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Note

Thin-layer chromatographic screening procedure for some drugs used in treatment of arthritis: "arthritic cures" prescribed in Mexico

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During the past two years approximately 170 samples of "secret" arthritis "cures", prescribed by physicians in Mexican arthritis clinics and obtained in Mexico, were submitted by U.S. residents to our laboratory for identification[1]. PharmChem Laboratories of Palo Alto, Calif., U.S.A. has also reported the receipt of a number of similar samples[2]. The increase in the number of alleged "arthritis cures" submitted to street-drug analysis programs[1,2] and reports[3–8] have lead us to develop a rapid screening procedure for tentative identification of these drugs.

Previously this laboratory has reported a rapid screening procedure using a single ethanolic extraction followed by thin-layer chromatography (TLC) for the tentative identification of certain common street drugs[9,10]. This basic procedure also has been found to be satisfactory for the extraction and tentative identification of certain of the commonly prescribed, non-steroid, Mexican "arthritic cures", *e.g.* indomethacin, phenylbutazone, trifluoperazine, chlordiazepoxide, meprobamate, and diazepam. The steroid drugs, triamcinolone, prednisolone, methylprednisolone, hydrocortisone, cortisone, prednisone, and paramethasone were extracted by 95% ethanol but TLC separation was not achieved when the basic developing system[9] was used. Thus, it was necessary to use another solvent system for satisfactory separation of such steroids.

EXPERIMENTAL

Materials and methods

The following standard solutions were prepared from solid dosage forms: 5 mg/ml in 95% ethanol each of triamcinolone (Lederle, Pearl River, N.Y., U.S.A.), prednisolone (Robinson, San Francisco, Calif., U.S.A.), methylprednisolone (Upjohn Co., Kalamazoo, Mich., U.S.A.), hydrocortisone (Robinson), prednisone (Robinson), paramethasone acetate (Eli Lilly & Co., Indianapolis, Ind., U.S.A.), cortisone acetate (Robinson), indomethacin (Merck Sharp & Dohme, West Point, Pa., U.S.A.), phenylbutazone (Ciba-Geigy, Summit, N.J., U.S.A.), trifluoperazine hydrochloride (Smith Kline & French Labs., Philadelphia, Pa., U.S.A.), chlordiazepoxide hydrochloride (Roche, Nutley, N.J., U.S.A.), meprobamate (Wyeth Labs., Philadelphia, Pa., U.S.A.), and diazepam (Roche).

TLC on silica gel layers with ethyl acetate–*n*-propanol–28% ammonium hydroxide solution (40:30:3) was carried out as previously described[9]. This procedure was used for the non-steroid compounds. Separation and identification of the steroid drugs were achieved by using the procedure developed by Hall[11]: silica gel layer, solvent was methylene chloride–*p*-dioxan–water (10:5:5), shaken well, allowed to separate into two layers and the lower layer was filtered through paper into the developing tank.

The reagents used for detection were: (A) 1% potassium permanganate in acetone; (B) 1% furfural in acetone, oversprayed with 10% sulfuric acid in acetone[12]; (C) acidic iodoplatinate[10]; (D) 5% 2,3,5-triphenyl-2*H*-tetrazolium chloride in methanol, mixed with an equal volume of 1 *N* sodium hydroxide solution just prior to use[12].

Procedures

Thin-layer chromatography. The developing solvents were mixed, poured into the developing tank and allowed to equilibrate for 1 h. The solvents were freshly prepared for each chromatographic separation. Amounts of 2–4 μl of standard solutions were applied to a plate and the spotted plates were developed at room temperature (23°) for 40 min. The developed plates were dried at 110° for 5 min, then scanned by short-wavelength (254 nm) UV light prior to spraying. The spray reagents were freshly prepared and used within 1 h. The developed TLC plate with the non-steroid drugs was sprayed sequentially by reagents A, B, and C. Color development was noted immediately after each spraying and then again (after heating at 110° for 5 min) before the next reagent was applied. The TLC plates with steroid drugs were developed by the methylene chloride–dioxan–water (10:5:5) system and sprayed with reagent D. The resulting R_F values and colors appearing after development and spraying for the non-steroid drugs are summarized in Table I and the characteristics of the steroid drugs are tabulated in Table II.

