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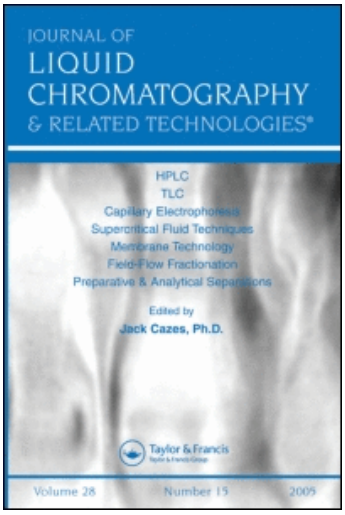

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
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To cite this Article Korte, Klaus and Casey, M. Linette(1983) 'Separation of Prostanoids by One-Dimensional Thin-Layer Chromatography', Journal of Liquid Chromatography & Related Technologies, 6: 1, 55 — 62

To link to this Article: DOI: 10.1080/01483918308066869

URL: http://dx.doi.org/10.1080/01483918308066869

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JOURNAL OF LIQUID CHROMATOGRAPHY, 6(1), 55-62 (1983)

SEPARATION OF PROSTANOIDS BY ONE-DIMENSIONAL THIN-LAYER CHROMATOGRAPHY

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ABSTRACT

We have developed a simple and rapid method for separation of 7 prostanoids and arachidonic acid by one-dimensional thin-layer chromatography. For this separation we employ commercially prepared thin-layer plates that have a preadsorbant (celite) area to which samples are applied. Due to the inert characteristics of the celite no separation occurs until the sample reaches the preadsorbant-silica gel junction. Since all the material moves with the solvent front as a sharp, narrow band to the preadsorbant (celite)-silica gel boundary, a high resolution of the separated compounds is achieved. The time required to apply one sample to the preadsorbant (celite) area is considerably less (2 min) than that required for application of a sample to silica gel (10 min). This method is suitable for the separation of major prostaglandins (PGE_2, $PGF_{2\alpha}$), the major enzymatically formed metabolites of these prostaglandins, and the stable, nonenzymatically formed product of thromboxane A_2 (TXB_2).

INTRODUCTION

To investigate the metabolism of arachidonic acid and prostanoids, thin-layer chromatography (TLC) on silica gel is used widely for the separation and identification of a number of prostaglandins (PG) and prostaglandin-like compounds. Many TLC systems have been described (1-5) in which a variety of

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prostanoids can be separated. According to the conventional method for TLC, samples are dissolved in small volumes (20 - 30 µl) of organic solvents which are applied to the silica gel in a successive series of aliquots (1 - 5 µl) overlaid at the origin. This process is extremely time consuming since the solvent must be removed completely between each application of a portion of the sample. Moreover, with a small volume, it is difficult to remove completely the compounds from the tubes. We have developed a technique for the separation of 7 major prostanoids and arachidonic acid by one-dimensional TLC in which we employ commercially available thin-layer plates with a preadsorbant (celite) area. With this method samples can be applied rapidly and very high resolution of the compounds is achieved. We developed this method to evaluate the formation of prostanoids in human endometrial stromal cells, which are maintained in monolayer culture in the presence of [^{14}C]arachidonic acid. These cells biosynthesize and metabolize prostanoids and secrete the products into the culture medium. This method has proved to be advantageous for these studies and will undoubtedly be applicable to a number of systems in which high resolution and quantification of prostanoids is desired.

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MATERIALS AND METHODS

Silica gel G preadsorbant thin-layer plates were purchased from Analtech, Newark, DE, USA, and were used without an activation or washing procedure. Prostanoid standards were gifts from Dr. John E. Pike, Upjohn Company, Kalamazoo, MI, USA. All solvents were of analytical grade from scientific supply houses. Glass tubes were siliconized using Aquasil (Pierce Chemical Company, IL, USA.)

The TLC plate was scored into 7 lanes (25 mm each). Each sample, in 150 µl chloroform:methanol (2:1, by vol), was applied with a micro-selectapette (Clay Adams, Parsipanny, NJ, USA) to the preadsorbant (celite) layer on an area (625 mm^2) 5 mm above the bottom edge of the plate and 5 mm below the preadsorbant

(celite)-silica gel boundary. To minimize oxidation of the compounds, the origin areas of the TLC plate were exposed to continuous nitrogen flow during the application process by use of a device supplied by Sindco Corp., Miami, FL, USA. The plates were placed into a plate conditioning apparatus (Analtech, Newark, DE, USA) and developed in a solvent system chloroform:methanol:acetic acid:water (95:5:1:0.2, by vol) to a height of 16 cm above the preadsorbant (celite)-silica gel limit. After removal of the plate from the TLC chamber, the solvent was evaporated under a stream of air for 5 min and thereafter the chromatogram was exposed to iodine vapor.

