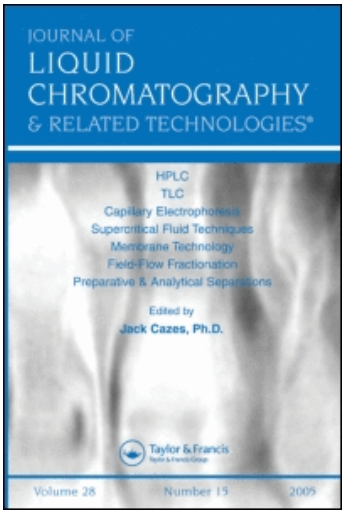

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SEPARATION OF PROSTAGLANDINS E_1 AND E_2 AND OTHER MAJOR EICOSANOIDS BY UNIDIMENSIONAL ARGENTATION THIN LAYER CHROMATOGRAPHY

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ABSTRACT

Prostaglandins (PG) E_1 and E_2 are important regulators of biologic functions, and can express different biological effects. In thin layer chromatography (TLC) systems which separate these compounds, comigration of other major eicosanoids is a problem. This paper describes a TLC system using a mobile phase of chloroform/methanol/acetic acid/H_2O (90:7.5:5:0.8) that separates PGE_1 and PGE_2, as well as other major eicosanoids, including dihomogammalinolenic acid (DHLA), the immediate fatty acid precursor of PGE_1.

INTRODUCTION

PGE_1 and PGE_2 have similar biological actions, but do have different effects on some cell functions. For example, PGE_1 inhibits whereas PGE_2 does not influence or increases aggregation of human platelets (1-3). In addition, PGE_1 but not PGE_2 reduces production of the lymphokine macrophage migration inhibitory factor (4). Furthermore, PGE_1 and PGE_2 express quantitative differences in their ability to resorb bone (5). Several thin-layer chromatography (TLC) techniques have been used to separate PGE_1

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and PGE_2 (6-11). We have examined the mobile phases used and find that they do not separate the major cyclooxygenase products of arachidonic acid (AA) from PGE_1 and PGE_2. This is a potential source of error if the major eicosanoids are to be quantified by bioassay or examined by scintillation counting or autoradiography. We therefore developed a simple TLC technique that separates PGE_1, PGE_2 and thromboxane B_2 (TxB_2), PGD_2, 6-Keto-PGF_{1a} (stable metabolite of prostacycline), and the immediate precursor of the 1 series eicosanoids, dihomogammalinolenic acid (DHLA).

MATERIALS AND METHODS

All chemicals were of high performance liquid chromatographic grade (Fisher Chemicals, King of Prussia, PA). Glass chromatography tanks (28 cm x 27 cm x 8 cm) with glass lids (Scientific Manufacturing Industries, Emeryville, CA) were used. Whatman LK5 silica-coated TLC plates (Whatman Chemical, Clifton, NJ) were scored into 2 cm channels with a 1 cm unused channel on each side. Plates were washed overnight in a mobile phase of chloroform/methanol (90:5), and air dried. They were coated with a 10% solution of silver nitrate (Kodak Chemicals, Rochester, NY) in distilled water and ethyl alcohol (14:86, v/v) in a 10" x 14" metal pan, air dried, activated in an oven (Boekel, Philadelphia, PA) at 110°F for 30 min, and then cooled at room air. Plates wrapped in aluminum foil and an air tight plastic bag could be kept for at least 1 week before use. All unlabeled eicosanoids were purchased from Sigma Chemical Co. (St. Louis, MO). Eicosanoids (5-10 ug) were spotted in the middle of the preadsorbant zone with a 20 ul (Rainen Instrument Co, Woburn, MA) or a 50 ul pipette (Hamilton Co., Reno, NV). After spotting, the eicosanoids were allowed to air dry, and were then run in the appropriate mobile phase until the solvent zone had reached 1 cm from the top of the plate. The plates were air dried, and then

dried at 110°F for 10 minutes. They were cooled at room air, and then sprayed with 3% cupric acetate (3 gm) in phosphoric acid/water (8:92, v/v) and air dried. Charring at 180°F for 10 minutes was often helpful in improving resolution of the bands.

RESULTS

Various solvent systems that have been used to separate PGE_1 and PGE_2 are listed in Table 1.

