

CHROM. 4008

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. GÄNSHIRT¹ and BÄUMLER AND RIPPSTEIN² used Silica Gel G to separate some antipyretics, while ŠARŠUNOVÁ AND SCHWARZ³ 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.* acids⁵, esters⁷, and heterocyclics⁸. 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 WANG⁹.

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 μ l of this solution was transferred to the polyamide layer.

Chromatography

The standard techniques of ascending thin layer chromatography were employed⁹.

Visualization

The developed chromatograms were irradiated with a U.V. lamp (254 m μ) (Mitsubishi 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 μ g were easily recognisable, except in the case of aspirin (limit 2-4 μ g). Salicylic acid and salicylamide show strong violet fluorescence under U.V. irradiation where as little as 0.05 μ g 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-gluc. acetic acid (4:5:1).

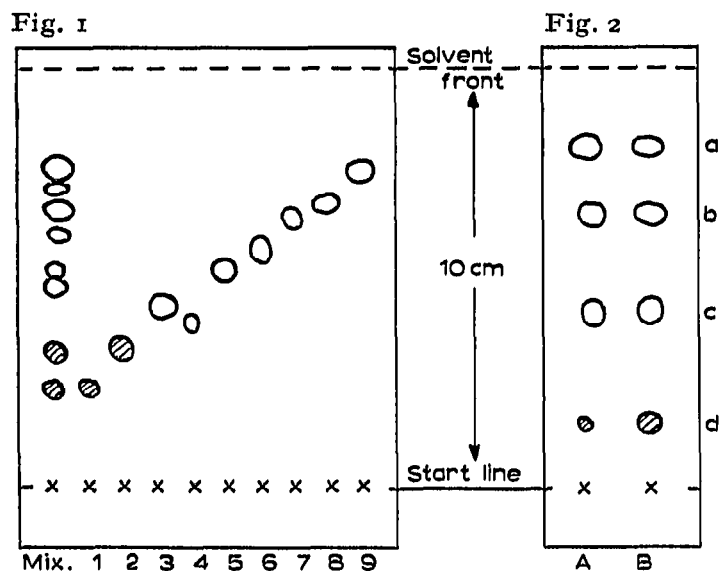


Fig. 1. 1 = salicylic acid; 2 = salicylamide; 3 = acetylsalicylic acid (aspirin); 4 = oxyphenbutazone; 5 = acetanilide; 6 = aminopyrin; 7 = phenacetin; 8 = indomethacin; 9 = antipyrin. Loading: 5 μg . Time for 10 cm run = 31 min. Solvent system: chloroform-benzene-90% formic acid (5:1:0.1). Detection: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

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. O: quenched spot; \odot : 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 WANG¹⁰, 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. tablets¹¹.

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Identification of LSD and other indole alkaloids by ultraviolet degradation products

Many forensic analysts use thin-layer chromatography (TLC) to identify LSD but have found two or more systems necessary to separate it from closely related compounds^{1,2}. In using TLC, some workers²⁻⁴ have observed degradation spots on the developed chromatograms. GENEST AND FARMILLO³ applied a degradation principle to the identification of LSD, using a two-hour acid hydrolysis followed by prolonged ultraviolet (U.V.) irradiation. Although this procedure is adequate for identification, it is quite time consuming. A relatively simple technique, using only one TLC system and requiring only one TLC plate, has been investigated for the identification of LSD.

The method involves controlled degradation of the alkaloids in a chloroform solution under U.V. irradiation. The degraded material is then chromatographed on a thin-layer plate by the procedure of ROMANO⁵. A series of colored spots on the thin-layer plate is then detected with an U.V. lamp. This series of spots, numbering as many as eight or nine, has been shown to be unique for each of the compounds tested.

Experimental

A 2-10 ml portion of chloroform solution containing 10 μ g of the alkaloid in question is placed in an open container (20 ml glass beaker is satisfactory) and the

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