Journal of Chromatography, 333 (1985) 115–122
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17 887

APPLICATION OF THIN-LAYER STICK CHROMATOGRAPHIC IDENTIFICATION TEST METHODS TO DRUGS CONTAINED IN PREPARATIONS IN THE PHARMACOPOEIA OF JAPAN

KOJI KAWANABE
Meiji College of Pharmacy, 1-22-1, Yato-cho, Tanashi-shi, Tokyo 188 (Japan)
(Received May 13th, 1985)

SUMMARY

A simple, precise technique was developed for identifying the drugs in preparations containing crude drugs listed in the Pharmacopoeia of Japan using thin-layer stick chromatography (TLSC), which is an advanced version of thin layer chromatography in a cylindrical form.

The target component in preparations and crude drugs could be detected easily by dipping the developed chromatographic stick in specific colour reagents or by exposure to ultraviolet rays. This technique was applied to the identification of drugs such as salicylic acid, phenol, acrinol and alkaloids and also vitamins contained in the 36 kinds of preparation.

INTRODUCTION

Thin-layer chromatography (TLC) is commonly used as the method for drug identification specified in Parts 1 and 2 of the Tenth Edition of the Pharmacopoeia of Japan (JPX)[1]. It is especially used as the method for the identification of the drugs contained in the 43 kinds of preparations given in Part 2 of the JPX.

Compared with other methods of analysis, TLC has several advantages, such as better separation capability and greater ease of handling. It is therefore one of the most appropriate analytical methods which conforms to the stipulations of drug identification test methods.

We have studied the applicability of thin-layer stick chromatography (TLSC)[2–6], which is an advanced version of TLC in a cylindrical form. In this method, a thin-layer stick is dipped in a colour reagent after development, whereas the usual TLC colour reagents require a complicated technique for spraying. It was found that detection was very much easier with this "dip" method. The detection sensitivity was also found to be excellent, requiring only 0.2 ml of solvent per development. With its ease of handling and its labour-saving characteristics, the method proved to be practical. The purpose of this work was to develop the application of TLSC to the identification of the drugs contained in the 36 kinds of preparations given in the JPX.

0021-9673/85/$03.30

EXPERIMENTAL

Preparation of thin-layer stick

Narrow glass tubes, 13 cm long and 2.3 mm O.D., were used for preparing thin-layer sticks. The absorbent was uniformly spread over the surface of the glass sticks, which were passed vertically through a funnel in which aqueous suspensions of the absorbent (2:1) were filled, as shown in Fig. 1. After heat activation of the absorbent at 90°C for 30 min, the layer of the absorbent on the stick had a smooth surface and the thickness of the layer, measured with a micrometer, was about 100 μm.

Absorbent

Silica gel containing a mixture of inorganic fluorescent materials (Wako FM-BO) (Wako, Tokyo, Japan) was mixed with microcrystalline cellulose (Abicel SF) (Funakoshi Pharmaceutical, Tokyo, Japan) in the proportions 5:2, and 3% of binder, hydroxyethylcellulose (HEC-LR 250) (Yoneyama Chemical, Osaka, Japan), was included.

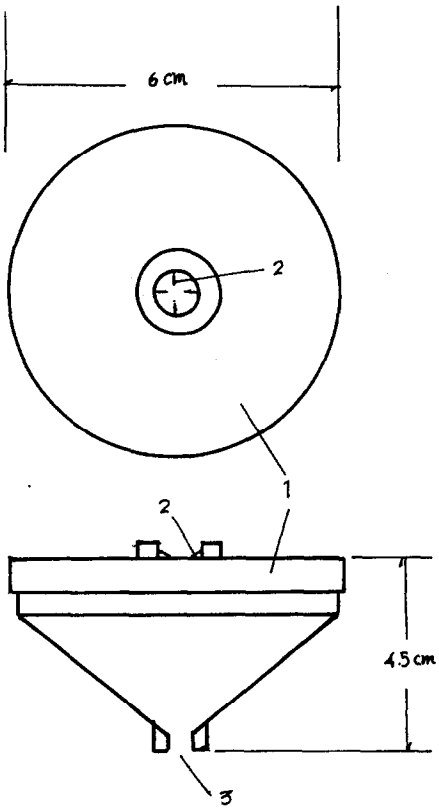

Fig. 1. Applicator for thin-layer stick chromatography. 1 = Cover; 2 = pin; 3 = hole.

