A rapid semiquantitative method for the determination of anticonvulsant drugs in blood and urine by thin-layer chromatography is described that is applicable in most laboratories. Utilization of SilicaAR TLC-7GF with benzene:acetone (4:1), chloroform:acetone (9:1), and carbon tetrachloride:acetone (7:3) provides separation of Dilantin, phenobarbital, Mysoline, Phenylethylmalondiamide, Mesantoin, and Nirvanol.

In the study and management of patients with epilepsy, a critical question is the relationship between the dosage of drugs ordered and their effectiveness for the patient. The quantitative determination of drugs in blood and urine has been a difficult procedure requiring highly specialized equipment and techniques; therefore, studies of anticonvulsant drug concentrations in blood and urine have not been conducted in most outpatient departments.

Thin-layer chromatographic techniques permit a rapid quantitative or semiquantitative method for drug assays which are applicable to most biologic specimens. Numerous thin-layer techniques for the determination of barbiturates and other drugs have been reported (1-5). In 1965 Olesen (6) described a method utilizing thin-layer chromatography for separation of 5,5 diphenylhydantoin (Dilantin) and phenobarbital.

Our report deals with a rapid semiquantitative method for the determination of anticonvulsant drugs in blood and urine that is applicable in most laboratories. The method is designed to provide the clinician with an approximate indication of the blood or urine levels of anticonvulsant drugs, as well as information related to the patient's ability to metabolize these drugs.
Materials and Method

Reagents

All reagents used are ACS reagent grade.

Conc. hydrochloric acid
Absolute ethanol
Chloroform:acetone, 9:1 (v/v)
Carbon tetrachloride:acetone, 7:3 (v/v)
Benzene:acetone, 4:1 (v/v)
Saturated sodium hydroxide
SilicAR TLC 7-GF (Mallinckrodt Chemical Works, St. Louis, Mo.)

Standards

Standard solutions were obtained:
Mesantoin (3-methyl-5-ethyl-5-phenylhydantoin; Sandoz Pharmaceuticals, Hanover, N. J.)
Nirvanol (5,5-phenylethylhydantoin; Sandoz Pharmaceuticals)
Phenylethylmalondiamide (PEMA; Ayerst Laboratories, New York, N. Y.)
Mysoline (5-ethyl-5-phenyl-hexahydropyrimidine-4,6-dione; Ayerst Laboratories)
Dilantin sodium (5,5-diphenylhydantoin sodium; Parke, Davis & Company, Detroit, Mich.)
Phenobarbital sodium (sodium 5-ethyl-5-phenylbarbiturate; Mallinckrodt Chemical Works, St. Louis, Mo.)

Preparation of Standards

Dilantin stock solution, 200 μg./ml. To a 25-ml. volumetric flask add 5 mg. of 5,5-diphenylhydantoin sodium and approximately 20 ml. of distilled water. Add one to two drops of saturated NaOH (until the solution is clear) and bring the volume to 25 ml. with distilled water.

Phenobarbital stock solution, 200 μg./ml. To a 25-ml. volumetric flask add 5 mg. of sodium phenobarbital and dilute to 25 ml. with distilled water. This solution is unstable and should be prepared freshly immediately prior to use.

Mysoline stock solution, 100 μg./ml. To a 25-ml. volumetric flask add 2.5 mg. of 5-ethyl-5-phenylhexahydropyrimidine-4,6-dione and dilute to 25 ml. with distilled water.

Mesantoin stock solution, 100 μg./ml. Prepare as Mysoline stock solution.

PEMA stock solution, 200 μg./ml. To a 25-ml. volumetric flask add 5 mg. of phenylethylmalondiamide (PEMA) and dilute to 25 ml. with distilled water.
Nirvanol stock solution 200, μg/ml. Prepare as PEMA stock solution.

All working standard solutions are prepared in concentrations equivalent to 5, 10, 20, and 40 μg, by appropriate dilution of the stock standards to 5 ml with distilled water. A mixed standard can be prepared readily by the addition of the appropriate volumes of the various stock standards to one tube with a final dilution to 5 ml.

Plate Preparation

Glass plates (14 by 19 cm.) are cleaned thoroughly, and a strip of autoclave tape, 0.6 by 19.0 cm. is placed on each side of the plate. A slurry of SilicAR TLC 7-GF is prepared by adding 9.0 ml. of water to 4.0 gm. of silica in a 17- by 150-mm. test tube. The mixture is shaken vigorously for 15 sec. and immediately poured between the tapes at one end of the plate. A glass stirring rod is placed across the top of the tape and pulled rapidly and smoothly across the plate, thus spreading the gel in an even layer over the surface of the plate. The plates are air dried at room temperature for 3–4 hr., and then may be stored until ready for use.

Extraction Procedure

To 2 ml. of plasma or serum, or 5.0 ml. of urine, in a 40-ml. glass- or polyethylene-stoppered centrifuge tube, add 0.1 ml. of concentrated HCl followed by 30 ml. of chloroform. Place on a vortex mixer for 2 min. or on a mechanical shaker for 10 min. Centrifuge (International Size, 2, 16 place head) at 1500 rpm for 5 min.; then transfer the chloroform (bottom) layer to a 50-ml. beaker, and evaporate the chloroform to a volume of less than 1 ml. Remove the beaker from the hot plate and complete the evaporation to dryness under a stream of dry air. The residue is resuspended in 0.4 ml. of absolute ethanol, and a 30-μl. aliquot spotted on the plate for each sample. A known standard mixture of the drugs under study and their metabolites should be carried through the extraction procedures as outlined above.