Although the TLC systems referenced in Table 1 separate PGE_1 from PGE_2, other major eicosaniods comigrate thus diminishing the utility of these systems (Table 2). However, separation of PGE_1,

TABLE 1

Solvent Systems Used to Separate Eicosanoids

TLC System	Ref.	Solvent Composition by Volume
SI	Present paper	chloroform/methanol/acetic acid/H_2O (90:7.5:5:0.8)
SII	12	ethyl acetate/2,2,4-trimethylpentane/acetic acid/methanol/H_2O (110:10:30:35:100)
SIII	6	ethyl acetate/2,2,4-trimethylpentane/acetic acid/H_2O (100:30:10:100)
SIV	6	ethyl acetate/2,2,4-trimethylpentane/acetic acid/H_2O (90:50:20:100)
SV	13	chloroform/methanol/acetic acid (90:5:5)
SVI	14	chloroform/methanol/acetic acid/H_2O (95:5:1:0.2)
SVII	9	0.0025 M phosphoric acid/acetonitrile with 0.2 M NaCl (52:48)
SVIII	8	organic (upper phase) of ethyl acetate/2,2,4-trimethylpentane/acetic acid/H_2O (110:50:20:100)
SIX	8	chloroform/methanol/acetic acid (80:10:10)
SX	15	ethyl acetate/isooctane/ethanol/acetic acid/H_2O (35:10:3:0.1:0.1)
SXI	11	ethyl acetate/methanol/acetic acid (100:10:1)
SXII	11	chloroform/methanol/acetic acid/H_2O (90:9:1:0.65)
SXIII	11	benzene/1,4-dioxane/acetic acid (20:20:1)
SXIV	11	ethyl acetate/acetone/acetic acid (90:10:1)

TABLE 2

Mobile Phases Used to Separate PGE_1 and PGE_2 But With Known Comigration of Major Eicosanoids[1]

TLC System	Ref.	Comigrating Eicosanoids
SII	12	TxB_2 and PGD_2
SIII	6	multiple tailing
SIV	6,11	PGE_1 and PGE_2 blur
SV	13	TxB_2 and PGD_2
SVI	14	6-Keto-PGF_{1a} and PGE_2
SVII	9	PGE_1 and PGD_2
SVIII	8	TxB_2 and PGD_2
SIX	8	TxB_2 and 6-Keto-PGF_{1a}
SX	15	6-Keto-PGF_{1a} tails
SXI	11	6-Keto-PGF_{1a} and PGF_{2a}, PGE_2 and PGD_2
SXII	11	PGE_2 and 6-Keto-PGF_{1a}
SXIII	11	6-Keto-PGF_{1a} and PGF_{2a}
SXIV	11	AA tailing

1 Comigration established as noted in References or by argentation technique as described in Materials and Methods.

PGE_2 and TxB_2, PGD_2, 6-Keto-F_{1a}, PGF_{2a}, AA and DHLA was achieved with chloroform/ methanol/acetic acid/H_2O (90:7.5:5:0.8) (Solvent I) as shown in Fig. 1. The mobilities of the compounds, expressed as R_f values, are shown in Table 3 with the preabsorbant-silica gel boundary as the origin. Development time is approximately 60 minutes. The method is reproducible (R_f standard error of the mean .5%) and reliable.

DISCUSSION

The method presented here allows separation by TLC of the monoenoic and dienoic PGE compounds and their immediate fatty acid precursors DHLA and AA respectively. Thus, metabolism of both PGE precursors may be studied simultaneously. In addition, the same system accommodates separation of the major cyclooxygenase products of AA: TxB_2, 6-Keto-F_{1a}, PGD_2, PGF_{2a}. Other investigators have separated PGE_1 from PGE_2. However, as shown here, comigration of other major eicosanoids with PGE_1 and PGE_2 occurs with several of the solvent systems used. Use of the Solvent I, a mobile phase of chloroform, methanol, acetic acid and water allows separation of PGE_1 from PGE_2 as well as separation of other major eicosanoids. This system is not only useful in

TABLE 3

Mobility of Separated Eicosanoids

Compound	Mobility (expressed as R_f)
PGF_{2a}	0.12
TxB_2	0.28
PGD_2	0.32
PGE_2	0.38
6-Keto-PGF_{1a}	0.51
AA	0.58
PGE_1	0.62
DHLA	0.83

