

Thin-layer Chromatography of the Common Barbiturates

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THIN-layer systems for barbiturates are of particular value to the clinical biochemist for the speedy differentiation of the different members of this class of hypnotics, and to the forensic chemist who has to detect therapeutic concentrations in small volumes of blood in "driving under the influence of drugs" cases.

We wish to report a system that is sensitive, rapid and has high resolving power. Merck-prepared cellulose plates with fluorescent indicator, or Kodak Chromatogram Sheet Type 6065 of 20 × 20 cm size, are cut to 4 × 10 cm size. By using smaller plates increased economy (about 1 shilling per plate) and decreased running time are obtained without significant loss of resolution. The plates are dipped in 10 per cent. w/v sodium orthophosphate, (Na₃PO₄).12H₂O, aqueous solution which is drained off and the plates are then blotted. They are oven-dried at 100° C for half an hour. After cooling they are dipped in acetone - water (75 + 25) and the acetone allowed to evaporate by gently warming the plate for about 1 minute in air. Water remaining on the back of the plate is removed by blotting. The spots of barbiturate containing about 1 μg of compound are applied to the plate or sheet which is developed in n-amyl methyl ketone in a beaker (or, for the sheet, preferably in an Eastman Chromatogram Developing Apparatus Type 104). The time of running is about 10 minutes. The spots are detected by examination in 254-nm light, and by spraying with saturated aqueous mercury(I) nitrate solution. Both methods are very sensitive; on the Kodak sheet 0.4 μg of barbiturate can easily be detected. Typical R_F values are shown in Table I; it is emphasised that known control spots should be run on each occasion as, although resolution is always excellent, R_F values are not absolutely reproducible.

TABLE I
R_F VALUES

Compound	R _F
Barbitone	0.2
Phenobarbitone	0.3
Cyclobarbitone	0.4
Butobarbitone	0.6
Nealbarbitone	0.7
Amylobarbitone	0.75
Pentobarbitone	0.80
Quinalbarbitone	0.85

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