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Note

Separation of prostaglandins A, B, D, E, F, thromboxane and 6-keto prostaglandin $F_{1\alpha}$ by thin-layer chromatography

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Thin-layer chromatography (TLC) has been widely used for the separation of prostaglandins (PGs) and various solvent systems have been proposed[1–4]. Recently Ubatuba[5] has compared the separation of nineteen PGs using seven solvent systems described by different authors[5]. None of the solvent systems so far reported can separate prostaglandin E (PGE), prostaglandin F (PGF), prostaglandin A (PGA), prostaglandin B (PGB), prostaglandin D (PGD), thromboxane B_2 (TxB_2) and 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$, a metabolite of prostacyclin) as a group on the same thin-layer chromatogram[6]. A method for the separation of these compounds would facilitate the study of the synthesis and metabolism of PGs *in vivo* and *in vitro*. The separation of PGE_1 from PGE_2 and $PGF_{1\alpha}$ from $PGF_{2\alpha}$ has been achieved by argentation TLC[1], but the removal of silver ions from prostaglandin extract poses a problem. The present communication describes a solvent system which separates the prostaglandins as a group, and a second solvent system which can separate PGE_1 from PGE_2 and $PGF_{1\alpha}$ from $PGF_{2\alpha}$.

MATERIALS AND METHODS

All chemicals used were of analytical grade. Pre-coated thin-layer silica gel 60 plates (10 × 20 cm, 0.25-mm thickness; E. Merck, Darmstadt, G.F.R., distributed by VWR Scientific, U.S.A.) and Eastman "chromatogram" plastic sheet (6061 silica gel) from Eastman-Kodak (Rochester, NY, U.S.A.), were used. Prostaglandin standards were gifts from Dr. John E. Pike, Upjohn Company, Kalamazoo, MI, U.S.A.

For the group separation of PGs, 10 × 20 cm pre-coated silica gel 60 plates were used. PGs were applied 1.5 cm above the bottom edge of the plate, allowed to dry with cold air and placed in a filter-paper-lined rectangular glass tank containing the solvent system chloroform–isopropanol–ethanol–formic acid (45:5:0.5:0.3). The plate was allowed to run at room temperature (23–25°C) up to 16 cm from the origin, dried by a stream of air and re-developed in the same direction in the same solvent system. It was removed from the chamber, dried and sprayed with 10% phosphomolybdic acid in ethanol and heated for visualization of the spots.

The separation of PGE_1, PGE_2, $PGF_{1\alpha}$, $PGF_{2\alpha}$, PGD_2 and TxB_2 was achieved on 10 × 20 cm Eastman "chromatogram" plastic sheet using the solvent system ethyl acetate–isooctane–ethanol–acetic acid–water (35:10:3:0.1:0.1). Double development

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upto 16 cm from the origin in an unlined glass tank was necessary for adequate separation.

RESULTS AND DISCUSSION

The positions of different PGs on a thin-layer plate after its development with the solvent system chloroform–isopropanol–ethanol–formic acid (45:5:0.5:0.3) are shown in Fig. 1. It is evident from the figure that PGs were clearly separated into groups, *e.g.*, PGE, PGF, PGA, PGB, PGD, TxB_2 and 6-keto $PGF_{1\alpha}$. Existing chromatographic methods generally fail to separate all of these on a single plate[5]. This solvent system is therefore unique and could be used for group separation of PGs.

The R_F values of PGs on two different solvent systems, *e.g.*, chloroform–isopropanol–ethanol–formic acid (45:5:0.5:0.3) (A) and ethyl acetate–isooctane–ethanol–acetic acid–water (35:10:3:0.1:0.1) (B) are shown in Table I. It is clear from the R_F values that $PGF_{1\alpha}$, $PGF_{2\alpha}$, TxB_2, PGE_2, PGE_1 and PGD_2 have been separated by

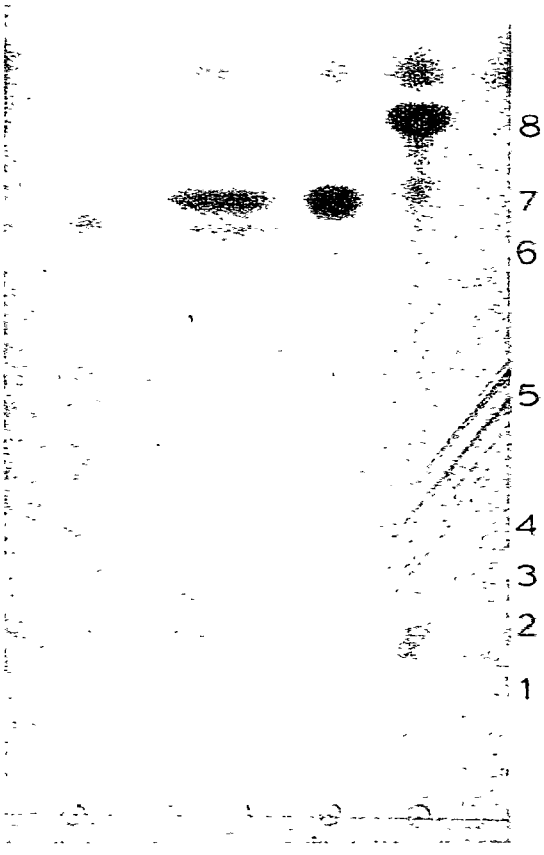

Fig. 1. Thin-layer chromatogram developed in chloroform–isopropanol–ethanol–formic acid (45:5:0.5:0.3) and sprayed with 10% phosphomolybdic acid in ethanol. 1 = $PGF_{1\alpha}$ or $PGF_{2\alpha}$; 2 = 6-keto $PGF_{1\alpha}$; 3 = TxB_2; 4 = PGE_1 or PGE_2; 5 = PGD_2; 6 = PGA_1 or PGA_2; 7 = PGB_1 or PGB_2; 8 = arachidonic acid.

solvent system B on Eastman "chromatogram" plastic sheet. But this solvent system could not separate PGA from PGB and the 6-keto $PGF_{1\alpha}$ tails in this solvent system. The solvent system used with glass TLC plates does not work as suitably with plastic sheets or *vice versa* and hence two-dimensional chromatography is not feasible on one plate.

TABLE I

R_F VALUES OF PROSTAGLANDINS USING TWO DIFFERENT SOLVENT SYSTEMS

Composition of solvent systems used: A, chloroform–isopropanol–ethanol–formic acid (45:5:0.5:0.3) with silica gel 60 plates and B, ethyl acetate–isooctane–ethanol–acetic acid–water (35:10:3:0.1:0.1) with Eastman "chromatogram" plastic sheets.

Compound	R_F	
	A	*B*
PGF_1	0.14	0.24
PGF_2	0.15	0.18
6-keto PGF_1	0.21	–
TxB_2	0.27	0.32
PGE_2	0.34	0.43
PGE_1	0.34	0.49
PGD_2	0.49	0.56
PGA_2	0.70	0.64
PGA_1	0.70	0.64
PGB_2	0.75	0.64
PGB_1	0.75	0.64
Arachidonic acid	0.84	0.79

The solvent systems so far reported can either separate PGF from 6-keto $PGF_{1\alpha}$, but fail to separate PGE from TxB_2 or *vice versa*[5,6] and so two different solvent systems are used when these four compounds are required to separate on a single sample[5–7]. Solvent A described here can separate PGE, PGF, TxB_2 and 6-keto $PGF_{1\alpha}$ on the same plate. This method is very simple, time saving and could be routinely used in PG research.

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