In a typical experiment human endometrial stromal cells were maintained in monolayer culture in the presence of [^{14}C]arachidonic acid (Amersham, Arlington Heights, IL, USA). At the end of the incubation period the medium (4 ml) was collected and acidified with 2 ml acetic acid (1N) and then extracted with 30 ml chloroform:methanol (2:1, by vol); the organic phase was removed and evaporated at room temperature under a stream of nitrogen. The residue was subjected to silicic acid column chromatography and the bulk of free [^{14}C]arachidonic acid was collected in the first fraction (13 ml chloroform). In the second fraction (chloroform:methanol, 10:1, by vol) PGs were obtained and the organic phase was concentrated at room temperature under a stream of nitrogen to approximately 0.5 ml. Ten µg each of $PGF_{2\alpha}$, PGE_2, 15-keto-PGE_2, 15-keto-$PGF_{2\alpha}$, 13,14-dihydro-15-keto-PGE_2 (PGEM), 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (PGFM), thromboxane (TX) B_2 and arachidonic acid were added as a mixture in 50 µl ethanol and the evaporation process was completed. The compounds were dissolved in 0.15 ml chloroform:methanol (2:1, by vol) and separated by TLC as described. After visualization of the standards and evaporation of the iodine, the areas of the chromatogram corresponding to the various compounds were scraped from the plate and eluted with 6 ml chloroform: methanol (2:1, by vol). The extracts were evaporated at room temperature under a stream of nitrogen and radio-

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activity was assayed in 16 ml Liquiscint (National Diagnostics, Somerville, NJ, USA) by liquid scintillation spectrometry.

RESULTS AND DISCUSSION

The positions of different prostanoids and arachidonic acid on a chromatogram after one-dimensional preadsorbant (celite)-silica gel chromatography are given in Fig 1. For purpose of

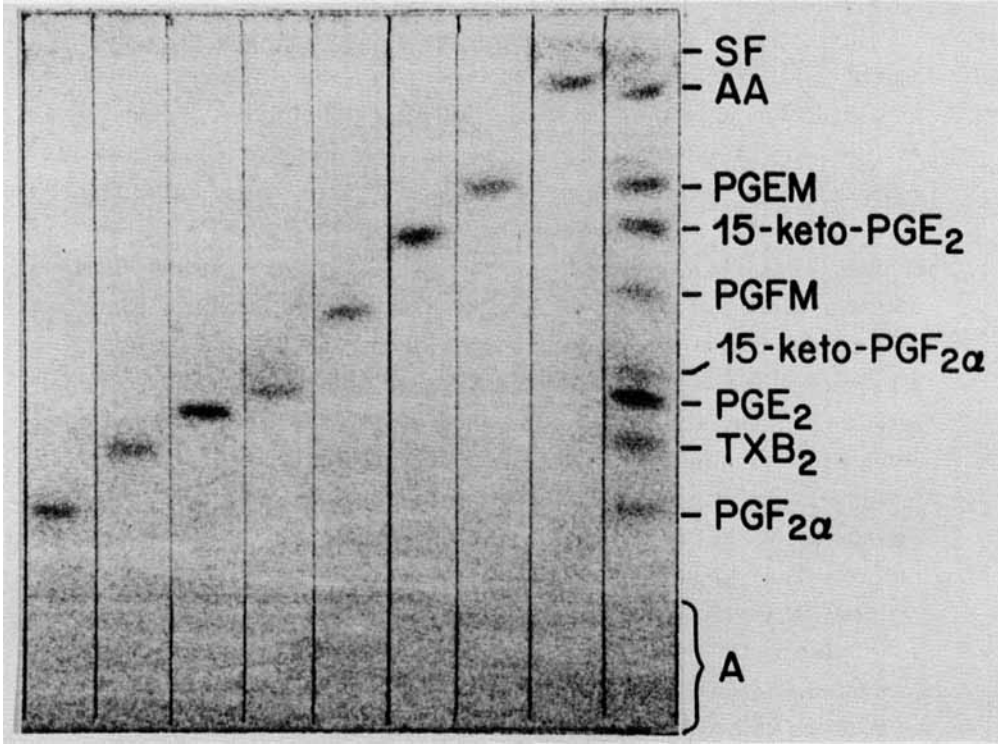

Figure 1. Prostanoid standard chromatogram after iodine staining. The compounds were applied either separately or as the mixture. Ten µg each of prostaglandin (PG)$F_{2\alpha}$, thromboxane B_2 (TXB_2), PGE_2, 15-keto-$PGF_{2\alpha}$, 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (PGFM); 15-keto-PGE_2; 13,14-dihydro-15-keto-PGE_2 (PGEM); and arachidonic acid (AA) were applied on the preadsorbant (celite) area. A = preadsorbant (celite) layer; SF = solvent front.

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TABLE 1

Mobility of Separated Prostanoids and Arachidonic Acid

Compound	Mobility (expressed as R_f)
$PGF_{2\alpha}$	0.11
TXB_2	0.20
PGE_2	0.25
15-keto-$PGF_{2\alpha}$	0.32
PGFM	0.46
15-keto-PGE_2	0.59
PGEM	0.69
Arachidonic acid	0.89

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illustration the scored lanes are 20 mm wide. The mobilities of the compounds, expressed as R_f values, are shown in Table 1. For computation of R_f values, the preadsorbant (celite)-silica gel boundary was taken as the origin.

