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Polyamide layer chromatography of some antipyretics

The antipyretics are so widely used in medicine that a simple, accurate and rapid analytical method is worthy of study. Recently, the uses of thin layer chromatography in the analysis of antiperetics was investigated in detail by several workers. GÄNSHIRT[1] and BÄUMLER AND RIPPSTEIN[2] used Silica Gel G to separate some antipyretics, while ŠARŠUNOVÁ AND SCHWARZ[3] used a loose layer of alumina for the determination of the R_F values of compounds having antipyretic and analgesic action, with ten different solvent systems. FUWA *et al.*[4] also applied stepwise chromatography to the analysis of the drugs included in Japanese Pharmacopeia VII (*cf.* ref. 5 for a detailed review).

The polyamide layer has been reported as suitable for the separation of several types of compounds, *e.g.* acids[6], esters[7], and heterocyclics[8]. The antipyretics are acids, esters or heterocyclic compounds, or combinations of these functional groups, so we applied polyamide layer chromatography to the separation of some antipyretic drugs.

Materials

All of the antipyretics were purchased on the local market and purified until they gave a sharp melting point as cited in the literature. The solvents were from Wako Pure Chemicals Ltd., Osaka, Japan, reagent grade. Polyamide layers were prepared according to WANG AND WANG[9].

The preparation of a sample from an antipyretic tablet was as follows. One quarter of a commercially available A.P.C. tablet (mixture of aspirin, phenacetin and caffeine according to British Pharmacopeia) was ground and extracted with 2.5 ml of methanol. The methanol solution was filtered and diluted to 25 ml with methanol. 1 μl of this solution was transfered to the polyamide layer.

Chromatography

The standard techniques of ascending thin layer chromatography were employed[9].

Visualization

The developed chromatograms were irradiated with a U.V. lamp (254 mμ) (Mitamura Riken, Tokyo, Japan) to locate the fluorescent or quenched spots.

Results and discussion

Table I shows the R_F values and the sensitivity of each of the antipyretics on different chromatograms. These were the mean values of six experiments. Fig. 1 is a typical chromatogram. It is clear from Table I that these antipyretics can be distinguished from each other by polyamide layer chromatography. The minimum amounts which can be detected by U.V. irradiation are shown in the last column of Table I. Usually 0.5 μg were easily recognisable, except in the case of aspirin (limit 2–4 μg). Salicylic acid and salicylamide show strong violet fluorescence under U.V. irradiation where as little as 0.05 μg are easily recognisable. This is excellent for the detection of free salicylic acid in aspirin.

TABLE I

R_F VALUES OF ANTIPYRETICS ON POLYAMIDE LAYERS

No.	*Name of sample*	*Solvent systems**				*Sensitivity to U.V. light detection (μg)*
		I	*II*	*III*	*IV*	
1	Salicylic acid	0.24	0.08	0.24	0.41	0.05
2	Salicylamide	0.32	0.24	0.33	0.37	0.05
3	Acetylsalicylic acid (aspirin)	0.44	0.32	0.47	0.61	3.0
4	Oxyphenbutazone	0.42	0.07	0.50	0.46	0.08
5	Acetanilide	0.50	0.38	0.57	0.48	0.2
6	Aminopyrin	0.53	0.95	0.73	0.85	0.2
7	Phenacetin	0.63	0.32	0.70	0.60	0.2
8	Indomethacin	0.68	0.01	0.71	0.77	0.1
9	Antipyrin	0.70	0.76	0.80	0.78	0.1

* Solvent systems: I = chloroform–benzene–90% formic acid (5:1:0.1); II = isopropanol–water–90% formic acid (1.5:6:0.1); III = chloroform–90% formic acid (20:0.1); IV = cyclohexane–chloroform–glac. acetic acid (4:5:1).

Fig. 1

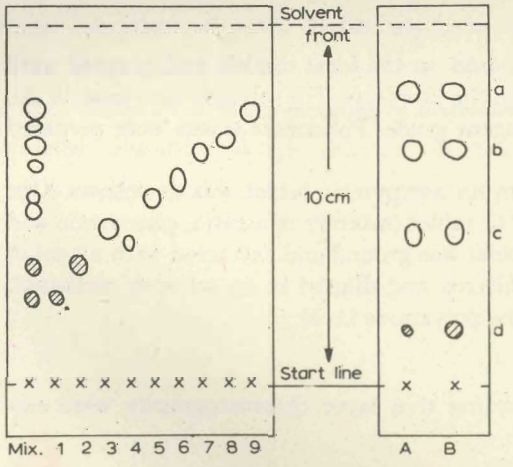

Fig. 2

Fig. 1. 1 = salicylic acid; 2 = salicylamide; 3 = acetylsalicylic acid (aspirin); 4 = oxyphenbutazone; 5 = acetanilide; 6 = aminopyrin; 7 = phenacetin; 8 = indomethacin; 9 = antipyrin. Loading: 5 μg. Time for 10 cm run = 31 min. Solvent system: chloroform–benzene–90% formic acid (5:1:0.1). Detection: U.V. irradiation (254 mμ).

Fig. 2. a = caffeine; b = phenacetin; c = aspirin; d = salicylic acid. Two different commercially available A.P.C. tablets. Products A and B responded with different spot sizes and fluorescence intensity due to free salicylic acid. Solvent III. ○: quenched spot; ●: fluorescent spot.

Solvent III is excellent for the analysis of medicinal preparations such as A.P.C. tablets. The developed chromatogram shows the presence of 3 compounds under U.V. irradiation (see Fig. 2). The presence of free salicylic acid appeared as fluorescent spot ($R_F = 0.21$). Tablets from different manufacturers show different degrees of contamination with free salicylic acid. If this method is combined with the direct fluorometric analysis of PATAKI AND WANG[10], it could be a more convenient and faster analytical

method than the column chromatographic method cited by A.O.A.C. for the analysis of A.P.C. tablets[11]

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