CHROM. 5007

Differentiation of phenothiazine derivatives by locating agent on thin-layer chromatographic plates*

There are many different phenothiazine-type drugs which may be encountered in toxicological analyses. Although a number of comprehensive articles dealing with the paper or thin-layer chromatographic behavior of phenothiazines have appeared in the literature[1-3], the authors felt a need for a fast and simple screening technique which would give the analytical toxicologist some clues as to which particular phenothiazine drug may be involved, so that its identity could be confirmed by additional specific tests. The following presentation describes an attempt to develop a single chromatographic system (one developing solvent and one locating agent) which could be routinely used for this purpose.

Experimental

The plates (20 × 20 cm or microscope slides) were prepared in the usual manner using Silica Gel GF as the adsorbent. The reference phenothiazines were spotted as alcoholic solutions of their salts. Development was carried out at room temperature, and a 15 cm running distance was used with the 20 × 20 cm plates. Following the development, the dried plates (or slides) were sprayed with a locating agent, and after drying at room temperature the identification of the phenothiazines was based on the different colours developed as well as on the relative R_F values. The compositions of the developing solvent and of the spray reagent are given as follows:

Developing solvent: ethyl acetate, 90 ml; acetone, 45 ml; ammonium hydroxide in ethanol (1:1), 4 ml.

Spray reagent: 10% phosphomolybdic acid (aqueous solution), 40 ml; 2.5% ferric chloride in ethanol, 10 ml; 6 *N* hydrochloric acid, 20 ml; distilled water, 20 ml.

The chromatographic behaviour of the 19 phenothiazine derivatives studied is summarised in Table I.

Discussion

The sensitivity of this technique was found to range between 5 and 10 μg for the compounds studied. Spotting of lesser amounts, in some cases, produced colours which were too weak to differentiate. The developed colours were found to be stable for several days.

If the presence of phenothiazine suphloxides is anticipated, spraying the developed chromatogram with sulphuric acid will demonstrate their presence, and may give additional analytical information to enable the differentiation. A spray reagent consisting of concentrated sulphuric acid (125 ml), ethanol (50 ml) and distilled water (75 ml), works quite well. Following this treatment, it is necessary to wait for at least 10 min before examining the chromatogram. The R_F values of the sulphoxides are lower than those of the corresponding parent compounds.

The technique has been successfully applied in toxicological analyses for phenothiazines. Following a positive general test for the presence of a phenothiazine,

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TABLE I

CHROMATOGRAPHIC DATA OF 19 PHENOTHIAZINE DERIVATIVES

No.	*Compound*	R_F *value*	*Colour developed*
1	Ethopropazine (Parsitan)	0.90	Greenish-brown
2	Levomepromazine (Nozinan)	0.87	Blue (intense)
3	Trimeprazine (Temaril)	0.85	Red
4	Triflupromazine (Vesprin)	0.79	Yellowish-brown
5	Thiopropazate (Dartal)	0.78	Reddish-violet
6	Chlorpromazine (Largactil)	0.72	Reddish-violet (plum)
7	Mepazine (Pacatal)	0.66	Reddish-brown
8	Promethazine (Phenergan)	0.65	Reddish-brown (light)
9	Thioridazine (Mellaril)	0.64	Turquoise
10	Aminopromazine (Lispamol)	0.60	Beige
11	Promazine (Sparine)	0.55	Reddish-brown (deep)
12	Acetylpromazine (Plegicil)	0.49	Orange-red
13	Trifluoperazine (Stelazine)	0.44	Yellowish-brown (tan)
14	Methdilazine (Tacaryl)	0.38	Reddish-brown
15	Thiethylperazine (Torecan)	0.38	Turquoise
16	Prochlorperazine (Compazine)	0.37	Reddish-violet
17	Fluphenazine (Prolixan)	0.35	Yellowish-brown
18	Perphenazine (Trilafon)	0.30	Reddish-violet
19	Thioproperazine (Majeptil)	0.23	Pink

such as a UV absorption curve, or the bromination–acidulation reaction[4], extracts of suitable biological materials, prepared according to the method of CURRY[5], were dissolved in as little ethanol as possible, and spotted with a mixture of phenothiazine drugs as the reference. Microplates were found very useful for screening, since the development takes only about 10 min. However, best separation of the different phenothiazines was accomplished on the 20 × 20 cm plates. In fatal cases, the drugs were detected in stomach contents, liver tissue and blood. In a case involving a patient receiving therapeutic doses of chlorpromazine, the chromatographic pattern of the urine extract gave a clear indication of the parent compound as well as its metabolites.

The technique makes it possible to achieve an adequate measure of differentiation between most of the commonly encountered phenothiazine derivatives, and has been found useful as a screening procedure in routine toxicological analyses.

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