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THIN-LAYER CHROMATOGRAPHIC SCREENING PROCEDURE FOR UNDECLARED SYNTHETIC DRUGS IN CHINESE HERBAL PREPARATIONS

S. YUEN* and C. A. LAU-CAM*
Food and Drug Administration, New York Regional Laboratory, 850 Third Avenue, Brooklyn, NY 11232 (U.S.A.)
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SUMMARY

A simple and rapid thin-layer chromatographic procedure was developed for the detection and presumptive identification of seventeen synthetic drugs previously reported as adulterants of Chinese herbal preparations. Depending on its complexity, the sample may be directly extracted into aqueous ethanol, or stepwise fractionated into acidic, basic, and neutral components. Extracts are analyzed on silica gel layers containing a fluorescent indicator with the aid of two solvent systems. Spots are visualized under short and long wavelength ultraviolet lights. The procedure was successfully tested on synthetic and commercial samples.

INTRODUCTION

Chinese herbal remedies are widely used by a segment of the American population for the treatment of a variety of disease states. In recent years there have been numerous reports indicating that these products, particularly those promoted for the treatment of arthritis, osteoarthritis, rheumatism, neuralgias, and related painful conditions, may contain undeclared, often potent, synthetic drugs.

Cognizant of the potential health hazards associated with the use of these adulterated products, the U.S. Food and Drug Administration (FDA) has issued repeated warnings addressed to the unknowing consumer, and state and government agencies have taken formal actions to prevent their importation into this country. However, because of their great popularity, Chinese herbal preparations continue to make their entry in many unorthodox ways, making their regulation a difficult, if not impossible, task.

In view of the frequent requests received by this laboratory to have Chinese herbal products analyzed for the presence of Western-type drug substances, we decided to develop a thin-layer chromatographic (TLC) procedure that could be used for screening purposes prior to more demanding techniques.

* Present address: St. John's University, College of Pharmacy and Allied Health Professions, Jamaica, NY 11439, U.S.A.

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EXPERIMENTAL

Materials and reagents

All solvents and reagents were of analytical reagent grade and were used as received from their manufacturers. Drug standards were purchased from commercial sources and analyzed for TLC purity prior to use. Samples of "Chuifong Toukuwan" pills were collected by U.S. FDA investigators in response to citizen complaints. Thin-layer plates were pre-coated, 20 × 20 cm, silica gel GF 0.25 mm (E. Merck, Darmstadt, F.R.G.). Samples were applied to the plates with 10-μl disposable micropipettes (Microcaps®, Drummond Scientific, Broomall, PA, U.S.A.). Chromatographic developments were carried out in a rectangular glass tank (Analtech, Newark, DE, U.S.A.), whose walls had been lined with a piece of Whatman No. 1 filter paper. Chromatographic spots were visualized using ultraviolet lamps emitting 254 and 365 nm radiation (Chromato-Vue®, Ultra-Violet Products, San Gabriel, CA, U.S.A.).

Solvent systems

The following solvent systems were used: (A) methylene chloride–methanol–water (183:27:5); (B) ethyl acetate–toluene–formic acid–dimethylformamide–water (75:75:2:4:4); (C) methylene chloride–methanol (183:27); (D) ethyl acetate–toluene–formic acid–dimethylformamide (75:75:2:4).

Standard preparations

Solutions of each of the compounds listed in Table I, or of their mixtures, were prepared in ethanol. All compounds were present in 1 mg/ml concentrations, except for chlorzoxazone which was added at 2 mg/ml.

Sample preparations

Direct extraction. The sample was ground to a fine powder with the aid of a mortar and pestle. The powder was mixed with 10 ml of ethanol–water (9:1), and the suspension was allowed to settle for a few minutes. The supernatant was filtered through Whatman No. 1 filter paper, and the filtrate was spotted on a thin-layer plate.

Fractionation. The sample was ground to a fine powder with the aid of a mortar and pestle. The powder was transferred to a glass stoppered 50-ml centrifuge tube and mixed with 10 ml of 0.1 *N* hydrochloric acid followed by 10 ml of ethyl acetate. After shaking for 5 min, the mixture was centrifuged at about 1100 *g* for 15 min, and the layers (A and B) were transferred to individual separatory funnels. The aqueous layer (B) was further extracted with ethyl acetate, and the combined organic layers (A) were shaken with 0.1 *N* sodium hydroxide.