TABLE I

LIST OF DRUGS SUBJECTED TO THIN-LAYER STICK CHROMATOGRAPHY

Ung. Acrinol et Zinc	Sp. Capsic. Salicyl.
Ol. Acrinol et Zinc	Sol. Thianthol et Acid Salicyl. Comp.
Ol. Acrinol et Zinc Comp.	Pulv. Vitamin B Comp.
Sol. Acrinol et Acid Boric	Ung. Hydrocortis. et Diphenhydramin
Pulv. Aspirin Phenacet et Coff.	Pulv. Phenacet. et Bromvalerylur.
Pulv. Aspirin Phenacet. et Coff. Comp.	Lin. Phenol et Zinc Oxyd
Past Arsenic.	Pulv. Methylephed. et Coff.
Ung. Sulfur Acid Salicyl. et Thianthol.	Sp. Iod. Acid Salicyl. et Phenol
Pulv. Phellod. Comp. ad Catapl.	Sp. Iod. et Caps. Comp.
Sp. Chloral. Salicyl.	Scopol. Rhiz.
Puilv. Chlorpheniramin Calc.	Ext. Scopol.
Pulv. Gentian. et Nat. Bicarb.	Pulv. Ext. Scopol.
Sp. Salicyl. Comp.	Pulv. Scopol. et Ethyl. Aminobenz.
Ung. Carb. Salicyl.	Supp. Scopol. Tann. Comp.
Ung. Phenol Salicyl.	Ung. Scopol. Tann. Comp.
Sp. Methyl. Salicyl. Comp.	Pulv. Scopol. Papaver. et Ethyl. Aminobenz.
Pulv. Alum. Salicyl.	
Pulv. Diphenhydramin Calc.	Vitamin A
Lin. Diphenhydramin Phenol et Zinc Oxyd.	

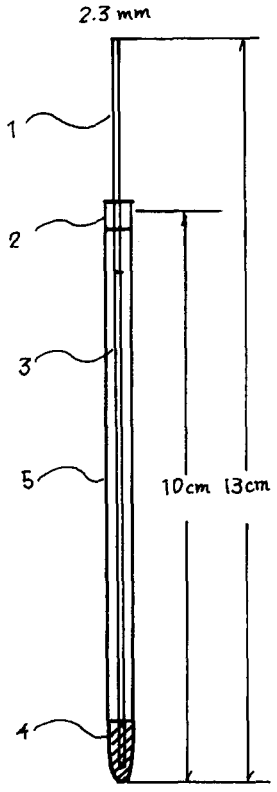

Fig. 2. Developing apparatus for thin-layer stick chromatography. 1 = Glass stick; 2 = rubber tube; 3 = adsorbent; 4 = developer; 5 = glass tube.

Sample

Samples were produced according to the JPX formula, but only 1% of the amount was produced. The samples were extracted and prepared according to the JPX method[1].

The preparations used in the study are listed in Table I.

Development

Volumes of 1–10 μl of sample solution were spotted 2 cm from the lower end of the thin-layer stick using a micropipette. Similarly, standard samples, which were prepared according to the methods specified in the JPX[1], were spotted on the reverse side to ensure more precise detection. A volume of 0.1–0.2 ml of developer was poured into a development tube before a thin-layer stick was inserted for development. The development distance was set at 5 cm for all the tests (Fig. 2).

Detection

After development, the thin-layer stick was dried by applying warm air with a drier. The stick was exposed to ultraviolet rays and the colour tone was observed and the R_F values were measured. The ultraviolet lamp was a PAN UV-Lamp (2600–3800 Å, PUV-1) (Tokyo Kogaku Kikai, Tokyo, Japan). The performance of the individual reagents was compared with that of standard samples for identification.

Developer

The test was performed on developing solvents as specified in Part 2, "Preparation", of the JPX[1] concerning "identification" using TLC:

(I) Chloroform–acetone–acetic acid (45:5:1).
(II) Diethyl ether–ethanol–acetic acid (30:10:1).
(III) Chloroform–acetone–methanol–concentration ammonia solution (73:10:15:2).
(IV) *n*-Butanol–water–acetic acid (7:2:1).
(V) Chloroform–methanol–water (30:10:1).
(VI) Isopropanol–acetic acid (9:1).
(VII) Ethyl acetate–methanol–12 *N* hydrochloric acid (18:2:0.05).
(VIII) Chloroform–acetone–concentrated ammonia solution (45:5:1).
(IX) *n*-Hexane–benzene (1:1).
(X) Chloroform–methanol–acetic acid (95:5:1).
(XI) Ethyl acetate diethyl ether (4:1).
(XII) Chloroform–ethanol–acetic acid (100:50:1).
(XIII) Benzene.

