brown after about 15 min, thus enhancing the identification of this drug. Phenformin hydrochloride cannot be detected by the nitroprusside–ferricyanide spray when solvent system $S_4$ is used. This may be attributed to the interference of residual formamide on the plate.

A mixture of all four compounds can be separated by solvent system $S_1$. Systems $S_2$ to $S_4$ will separate tolbutamide from phenformin hydrochloride and either acetohexamide or chlorpropamide and are intended as alternatives to system $S_1$. A mixture of acetohexamide and chlorpropamide will not separate in systems $S_2$ to $S_4$ and should a spot be detected which may correspond to either of these compounds alone or in combination, system $S_5$ will separate a mixture of the two efficiently and in conjunction with the differing colour reactions will confirm which substance is present.

This chromatographic method presents a rapid and convenient method of differentiating between the four hypoglycaemic agents and may find application in pharmaceutical or forensic work.

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## Preparative polyamide layer chromatography

The rapidity of analysis, only slight broadening of the spots and easy handling in diagnostic polyamide layer chromatography[1–6] encouraged us to use preparative polyamide layer chromatography in the purification of dinitrophenyl amino acids from dinitrophenylation products. Satisfactory results were obtained and various advantages over the recrystallization method were observed in our laboratory[7]. In order to test the characteristics of the polyamide layer for preparative scale work, we selected isomeric nitroanilines for this evaluation because these compounds were brightly coloured, quite stable on handling, easily available in pure form and had large differences in $R_F$ value.

As in previous experiments[6], we prepared the polyamide layer by spreading 15 ml of polyamide solution (20 g polycaprolactam in 100 ml of 75% formic acid) on

15 × 15 cm glass plates which were kept horizontally in a chromatographic cabinet (50 × 45 × 45 cm), saturated in advance with water vapour, where the slow evaporation of the formic acid was allowed to proceed. The resulting layer had a thickness of 0.07 cm. In order to increase the loading capacity, we tried to make thicker layers but this often resulted in cracked layers. The upper limit seemed to be 0.10 cm (30 ml polyamide solution on a 15 × 15 cm plate). For quantitative work, as well as for the recovery of separated substances, the layer had to be prewashed with a polar solvent, usually the solvent used in the later extraction of the substances.

Because of the durability of the polyamide layer, the application of sample solution was so easy that no special equipment was necessary. With the help of a tapered capillary, several ml of a solution of a mixture of nitroanilines could be applied on a 15 × 15 cm layer in less than 30 min. The applied mixture formed a band less than 0.5 cm in width. The start line was set 1.5 cm from the bottom edge and was 13 cm long in order to avoid the edge effects.

The loading capacity was about 15 mg per substance on a 15 × 15 cm layer of 0.07 cm thickness for nitroanilines whose $R_F$ values were 0.57 (*o*-nitroaniline), 0.35 (*m*-nitroaniline) and 0.16 (*p*-nitroaniline) in a carbon tetrachloride–glacial acetic acid (9:1) system. The ratio of adsorbent to a single substance was 200:1 by weight. Of course, the loading varied greatly with the substances to be separated, but this ratio was distinctly better than with other adsorbents. After development, each separated band which was *ca.* 2 cm in width was scraped into a flask and extracted several times with ethanol until no more of the substance could be detected in the extract. The yields were always above 90%. The purity of the recovered substances was checked by polyamide layer chromatography and melting point.

This experiment shows that polyamide layer chromatography is excellent for the separation and purification of small amounts of substances.

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