The organic layer (A) was evaporated to dryness with the aid of a stream of air, the residue was dissolved in 1 ml of ethanol, and the solution (neutral components) was spotted on a thin-layer plate. The alkaline aqueous layer (A-1) was transferred to a separatory funnel, made acidic with hydrochloric acid, and extracted with ethyl acetate. The organic layer (A-2) was evaporated to dryness with the aid of a stream of air, the residue was dissolved in 1 ml of ethanol, and the solution (acidic components) was spotted on a thin-layer plate. The aqueous layer (B) was made

alkaline with ammonium hydroxide solution, and extracted with ethyl acetate. The organic layer (B-1) was evaporated to dryness with the aid of a stream of air, the residue was dissolved in 1 ml of ethanol, and the solution (basic components) was spotted on a thin-layer plate. The fractionation pattern of the seventeen drugs studied is described under Results and discussion.

Thin-layer chromatography

Two plates were used for each analysis. Standard and sample preparations were spotted on the same plate, 1.5–2.0 cm apart, and 2 cm away from one of the edges of the plate. Following air-drying at ambient temperature, the plates were developed to a distance of about 15 cm, in a tank that had been equilibrated with the solvent system for at least 30 min. One plate was developed with solvent system A and the other with solvent system B. After marking the solvent fronts, the plates were removed from the chromatographic tanks and allowed to air-dry inside a hood. Spots were visualized under short and long wavelength ultraviolet lights, or by exposing the plates to iodine vapors inside a closed chamber.

RESULTS AND DISCUSSION

Up to seventeen synthetic drugs are known to occur as adulterants of Chinese herbal products[1–11]. Mixtures of all these drugs were effectively resolved by combining the use of silica gel plates with two solvent systems (Table I, Fig. 1). Chromatographic separations were found to be highly reproducible from plate to plate, and from day to day.

TABLE I

hR_F VALUES ($= R_F \times 100$) FOR SEVENTEEN SYNTHETIC DRUGS REPORTED TO OCCUR IN CHINESE HERBAL PRODUCTS

For composition of solvent systems see text. Values represent the average ± S.D. of four plates.

Drug	*Solvent system*			
	A	*B*	*C*	*D*
Acetaminophen	19.5 ± 1.3	13.8 ± 0.5	31.8 ± 1.3	16.5 ± 0.6
Aminopyrine	54.8 ± 1.3	5.3 ± 0.5	51.3 ± 2.2	5.8 ± 0.5
Antipyrine	48.5 ± 0.6	10.3 ± 0.5	48.8 ± 1.0	12.0 ± 0.0
Betamethasone	25.8 ± 1.5	15.0 ± 0.0	39.5 ± 2.4	17.3 ± 0.5
Caffeine	55.3 ± 0.5	11.8 ± 0.5	52.8 ± 1.7	14.5 ± 0.6
Chlordiazepoxide	50.8 ± 1.0	8.0 ± 0.0	49.8 ± 2.5	6.0 ± 0.0
Chlorzoxazone	56.5 ± 0.6	50.3 ± 0.5	57.0 ± 2.0	49.8 ± 1.3
Dexamethasone	27.5 ± 1.3	15.0 ± 0.0	41.3 ± 1.7	17.3 ± 0.5
Diazepam	70.8 ± 0.5	40.0 ± 0.0	68.0 ± 1.4	35.3 ± 0.5
Hydrochlorothiazide	11.3 ± 1.0	9.0 ± 0.0	20.5 ± 0.6	10.0 ± 0.0
Indomethacin	41.0 ± 1.2	44.0 ± 0.8	43.0 ± 1.8	38.8 ± 1.0
4-Isopropylantipyrine	66.0 ± 0.0	34.8 ± 0.5	65.0 ± 1.4	32.5 ± 1.0
Methyltestosterone	61.3 ± 1.0	33.5 ± 0.6	59.3 ± 1.0	31.5 ± 0.6
Phenacetin	52.3 ± 0.5	22.8 ± 0.5	53.8 ± 1.5	23.5 ± 0.6
Phenylbutazone	76.5 ± 0.6	65.0 ± 0.0	72.3 ± 1.7	64.0 ± 0.8
Prednisolone	23.3 ± 1.0	12.0 ± 0.0	35.8 ± 0.5	15.0 ± 0.8
Theophylline	33.0 ± 0.0	9.8 ± 0.5	42.0 ± 1.2	11.8 ± 0.5