Detection reagent

Iron(III) chloride solution, 1%. Iron(III) chloride (1 g) was dissolved in water and diluted to 100 ml.

Antimony trichloride. Antimony trichloride was washed with refined and distilled chloroform until the washings became transparent. Chloroform was added to make a saturated solution.

Dragendorff's reagent. Basic bismurth nitrate (2 g) was dissolved in a small

volume of hydrochloric acid, 20% ammonia solution was added to produce bismuth hydroxide, which was then filtered and washed before being dissolved in 50 ml of 10% hydrochloric acid. (A) Potassium iodide (3 g) was dissolved in 80% ethanol to a volume of 100 ml. (B) Three drops of A and 4 ml of B were mixed together at the time of use.

TABLE II

ANALYTICAL RESULTS FOR DRUGS USING THIN-LAYER STICK CHROMATOGRAPHY

Thin-layer stick: Wakogel B-O FM–microcrystalline cellulose (5:2), HEC-LR250.

Preparation	*Drug detected*	*Reagent**	*Colour***	R_F	*Sensitivity (μg)*	*Developer*
Ung. Sulfur Acid Salicyl. et Thianthol	Salicylic acid	UV	B	0.49	0.5	I
		$FeCl_3$	RV			
Ung. Phenol. Salicyl.	Salicylic acid	UV	B	0.45	0.6	I
		$FeCl_3$	RV			
	Phenol	UV	RV	0.62	2.0	
Sol. Thianthol et Acid Salicyl. Comp.	Salicylic acid	UV	B	0.40	1.0	I
		$FeCl_3$	RV			
	Phenol	UV	RV	0.71	2.0	
Sp. Iod. Acid Salicyl. et Phenol	Salicylic acid	UV	B	0.48	0.25	I
		$FeCl_3$	RV			
	Phenol	UV	RV	0.69	2.0	
	Benzoic acid	UV	RV	0.52	8.0	
Sp. Chloral. Salicyl.	Salicylic acid	UV	B	0.44	1.0	I
		$FeCl_3$	RV	0.44	1.0	
Sp. Salicyl. Comp.	Salicylic acid	UV	B	0.46	1.0	I
		$FeCl_3$	RV	0.46	1.0	
	Phenol	UV	RV	0.64	2.0	
Ung. Carb. Salicyl.	Salicylic acid	UV	B	0.43	1.0	I
		$FeCl_3$	RV	0.43	1.0	
Pulv. Alumn. Salicyl.	Salicylic acid	UV	B	0.45	1.0	I
		$FeCl_3$	RV	0.45	1.0	
Sp. Capsic. Salicyl.	Salicylic acid	UV	B	0.48	1.0	I
		$FeCl_3$	RV	0.48	1.0	
Sp. Methyl. Salicyl.	Methyl salicylate	UV	BV	0.55	8.0	IX
		$FeCl_3$	RV	0.55	2.0	
Sp. Iod. et Caps. Comp.	Phenol	UV	RV	0.65	5.0	I
	Methyl salicylate	UV	B	0.82	1.2	
		$FeCl_3$	RV	0.82	2.5	
Ung. Acrinol et Zinc	Acrinol	UV	YG	0.27	0.1	II
Ol. Acrinol et Zinc	Acrinol	UV	YG	0.31	0.1	II
Ol. Acrinol et Zinc Comp.	Acrinol	UV	YG	0.28	0.1	II
	p-Ethyl amino-benzoate	UV	V	0.70	0.24	
Sol. Acrinol et Acid Boric.	Acrinol	UV	YG	0.28	0.1	II

* UV = ultraviolet light, 2600–8800 Å. $FeCl_3$ = 1% $FeCl_3$ reagent.

** B = blue; RV = reddish violet; BV = bluish violet; V = violet; YG = yellowish green.

RESULTS AND DISCUSSION

Identification of salicylic acid, phenol and acrinol in preparations

There are a large number of preparations containing salicylic acid and phenol and tests were conducted to identify these drugs. Chloroform–acetone–acetic acid (45:5:1) was effective for development.

When the thin-layer stick after development was exposed to ultraviolet rays, salicylic acid showed a blue and phenol a reddish purple colour. Because of the difference in colour tone, detection was made more precisely, *e.g.*, with Sp. Salicyl. Comp. (compound salicylate spirit), R_F values of 0.45 and 0.62 were observed. In the next step, the thin-layer stick was immersed in the iron(III) chloride solution. Salicylic acid, which developed a reddish purple colour, was identified.