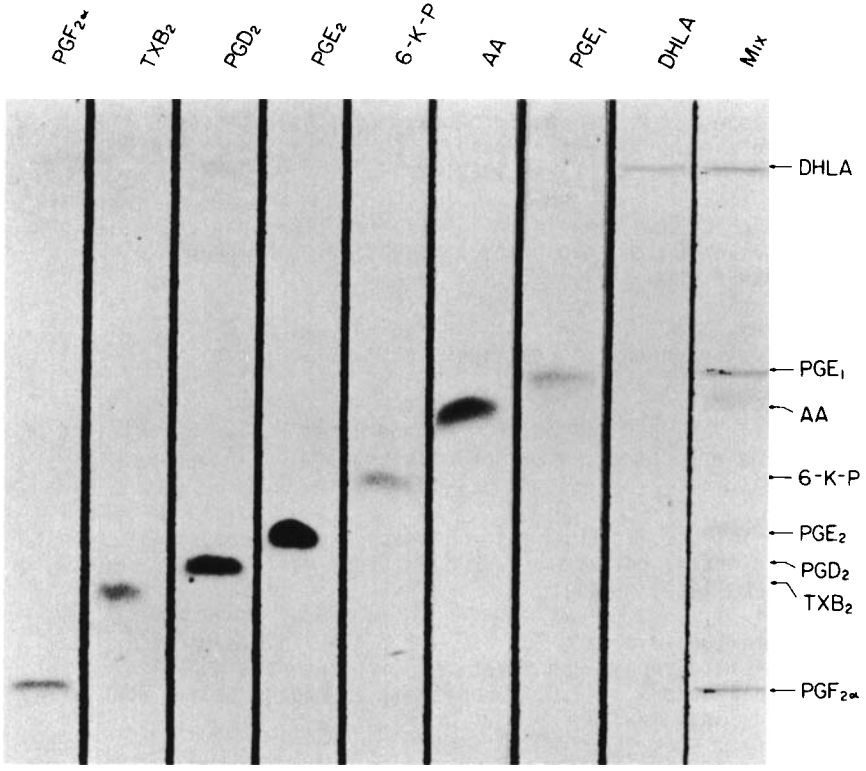

FIGURE 1

Chromatogram showing eicosanoid separation with a mobile phase of chloroform/methanol/acetic acid/H_2O (90:7.5:5.0:0.8). 6-Keto-PGF_{1a} is abbreviated as 6-K-P.

examining AA and its metabolites, but may also be used in experiments where DHLA and PGE_1 are to be examined.

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RERERENCES

1. Willis, A.L., Comai, K., Kuhn, D.C. and Paulsrud, J., Dihomo-gamma-linolenate suppresses platelet aggregation when administered in vitro or in vivo. Prostaglandins 1974:509, 1974.

2. Kloeze, J.,Relationship between chemical structure and platelet-aggregation activity of prostaglandin. Biochim. Biophys. Acta 187:285,1969.

3. Shio, M. and Ramwell, P., Effects of prostaglandin E_2 and aspirin on the secondary aggregation of human platelets. Nature (New Biol.) 236:45, 1972.

4. Bray, M.A., Gordon, D., and Morley, J., Role of prostaglandins in reactions of cellular immunity. Br. J. Pharmac. 52:453P, 1974.

5. Dietrich, J.W., Goodson,J.M. and Raisz L.G., Stimulation of bone resorption by various prostaglandins in organ culture. Prostaglandins 10:231, 1975.

6. Daniels, E.G. In: Lipid Chromatographic Analysis. (G.V. Marinetti, ed.) Vol. 2, 2nd edition, Marcel Dekker, New York, 1979, p 611.

7. Hamilton, J.G. and Tobias, L.D., In: Thin Layer Chromatography: Quantitative, Enviornmental and Clinical Applications. (J.C. Touchstone, D. Rogers, eds.) John Wiley and Sons, New York, 1980. p.478.

8. Salmon, J.A. and Flower, R.J., Extraction and thin-layer chromatography of arachidonic acid metabolites. Methods Enzymol. 86:477, 1982.

9. Rydzik, R., Terragno, N.A. and Terragno, A., Separation of eicosanoids by reverse-phase thin-layer chromatography. J. Liq. Chromat. 7:1313, 1984.

10. Sun, F.F., Chapman, J.P. and McGuire, J.C., Metabolism of protaglandin endoperoxide in animal tissues. Prostaglandins 14:1055, 1972.

11. Ubatuba, F.B. Use of a novel colour reaction in the chromatography of 6-oxo-prostaglandin F_{1a} and related prostaglandins. J. Chromatog. 161:165, 1978.

12. Korte, K., and Casey, M.L., Separation of prostanoids by one-dimensional thin-layer chromatography. J. Liq. Chrom. 6:55, 1983.

13. Green K. and Samuelsson, B., Prostaglandins and related factors: XIX: Thin-layer chromatography of prostaglandins. J. Lipid Res. 5:118, 1964.

14. Croshaw, K. The incorporation of 1-^{14}C arachidonic acid into the lipids of rabbit renal slices and conversion to prostaglandins E_2 and F_{2a}. Prostaglandins 3:607, 1973.

15. Goswami, S. and Kinsella, J.E., Separation of prostaglandins A, B, D, E, F, thromboxane and 6-Keto-prostaglandins F_{1a} by thin-layer chromatography. J. Chromatogr. 209:334, 1981.