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SIMULTANEOUS DETECTION OF PHENOBARBITONE, PHENYTOIN AND THEIR MAJOR METABOLITES IN URINE BY THIN LAYER CHROMATOGRAPHY

PHENOBARBITONE and phenytoin are widely-used anti-convulsants[1]. The drugs are metabolised in the body primarily *via* oxidative hydroxylation. The principal metabolite of phenobarbitone is hydroxyphenobarbitone[2, 3] (5-*p*-hydroxyphenyl-5-ethyl barbituric acid), while that of phenytoin is hydroxydiphenylhydantoin[4] (5-*p*-hydroxyphenyl-5-phenylhydantoin). Both the hydroxy-metabolites appear in the human urine partly free and partly in the conjugated form.

The detection of the unchanged drugs together with their metabolites in urine is of value to the clinician in assessing the absorption and metabolism of the drugs prescribed. In cases of poisoning by accidental overdosage, the detection of the metabolites provides valuable additional information to the toxicologist.

Although several chromatographic methods[5–8] have been reported for the analysis of mixtures of phenobarbitone and phenytoin, a rapid, simple and inexpensive procedure for the simultaneous detection of the drugs and their metabolites does not seem to be currently available. In this paper, we present a thin-layer chromatographic (TLC) method for the separation and detection of phenobarbitone, phenytoin and their major hydroxy-metabolites on thin layers of Silica Gel GF_{254}, which affords a simple means of detecting them in short-wave ultraviolet light as spots of quenched fluorescence. Further detection is possible by the use of the mercuric-diphenylcarbazone[9] spray reagent or the universal visualisation reagent for drugs N,2,6-trichloro-*p*-benzoquinone imine[10].

The drugs were ether-extracted from urine together with their metabolites in acid medium (pH 2). The acid-ether extract was dried and a solution of the residue in ethanol used for TLC.

Suitable aliquots of the drugs and their metabolites (5 to 10 μg each from ethanolic solutions) were spotted alongside the extract from urine, on thin-layer (0·25 mm) plates previously activated at 110° C for half an hour. Development was carried out in the solvent systems shown in Table I by the ascending technique, the solvent migration being 10 cm. After development, the chromatograms were air-dried and examined under short-wave ultraviolet light (254 nm) in order to observe the spots of quenched fluorescence. The plates were then sprayed with the mercuric-diphenylcarbazone spray reagent or with the N, 2,6-trichloro-*p*-benzo quinone imine visualising agent.

TABLE I

Chromatographic behaviour of phenobarbitone (PB), *hydroxyphenobarbitone* (HPB), *phenytoin* (DPH) *and hydroxy-diphenylhydantoin* (HDPH) *in several solvent systems*

Solvent system	Time (min)*	R_f values			
		PB	HPB	DPH	HDPH
Ethyl acetate-*n*-butanol-ammonia (8 : 2 : 1)	20	0·35	0·19	0·72	0·61
Benzene-acetone-ammonia (50 : 50 : 2)	20	0·55	0·29	0·79	0·42
Chloroform-acetone-ethyl acetate (8 : 2 : 1)	25	0·73	0·33	0·67	0·27
Carbon tetrachloride-ether (70 : 30)	35	0·40	0·09	0·21	0·04

* Time for the solvent front to move 10 cm.

Table I gives the R_f values of the drugs and their metabolites in the four different solvent systems and the time for the solvent front to advance 10 cm in each instance. Although all of the solvent systems were found to effect satisfactory separations of the four compounds, the system ethyl acetate-*n*-butanol-ammonia

(8:2:1) was found to be the best, yielding compact round spots with good separation (Fig. 1). It may be noted that in the first two solvent systems, the R_f values of phenytoin and its metabolite are higher than those of phenobarbitone and its metabolite; the reverse is the case in the other two solvent systems.

