[6]. Hydrolysis was accomplished by spraying the developed plates with 10 *N* HCl and heating them for 10 min at 100° in an oven. The colors noted are reported in Table II.

The sensitivity limits for the detection reagents were approximately: 5 μg for

TABLE I

R_F VALUES OF THIAZIDES USING VARIOUS SOLVENT SYSTEMS

Each value represents the average of at least three determinations.

Thiazide	*R_F value*			
	Methyl ethyl ketone–n-hexane			*Chloroform–acetone–triethanolamine (50:50:1.5)*
	1:1	*2:1*	*3:2*	
Cyclothiazide	0.41	0.63	0.63	0.62
Methyclothiazide	0.36	0.60	0.54	0.56
Hydrochlorothiazide	0.22	0.47	0.37	0.40
Acetazolamide	0.18	0.42	0.40	0.39
Quinethazone	0.13	0.33	0.25	0.30
Benzthiazide	0.45	0.60	0.53	0.56
Trichloromethiazide	0.50	0.65	0.59	0.57
Hydroflumethiazide	0.32	0.56	0.53	0.46
Polythiazide	0.46	0.67	0.70	0.65
Bendroflumethiazide	0.57	0.68	0.69	0.66
Chlorothiazide	0.16	0.39	0.36	0.31
Ethacrynic acid	0.14	0.37	0.40	0.06
Chlorthalidone	0.21	0.50	0.38	0.43
Triamterene	0.12	0.19	0.21	0.36
Furosemide	0.30	0.57	0.50	0.10
Diazoxide	0.12	0.19	0.42	0.23
Propranolol	0.00	0.00	0.00	0.00
Hydralazine	0.72	0.62	0.00	0.68
Reserpine	0.10	0.25	0.05	0.63
Metolazone	0.31	0.60	0.50	0.63

Dragendorff's reagent and mercurous nitrate; 2 μg for anisaldehyde, chloroplatinic acid and diphenylcarbazone reagents; 1 μg for alkaline DMAB and potassium permanganate; and 0.5 μg for the Bratton–Marshall reagent.

Preparation of thin-layer plates

The silica gel G (30 g) was shaken vigorously with 65 ml water for 60–90 sec and the slurry was spread over five 20 × 20 cm glass plates to a thickness of 250 μm with a Kensco (Kensington Scientific) apparatus. The coated plates were air-dried at room temperature, then activated for 1 h at 110° and stored in a desiccator.

Procedure

Clean, dry chromatography tanks were prepared with each of the developing solutions. The tanks were allowed to equilibrate by covering one wall of each tank with a sheet of Whatman No. 1 filter paper and allowing the solvents to stand in the tanks for 24 h at room temperature. The materials to be chromatographed were routinely spotted approximately 1 cm apart and 1.5 cm from the bottom of the plate. The solutions of the drugs used were prepared in methylene chloride–methanol (3:2) at 2 mg/ml, except quinethazone, which was dissolved in chloroform–methanol–dimethylsulfoxide (1:1:1). Aliquots of 10–20 μl were applied to the plates. The plates were developed once to the top of the plate in a solvent system, and allowed to air dry before spraying with one of the detecting systems.

TABLE II

DETECTING COLORS OF THIAZIDES USING VARIOUS SPRAY REAGENTS

Y = yellow; Br = brown; O = orange; Pn = pink; G = green; W = white; V = violet; Pu = purple; Bl = blue; R = red; L = light; D = dark; UV = visible under ultraviolet; – = not detected.

Thiazide	*Color*									
	Dragendorff's	*Sulfuric acid*	*Alkaline DMAB*	*Anisaldehyde*	*Potassium permanganate*	*Chloroplatinic acid*	*Diphenylcarbazone*	*Mercurous nitrate*	*BMR with hydrolysis*	*BMR without hydrolysis*
Cyclothiazide	–	G	Y	Y	W	Pu	Pu	W	R	O
Methyclothiazide	Y	–	DY	Y	W	Pu	Pu	W	R	G
Hydrochlorothiazide	Y	–	Y	Y	W	O	Pu	Y	LPu	W
Acetazolamide	–	–	Y–O	–	–	W	–	W	Pu	–
Quinethazone	Y	–	LY	Y	W	Y	Pu	W	Pu	W
Benzthiazide	–	–	O	–	W	W	Pu	W	W	–
Trichloromethiazide	Y	–	Y	Y	W	O	Pu	W	LPn	Pn
Hydroflumethiazide	Y	–	Y	Y	W	W	Pu	W	LPu	–
Polythiazide	Y	–	LY	Y	W	W	Pu	W	R	O
Bendroflumethiazide	Y	–	DY	Y	W	–	Pu	Y	Pn	LY
Chlorothiazide	–	–	LY	V	–	W	–	W	UV	–
Ethacrynic acid	Br	–	DY	LPu	W	O	Pu	W	–	–
Chlorthalidone	Y	–	Y	LY	–	–	Pu	W	UV	–
Triamterene	LY	–	Y–O	LBl	W	Br	Pu	Y	Y	LBl
Furosemide	–	–	Y	Br	W	W	Pu	Y	DR	Pn
Diazoxide	LY	–	W	–	W	W	Pu–Y	W	–	LBl
Propranolol	O	–	–	LV	–	–	–	–	W	G
Hydralazine	Pn	–	O	Y	–	–	–	–	O	Pn
Reserpine	O	G	–	R	W	W	Pu	W	Y	Y
Metolazone	LY	–	LY	–	Y	LPu	LPu	–	Pn	LPn

RESULTS

By using any one of the four solvent systems reported, it is possible to separate most of the twenty drugs. By developing one plate in one of the MEK–*n*-hexane systems and a second plate in the chloroform–acetone–triethanolamine system, separation and identification of all of the drugs can be achieved.

Of all the detecting reagents examined, the alkaline DMAB and the BMR with hydrolysis gave the greatest variety of colors and therefore proved to be most useful. The diphenylcarbazone gave purple spots with most thiazides, while the potassium permanganate and mercurous nitrate yielded white spots with most of the drugs. Sulfuric acid with heat is not useful. Chloroplatinic acid can aid in identification of most of the drugs, but does not detect bendroflumethiazide, chlorothalidone, propranolol and hydralazine. Dragendorff's reagent will detect all of these latter compounds. The DMAB reagent failed to detect propranolol and reserpine, while the BMR with hydrolysis would not visualize bendroflumethiazide and furosemide.

The above systems and combinations of the detecting methods will be most useful in studies on the identification of thiazides and antihypertensive drugs in biological fluids and in various dosage forms.

ACKNOWLEDGEMENTS

The authors wish to thank the various pharmaceutical manufacturers for generously providing the drugs used in this investigation. We also wish to thank Ms. Jeanne Hassing and Laura Pape for technical assistance. This investigation was supported in part by a grant from the Nebraska Heart Association.

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