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Note

The simultaneous separation of individual prostaglandins by thin-layer chromatography on an unmodified support

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Many solvent systems have been devised for the thin-layer chromatographic (TLC) separation of prostaglandins (PGs)[1]. These systems usually separate PGs into groups E, F and A/B, although Amin[2] has reported a simultaneous separation of A_2 from B_2 as well as from E_2 and $F_{2\alpha}$. Separation of the individual members of each group, which only differ in their degree of saturation (*e.g.*, PGE_1 from PGE_2) have previously necessitated the use of silver nitrate[3] or ferric chloride[4] impregnated silica layers, or specially prepared silica gel impregnated glass fibre sheets[5]. We are unaware of any system which will separate these individual PGs on unmodified TLC supports.

Our interest in a simple system for PG separation stems from an investigation at this Institute of their involvement in the stimulation of bone resorption by breast tumours[6]. Radioimmunoassays have recently been established in our laboratories for the assay of PGs released by these tumours. The antisera used, although specific for each group of PGs, do not distinguish between members within each group. Hence, an initial separation of the closely related PGs is essential before the contribution of each may be assessed. This communication reports a simple system using unmodified, commercially available TLC supports for the simultaneous separation of PGE_1, PGE_2, $PGF_{1\alpha}$ and $PGF_{2\alpha}$.

EXPERIMENTAL

Materials

Unlabelled prostaglandins were the generous donation of Dr. J. E. Pike (Upjohn Co., Kalamazoo, Mich., U.S.A.). [^{3}H]Prostaglandins were obtained from New England Nuclear, Boston, Mass., U.S.A., (5,6(n)-[^{3}H]-PGE_1 and 5,6(n)-[^{3}H]-$PGF_{1\alpha}$) and from the Radiochemical Centre, Amersham, Great Britain, (5,6,8,11,12,14,15(n)-[^{3}H]-PGE_2 and 5,6,8,11,12,14,15(n)-[^{3}H]-$PGF_{2\alpha}$). These were purified before each experiment by chromatography in the system described below. The scintillation fluid used for assessing radioactivity consisted of 7 g/l butyl PBD (2-[4′-*tert.*-butylphenyl]-5-[4″ biphenylyl]-1,3,4-oxadiazole) and 80 g/l naphthalene dissolved in a mixture of toluene–dioxane–ethanol (5:5:3, by vol.).

The TLC plates employed were DC-Alufolien Kieselgel 60 F_{254} (Merck, Darmstadt, G.F.R.), Polygram SIL G/UV_{254} (Camlab., Cambridge, Great Britain) and ITLC (Gelman, Ann Arbor, Mich., U.S.A.).

Procedures

The PGs were visualised after chromatography by spraying with dodecamolybdophosphoric acid in ethanol (20% w/v) and heating at 100° until spots appeared. When [^{3}H]PGs were chromatographed, their subsequent locations were determined by scraping 2-mm bands from the plates, and eluting with 0.5 ml absolute ethanol in glass scintillation vials. Scintillation fluid (10 ml) was added to each vial after 1 h. Radioactivity was assessed in a Packard Model 3380 liquid scintillation counter ([^{3}H]efficiency approx. 40%).

RESULTS

Merck TLC plates were found to be suitable for the present purposes. Single solvents (Analytical Reagent Grade) were tested initially. Chloroform or ethyl acetate gave incomplete separation with marked tailing after continuous development for at least 20 h. The combination of either with a more polar solvent decreased both development time and tailing. Ethanol was effective but reduced the degree of separation. Isopropanol or *n*-butanol, when present in greater proportions, was almost as efficient at reducing tailing and this could be further improved by the addition of a small proportion of ethanol. The effect of the high proportion of polar solvents to carry the PGs too far was counteracted by the addition of benzene.

The system eventually devised was benzene–chloroform–*n*-butanol–ethanol (4:10:5:1) which could simultaneously separate PGE_1, PGE_2, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ in a single, 2-h development (Fig. 1). PGA_1 ran close to the solvent front. An objective assessment of the separations was obtained by chromatographing [^{3}H]PGs adjacent to each other on the same plate in the presence of less than 1 μg unlabelled carrier PGs (Fig. 1).

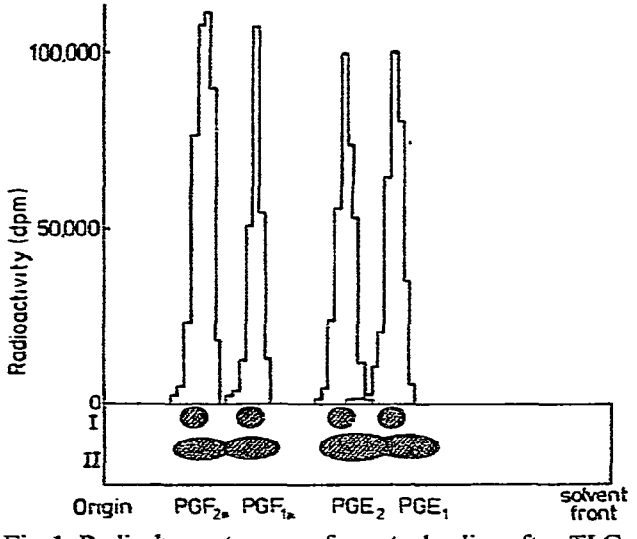

Fig. 1. Radiochromatogram of prostaglandins after TLC. Separation on silica gel plates (Merck) in the solvent system; I and II are facsimiles of visualised prostaglandins after 1 and 10 μg, respectively, of each were chromatographed.

The use of the other TLC plates (Polygram and ITLC) resulted in unacceptable tailing of the PG spots when chromatographed in the above solvent system. Heat activation of these supports did not improve the separation or decrease the degree of tailing.

DISCUSSION

This appears to be the first report of TLC on unmodified commercially available plates which gives separation of PGs differing only in their degree of saturation. It has been demonstrated by re-chromatography that the resulting PGs have mobilities identical to those expected, therefore indicating that they have undergone no alteration during the procedure. The use here of a neutral solvent system does not encourage oxidation as may occur in the presence of mild acidic solvents. The devised solvent system was applicable to only one type of TLC plate tested and was also dependent upon the mass of each PG applied (Fig. 1). It is possible that the type of analyses used in devising this system could be successfully applied to those TLC plates upon which separations were obliterated by tailing.

The necessity for devising this system was due to our requirement for a separation technique for the individual PGs. Such a system needed to be applicable prior to radioimmunoassay as the antisera cross-reacted with closely related PGs. Work is now proceeding in our laboratories using this system in conjunction with radioimmunoassays and the initial results have demonstrated the validity of this technique. It would seem probable that the system described here could equally be used in conjunction with Amin's method[2] for direct quantitative determination of PGs or with most other techniques for quantitation.

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