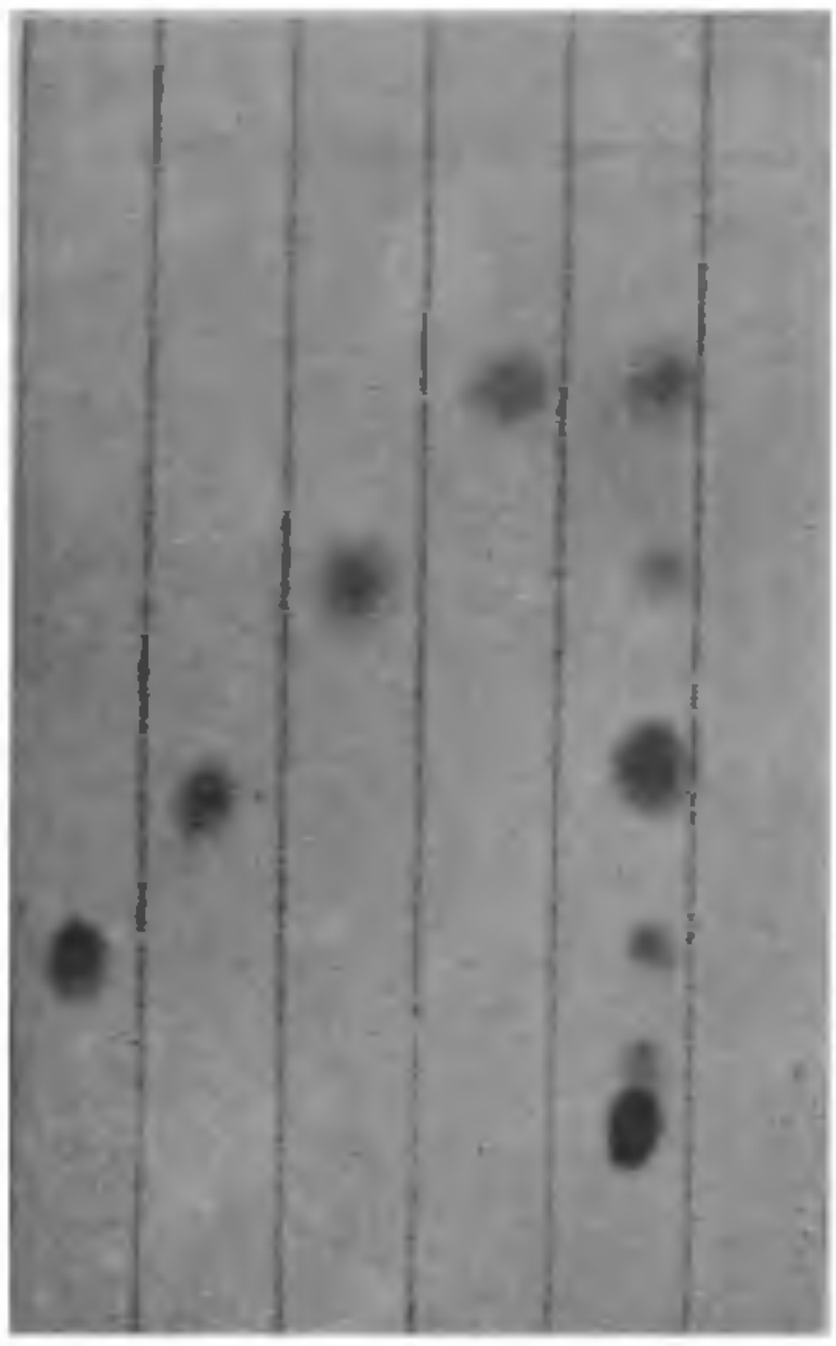

FIG. 1. Thia-layer chromatogram developed in ethylacetate-*n*-butanol-ammonia (8:2:1) and photographed after spraying with the mercuric-diphenyl carbazone reagent. Spotting (left to right): hydroxyphenobarbitone; phenobarbitone; hydroxydiphenylhydrantonin; phenytoin; and extract from urine in a casn of poisoning

When the chromatograms were viewed in short-wave ultraviolet light, all the four compounds showed up as dark spots (at 5 μg levels or more) against the greenish fluorescent background and were readily identified with the help of reference substances, thus affording a non-destructive method of detection.

Table II describes the response of the drugs and their metabolites to the two spray reagents that could be used for their detection. With the mercuric-diphenylcarbazone reagent, purple to bluish-violet spots are obtained with a detection limit of about 1 μg. In the case of the N,2,6-trichloro-*p*-benzoquinone imine reagent, blue to greyish-blue spots appear after heating the plate to 110° C for one or two minutes. This spray reagent is more sensitive, capable of detecting down to 0·05 μg. The spots at low levels are better seen by viewing from the back of the plate with transmitted light.

TABLE II

Colour reactions of phenobarbitone, phenytoin and their hydroxy-metabolites with spray reagents

Compound	Mercuric-diphenyl-carbazone	N,2,6-tri-chloro-p-benzoquinone imine
Phenobarbitone	Purple	Blue
Hydroxyphenobarbitone	Bluish-violet	Blue
Phenytoin	Violet	Greyish-blue
Hydroxydiphenylhydantoin	Bluish-violet	Greyish-blue

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