Each standard is separated distinctly and application of the compounds as the mixture does not lead to alterations in R_f values. In the TLC system used the mobility of 6-keto-$PGF_{1\alpha}$ [the more stable, nonenzymatically formed product of prostacyclin (PGI_2)] is the same as that of PGE_2 (not shown). However, if separation of these two compounds is desired, they may be eluted from the silica gel with chloroform:methanol (2:1, by vol), and separated by TLC in a solvent system of chloroform:isopropanol:ethanol:formic acid (45:5:0.5:0.3, by vol) as described previously by Goswami et al. (4).

An example of the results obtained with the application of the methods described is shown in Fig. 2. Human endometrial stromal cells maintained in monolayer culture were incubated with [^{14}C]arachidonic acid for 24 h to evaluate the biosynthesis of radiolabeled prostanoids. Since the radioactivity in the medium extract may be limited, it is necessary to apply the entire extract residue on TLC to achieve accuracy when assaying radioactivity by liquid scintillation spectrometry. The conventional means of

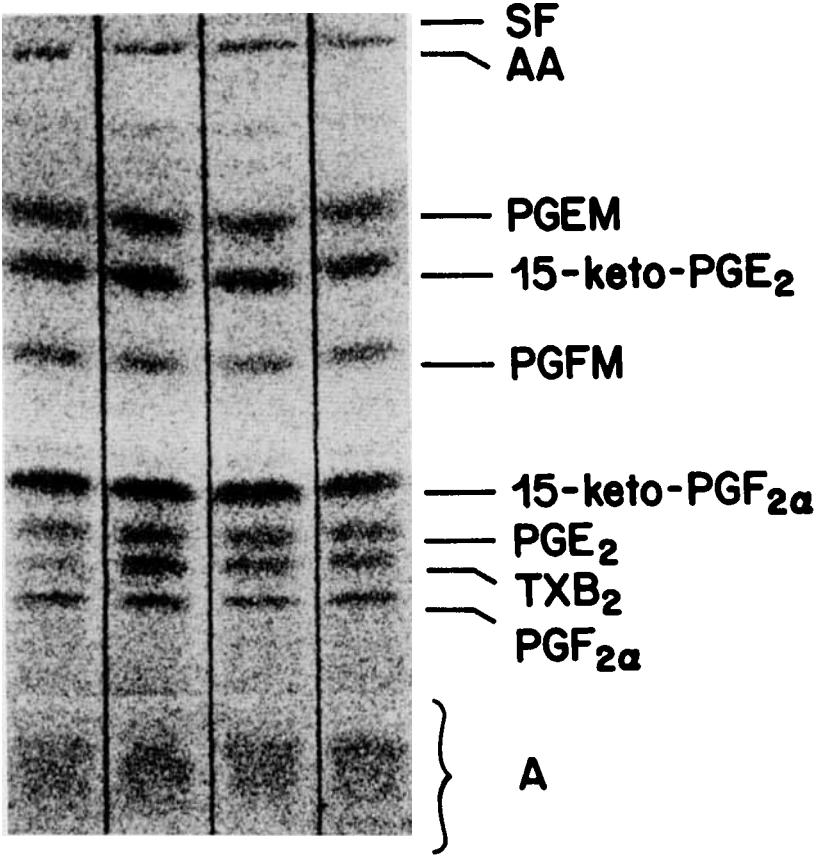

Figure 2. Prostanoid chromatogram of human endometrial stromal cell monolayer culture medium extracts after iodine staining. The culture medium was extracted, the standards were added and the compounds were applied to the preadsorbant (celite) area in 0.15 ml chloroform:methanol (2:1, by vol) as described in the text. For abbreviations see legend of Fig. 1.

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sample application on TLC is very time consuming because of the necessity for complete solvent removal between repeated application of aliquots of the sample in a single band at the origin. Moreover, the capacity of the silica gel is limited. Using the method we describe here the spotting time per sample was 2 min compared to 10 min with conventional methods. By virtue of the characteristics of the preadsorbant (celite) layer, all of the applied material moves with the solvent front as a sharp band to the preadsorbant (celite)-silica gel boundary and no separation occurs until the sample reaches the silica gel layer. For this reason, sample application in a single band is not necessary. The method we describe provides for a more rapid means of sharp separation of most major prostanoids and arachidonic acid and is applicable to many systems in which identification of these compounds by TLC is to be achieved.

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ACKNOWLEDGEMENTS

The authors thank Joe Dixon and Jess Smith for skilled technical assistance and are appreciative of the expert editorial assistance of Lydia Morris.

The investigation was supported, in part, by USPHS Grant No. 5-P50-HD11149.

Dr. Korte is a postdoctoral trainee supported, in part, by DFG Grant No. 1-3, Ko 772/1-2 (G.F.R.). Dr. Casey is a post-doctoral trainee supported, in part, by USPHS Training Grant No. 1-T32-HD07190.

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