Table II lists the preparations, the drugs to be identified, detection reagents, colour tones, R_F values and individual detection limits.

Salicylic acid showed a higher detection sensitivity level than phenol. Ung. acrinol et zinc [acrinol (ethacridine lactate; 6,9-diamino-2-ethoxyacridine lactate) and zinc oxide ointment] and three other preparations contained acrinol. By using as the developer diethyl ether–ethanol–acetic acid (30:10:1), acrinol was separated, showing a R_F value of 0.3. Then, on applying ultraviolet rays, acrinol was identified from its yellowish green fluorescence. The detection limit was 0.1 μg. The R_F value of the *p*-ethylaminobenzoate contained in compound acrinol zinc oxide oil was 0.70 and, therefore, it was completely separated from acrinol. On applying ultraviolet rays, the *p*-ethylaminobenzoate developed a purple colour and was thus clearly identified.

Identification of alkaloids in crude drugs including preparations

Many of the preparations contained alkaloids such as atropine or scopolamine. As the alkaloids could not be detected using ultraviolet rays, Dragendorff's reagent

TABLE III

ANALYTICAL RESULTS FOR DRUGS USING THIN-LAYER STICK CHROMATOGRAPHY

Thin-layer stick: Wakogel B-O FM–microcrystalline cellulose (5:2), HEC-LR250. Dragendorff's reagent used throughout. Colours: all orange.

Preparation	*Drug detected*	R_F	*Sensitivity (μg)*	*Developer*
Ung. Scopol. Tann. Comp.	Atropine · H_2SO_4	0.30	1.0	III
	Scopolamine · HBr	0.58	1.0	
Scopol. Rhiz.	Atropine · H_2SO_4	0.32	0.2	III
	Scopolamine · HBr	0.64	4.0	III
Ext. Scopol.	Atropine · H_2SO_4	0.35	0.2	III
	Scopolamine · HBr	0.64	4.0	
Pulv. Ext. Scopol.	Atropine · H_2SO_4	0.31	0.2	III
	Scopolamine · HBr	0.64	4.0	
Pulv. Scopol. et Ethyl. Aminobenz.	Atropine · H_2SO_4	0.31	0.2	III
	Scopolamine · HBr	0.64	4.0	
Pulv. Scopol. Papaver. et Ethyl Aminobenz.	Atropine · H_2SO_4	0.30	0.2	III
	Scopolamine · HBr	0.64	4.0	
	Papaverine · HCl	0.91	2.5	

was used for their identification. For the separation of each of the alkaloids, chloroform–acetone–methanol–concentrated ammonia solution (73:10:15:2) was used as the developer. After development, the thin-layer stick was dried and then immersed in Dragendorff's reagent. Each of the alkaloids was identified from their orange chromatogram. The R_F values of atropine and scopolamine were 0.30–0.32 and 0.64, respectively. The papaverine contained in Pulv. Scopol. Papaver. et Ethyl

TABLE IV

ANALYTICAL RESULTS FOR DRUGS USING THIN-LAYER STICK CHROMATOGRAPHY

Thin-layer stick: Wakogel B-O FM–microcrystalline cellulose (5:2), HEC-LR250.