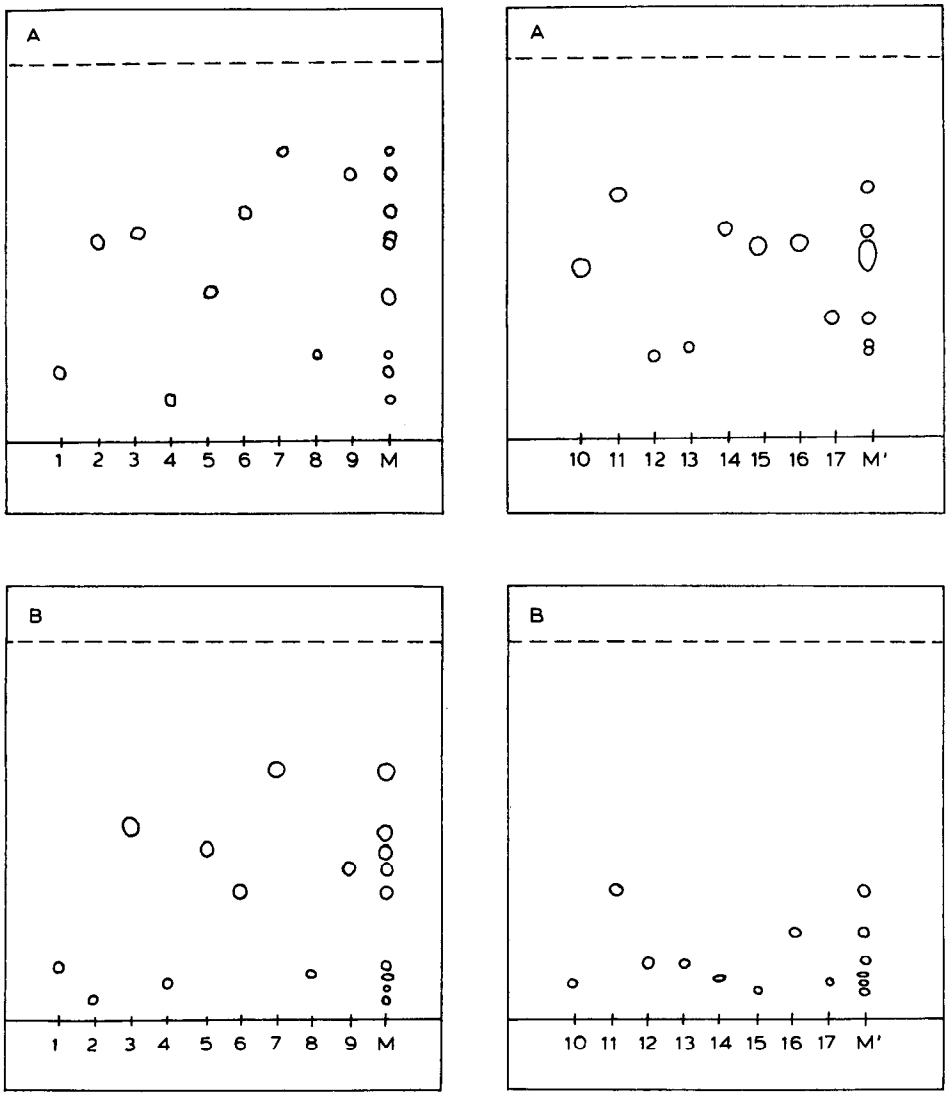

Fig. 1. Thin-layer chromatograms of synthetic drugs found as adulterants of Chinese herbal preparations. (A) Silica gel developed with methylene chloride–methanol–water (183:27:5, v/v), and (B) silica gel developed with ethyl acetate–toluene–water–formic acid–dimethyl formamide (75:75:4:2:4, v/v). Spots were visualized under short wavelength ultraviolet light. 1 = Acetaminophen, 2 = aminopyrine, 3 = chlorzoxazone, 4 = hydrochlorothiazide, 5 = indomethacin, 6 = methyltestosterone, 7 = phenylbutazone, 8 = prednisolone, 9 = diazepam, M = mixture of 1–9, 10 = antipyrine, 11 = 4-isopropyl antipyrine, 12 = betamethasone, 13 = dexamethasone, 14 = caffeine, 15 = chlordiazepoxide, 16 = phenacetin, 17 = theophylline, M' = mixture of 10–17.