Development and Quantitation

The samples are spotted 3.0 cm. from the bottom of the plate and 1.2 cm. apart. Four spots equivalent to 5, 10, 20 and 40 μg. of the standard mixture are spotted alternately with the 20-μl. spots of the unknown samples. After spotting, the tape is removed prior to developing. Three plates are spotted for each series of samples. The first plate is developed in chloroform:acetone, 9:1 (v/v); the second plate in carbon tetrachloride:acetone, 7:3 (v/v); and the third plate in benzene-acetone,
(v/v), until the solvent front has migrated 10 cm.; this requires approximately 30–45 min. The plates are removed from the developing chamber, dried at room temperature, and examined under short-wavelength ultraviolet light (250 m\(\mu\)).

The spots demonstrated by ultraviolet absorption are outlined with a soft lead pencil and compared with those of the known standards. The \(R_t\) and \(R_p\) values of the unknown spots are determined and compared to those of the standards.

\[
R_t = \frac{\text{distance drug traveled (cm.)}}{\text{distance solvent traveled (cm.)}}
\]

\[
R_p = \frac{\text{distance drug traveled (cm.)}}{\text{distance phenobarbital traveled (cm.)}}
\]

**Results and Discussion**

Each solvent system yields different \(R_t\) and \(R_p\) values for various drug combinations (Table 1). For example, the best separation of Dilantin and phenobarbital is obtained in benzene:acetone, whereas chloroform:acetone provides a poor separation of the two compounds. If the patient’s medication is known before analysis, it is possible to select the most appropriate system and eliminate those which do not give good separation of the drugs in question.

Recoveries range from 80 to 105\%, depending upon the initial concentration in the sample. The lower limit of detection is approximately 1 \(\mu\)g. for Dilantin, phenobarbital, Mesantoin, and Nirvanol, and 2 \(\mu\)g. for Mysoline and PEMA.

The best separations of drugs in this procedure generally are obtained by developing unactivated plates in unsaturated chambers. This is true particularly of the chloroform:acetone system. If the plates in the chloroform system are developed in a saturated chamber, Dilantin and phenobarbital will not separate.

Most epileptics receive two or more anticonvulsant drugs and, often, other drugs, such as tranquilizers, in an attempt to provide adequate

<table>
<thead>
<tr>
<th>Drug</th>
<th>Benzen</th>
<th>Acetone, 4:1</th>
<th>Chloroform</th>
<th>Acetone, 8:1</th>
<th>Chloroform</th>
<th>Acetone, 8:1</th>
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<tr>
<td></td>
<td>(R_t)</td>
<td>(R_p)</td>
<td>(R_t)</td>
<td>(R_p)</td>
<td>(R_t)</td>
<td>(R_p)</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>0.65</td>
<td>1.00</td>
<td>0.78</td>
<td>1.00</td>
<td>0.66</td>
<td>1.00</td>
</tr>
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<td>Dilantin</td>
<td>0.52</td>
<td>0.80</td>
<td>0.71</td>
<td>0.91</td>
<td>0.52</td>
<td>0.79</td>
</tr>
<tr>
<td>Mysoline</td>
<td>0.11</td>
<td>0.17</td>
<td>0.26</td>
<td>0.33</td>
<td>0.19</td>
<td>0.29</td>
</tr>
<tr>
<td>PEMA</td>
<td>0.16</td>
<td>0.25</td>
<td>0.20</td>
<td>0.26</td>
<td>0.16</td>
<td>0.24</td>
</tr>
<tr>
<td>Mesantoin</td>
<td>0.77</td>
<td>1.18</td>
<td>0.89</td>
<td>1.14</td>
<td>0.84</td>
<td>1.27</td>
</tr>
<tr>
<td>Nirvanol</td>
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<td>1.01</td>
<td>0.80</td>
<td>1.03</td>
<td>0.65</td>
<td>0.98</td>
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</table>
seizure control. Therefore, it is advisable, whenever possible, to utilize a standard containing all of the medications such a patient might be receiving.

Our laboratory has screened 174 compounds for possible interference in this procedure (7). Even if all of the parent compounds a patient is receiving are carried through the procedure, it is possible to obtain additional unknown spots using the patient’s blood. Generally, these spots represent metabolic products of the parent compounds, as well as other drugs the patient is receiving, which are not identified readily on the chromatogram.

The therapeutic levels of these drugs may be difficult to assess because of their metabolism. For example, Mesantoin is demethylated rapidly to Nirvanol in the liver, and Mysoline is metabolized to both PEMA and phenobarbital (8, 9). Therefore, the amount of parent drug present in the samples may be small, whereas there may be a large amount of metabolites present. Individual variability in the utilization of anticonvulsant drugs is very common; thus, the seizures of one epileptic may be controlled with a low level of the drug(s) in the blood, whereas in another individual with the same blood concentration(s), the seizures would not be controlled.

Interpretation of the significance of a given level in the blood must be based on the clinical evaluation of the patient in conjunction with the laboratory findings. The interpretation of anticonvulsant drug concentrations in blood and urine and their interrelationships has been reviewed elsewhere (8–11).

The method described in this paper is designed only as a rapid screening procedure to assist the clinician in his evaluation of the patient’s status on his present drug regimen. Quantitative determinations are necessary for complete evaluation of possible defects in the patient’s ability to utilize adequately the drugs in a given anticonvulsant regimen.

References