Preparation	*Drug detected*	*Reagent**	Color**	R_F	*Sensivivity (μg)*	*Developer*
Pulv. Phellod. Comp. ad Catapl.	Berberin	UV	Y	0.45	0.05	IV
Pulv. Gentian. et Nat. Bicarb.	Gentian	UV	DV	0.50	(+)	V
Supp. Scopol. Tann. Comp.	*p*-Ethyl aminobenzoate	UV	V	0.72	0.15	VI
Pulv. Aspirin Phenacet. et Coff.	Aspirin	UV	B	0.27	5.0	VII
	Phenacetin	UV	RV	0.77	0.5	
		D	OR	0.77	5.0	
	Caffeine	UV	RV	0.54	1.0	
		D	OR	0.54	4.0	
Pulv. Aspirin Phenacet. et Coff. Comp.	Chlorpheniramine · maleic acid	UV	V	0.00	1.0	VII
		D	OR	0.00	1.0	
Past. Arsenic	Procaine · HCl	UV	BV	0.49	1.0	VIII
Pulv. Diphenhydramin	Diphenhydramine	UV	RV	0.40	20	VIII
		D	OR	0.40	10	
Lin. Phenol et Zinc Oxyd.	Phenol	UV	RV	0.70	2.0	VII
Lin. Diphenhydramin Phenol et Zinc Oxyd.	Phenol	UV	RV	0.64	8.0	VII
	Diphenhydramine	UV	RV	0.40	10.0	VII
		D	OR	0.40	6.0	
Pulv. Chlorpheniramin Calc.	Chlorpheniramine · maleic acid	UV	RV	0.24	1.0	VIII
		D	OR	0.24	1.0	
Pulv. Phenacet. et Bromvalerylur.	Bromovalerylurea	UV	V	0.45	10	I
Ung. Hydrocortis. et. Diphenhydramin	Hydrocortisone · HAc	UV	RV	0.77	5.0	XI
	Diphenhydramin	UV	RV	0.37	25	
		D	OR	0.37	10	
Pulv. Methylephed. et Coff.	Sodium benzoate	UV	RV	0.59	4.0	I
	Caffeine	UV	BV	0.44	2.0	
		D	OR	0.44	4.0	
Pulv. Vitamin B Comp.	Thiamine	UV	RV	0.00	5.0	XII
	Rivoflavin	UV	Y	0.36	1.0	
	Pyridoxine · HCl	UV	B	0.47	5.0	
	Nicotinamide	UV	RV	0.57	5.0	
Vitamin A	Retinol · palmitic acid	$SbCl_3$	B	0.84	1.5U	XIII
	Retinol · HAc	$SbCl_3$	B	0.51	1.5U	

* UV = ultraviolet light, 2600–3800 Å. D = Dragendorff's reagent.

** Y = Yellow; DV = dark violet; B = blue; RV = reddish violet; OR = orange red; V = violet; BV = bluish violet.

aminobenz. (scopolia extract, papaverine and ethyl aminobenzoate powder) showed the highest R_F value and was clearly separated. Table III lists the detection reagents, colours, R_F values and detection limits for the preparations given above.

Identification of other drugs in preparations including crude drugs

Table IV shows the results of separations carried out on preparations including crude drugs, antipyretic analgesics such as aspirin and preparations containing caffeine, phenacetin, diphenhydramine, etc. For all these preparations, a combination of the ultraviolet detection method and Dragendorff's test was effective.

With regard to Pulv. Gentian. et Nat. Bicarb. (gentian and sodium hydrogen carbonate powder), the detection sensititity could not be determined because its standard, gentiopicroside glycoside, could not be obtained. As developers, the following were all effective: chloroform–acetone–concentrated ammonia solution (45:5:1), ethyl acetate–methanol–12 *N* hydrochloric acid (18:2:0.05), *n*-butanol–water–acetic acid (7:2:1) and isopropanol–acetic acid (9:1). The detection sensitivity of the berberine contained in Pulv. Phellod. Comp. ad Catap. (compound phellodendron powder for cataplasm) and of the *p*-aminoethyl aminobenzoate contained in Supp. Scopol. Tann. Comp. (compound scopolia extract and tannic acid suppositories) was especially high (0.05 and 0.15 μg, respectively), when determined by the ultraviolet method (Table IV).

For identifying vitamin preparations containing thiamine, riboflavin, pyridoxine, etc., and vitamin A, the ultraviolet method was effective, with each spot showing a different colour (Table IV). Chloroform–ethanol–acetic acid (100:50:1) was the most effective developer.

CONCLUSION

In thin-layer stick chromatography, only a small amount of solvent is required and development can be carried out at complete solvent saturation, as the support is a rod-like narrow glass tube. The reproducibility with this method is therefore excellent. Moreover, the colours can be developed simply by immersion. Compared with the conventional spray detection method, this type of chromatography is much easier and the detection sensitivity is higher. The method has been found to be useful for the identification of pharmaceuticals.

REFERENCES

1 Ministry of Health and Welfare, *The Pharmacopoeia of Japan*, 10th ed., Hirokawa, Japan, 1981.
2 K. Maruyama and K. Kawanabe, *Bunseki Kagaku (Jap. Anal.)*, 22 (1973) 1295.
3 K. Kawanabe and K. Maruyama, *Bull. Meiji Coll. Pharm.*, 10 (1980) 25.
4 K. Maruyama and K. Kawanabe, *J. Food Hyg. Soc. Jap.*, 15 (1974) 226.
5 K. Kawanabe and K. Maruyama, *Yaakaugaku Zasshi*, 101 (1981) 912.
6 K. Kawanabe, *Yakugaku Zasshi*, 102 (1982) 335.