Preliminary experiments indicated that solvent systems C and D were able to effect clean separations among the majority of drugs studied. Upon further experimentation, we found that the resolution of the pairs diazepam–methyltestosterone and acetaminophen–prednisolone with solvents D and C, respectively, was directly

dependent on the relative ambiental humidity. To off-set this variable we decided to add a certain volume of water to each of these solvents. For comparative purposes, however, the migration behavior of all seventeen drugs in solvent systems with and without water are shown alongside in the table. Formic acid was included in solvent systems B and D to preclude the tailing of the indomethacin spot. Pre-saturation of the chromatographic tank was found to be essential for insuring optimum resolutions, and for keeping compounds with high R_F values from migrating with or near the solvent front.

All seventeen drugs yielded chromatographic spots which were clearly visible under a short wavelength ultraviolet lamp against the fluorescent background. Of these, four compounds were also visible under long wavelength ultraviolet light as blue (chlordiazepoxide) or bluish white (aminopyrine, diazepam, indomethacin) spots. Visualization of the spots under ultraviolet light offered greater sensitivity than by exposing the plates to iodine vapors. As little as 0.25 μg (about 2 μg for chlorzoxazone) of drug was detectable under short wavelength ultraviolet light.

Two lots of Chinese herbal pills sold under the name of "Chuifong Toukuwan" were submitted to this laboratory for chemical analysis. The samples appeared as dark brown, round masses, about 0.50 to 0.75 cm in diameter, and with a pungent aroma. The labeling for these samples only listed ingredients of plant origin. The samples were subjected to the two methods of sample preparation described. The direct method is intended for products containing from one to a few synthetic drugs or having a non-interfering matrix. The fractionation scheme, representing a modification of one reported by Clarke[12], is reserved for more complicated samples. By using this fractionation scheme, complex mixtures of synthetics can be broken down into three fractions labeled acidic (acetaminophen, antipyrine, caffeine, chlorzoxazone, hydrochlorothiazide, indomethacin, phenylbutazone, theophylline), basic (aminopyrine, chlordiazepoxide), and neutral (betamethasone, dexamethasone, diazepam, 4-isopropylantipyrine, methyltestosterone, phenacetin, prednisolone) components.

Using the proposed screening procedure, one lot of "Chuifong Toukuwan" pills was found to contain hydrochlorothiazide, whereas the other contained hydrochlorothiazide and indomethacin. The presence of this diuretic agent was confirmed by reversed-phase high-performance liquid chromatography[11]. Our findings are in accord with those previously obtained at other FDA laboratories.

As early as 1974, the same product was found to contain the combinations phenylbutazone with aminopyrine[1-3] or acetaminophen, aminopyrine, chlorzoxazone, diazepam, methyltestosterone, phenylbutazone, and prednisolone[1]. Later chlordiazepoxide[4], indomethacin[4], and indomethacin with hydrochlorothiazide[4] were also identified in other samples. Investigations carried out abroad have disclosed the presence of additional drugs and drug-combinations. In Holland, a herbal preparation sold as "Chuei-Fong-Tou-Geu-Wan" yielded dexamethasone and indomethacin[5] and the same drugs in combination with diazepam and hydrochlorothiazide[6]. A batch of "Chuifong Toukuwan" pills sold in Australia contained varying amounts of phenylbutazone, phenacetin, aminopyrine, and mercuric sulfide[7]. Analyses conducted on products available in Japan as "Tsuifutokotsugan", "Fushitsuneitsugan", "Saizogan", and "Kenpogan" indicated the presence of the same drugs found in the Australian samples but in addition showed corticosteroids[8,9].

From the published reports and our own experience, adulterated Chinese herbal products can be considered as very unique and challenging analytical samples. They are sold under names that are frequently spelled differently, reflecting their multiple commercial origins. Their content of synthetic drugs is variable, ranging from single entities to complex mixtures. Different adulterants may appear in different lots of the same product. Even when the same synthetic is present, its concentrations may differ from lot to lot. The screening procedure proposed here should prove useful for the detection and presumptive identification of all those adulterants thus far reported. The procedure is simple, rapid, and possesses a good degree of sensitivity.

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