TABLE I

CHARACTERISTICS OF INDOMETHACIN, PHENYLBUTAZONE, TRIFLUOPERAZINE, CHLORDIAZEPOXIDE, MEPROBAMATE, AND DIAZEPAM AFTER TLC

Drug	*Minimum amount for detection (μg)*	R_F	*Color with reagent*		
			A	*B*	*C*
Indomethacin	5	0.10	Ye*	Pu*	LtBr**
Phenylbutazone	5	0.53	Ye*	YeBr*	YeBr**
Trifluoperazine	2	0.35	—	—	DkBl* to Pu**
Chlordiazepoxide***	5	0.56	—	—	DkBr*
Meprobamate	5	0.71	—	PuBr*	DkBr**
Diazepam§	5	0.77	—	—	DkBr*

* Color immediately after spraying with reagent (no change with heat). Ye = yellow, Pu = purple, LtBr = light brown, DkBl = dark blue, YeBr = yellow-brown, DkBr = dark brown, PuBr = purple-brown.

** Color after heating at 110° for 10 min.

*** Green fluorescence with UV light (254 nm).

§ Yellow fluorescence with Uv light (254 nm).

TABLE II

CHARACTERISTICS OF SOME STEROID ANTI-INFLAMMATORY DRUGS AFTER TLC

Solvent: Methylene chloride–*p*-dioxan–water (10:5:5) (lower layer).

Drug	*Minimum amount for detection (μg)*	R_F	*Color* with reagent D*
Triamcinolone	10	0.30	Red-pink
Prednisolone	5	0.35	Red-pink
Methylprednisolone	5	0.38	Red-pink
Hydrocortisone	5	0.43	Red-pink
Prednisone	5	0.48	Red-pink
Paramethasone	5	0.63	Red-pink
Cortisone	10	0.67	Red-pink

* Developed TLC plates were heated at 110° for 10 min; colors evaluated 5 min after removal from heat.

RESULTS AND DISCUSSION

The solvent systems used for the separation of these compounds were effective; the R_F values were sufficiently different (Tables I and II); and the spots which appeared after spraying with the detecting reagents were discrete and well defined. The sequential spraying of the three reagents A, B and C gave constant results. The colors resulting from the sequential spraying of the developed TLC plate were useful in identifying the non-steroid drugs (Table I). The distinctive TLC-derived characteristics of each of the non-steroid drugs are summarized below.

Indomethacin

The R_F value, the immediate yellow color when sprayed with reagent A, turning to purple when oversprayed reagent B and then changing to light brown after spraying with reagent C and heating at 110° for 10 min.

Phenylbutazone

The R_F value, the immediate yellow color when sprayed with reagent A, turning yellow-brown when treated with reagent B and no change in color when oversprayed with C and heated.

Trifluoperazine

The R_F value and the distinctive dark blue color immediately upon spraying with reagent C and no reactions with the other two detecting reagents (A, B).

Chlordiazepoxide

Characteristic R_F value, green fluorescence with short-wavelength (254 nm) UV light, dark brown color with reagent C and no reaction with reagent A or reagent B sprays.

Meprobamate

The R_F value, no color reaction with reagent A, purple-brown when over-

sprayed with reagent B, and the turning to dark brown when treated with reagent C and heated.

Diazepam

Characteristic R_F value, yellow fluorescence with short wavelength (254 nm) UV light, no color reaction with reagent A or reagent B but an immediate dark brown color when oversprayed with reagent C.

Steroids

These compounds were effectively separated (Table II) by the methylene chloride–dioxan–water system. The spots which appeared after spraying with the detecting reagent were discrete and well defined. Each steroid gave a similar reddish-pink spot when sprayed with reagent D; color intensity varied with the amount of steroid applied to the thin-layer plate. The characteristic R_F value (Table II) of each steroid and the reddish-pink color of the spot after spraying with reagent D made possible tentative identification of the corticosteroids under study.

CONCLUSIONS

The qualitative analysis of certain "secret" arthritis "cures" frequently prescribed by physicians of the popular Mexican arthritis clinics, can be effected by single extraction with 95% ethanol and TLC analysis of the extract.

The described procedure is satisfactory for screening solid dosage forms containing indomethacin, phenylbutazone, trifluoperazine, chlordiazepoxide, meprobamate, diazepam, or the common corticosteroids. The judicious use of the TLC-derived information provides tentative identification of these compounds in medical emergencies. All the active ingredients of these drugs seen to date are commonly available in the U.S.A.

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