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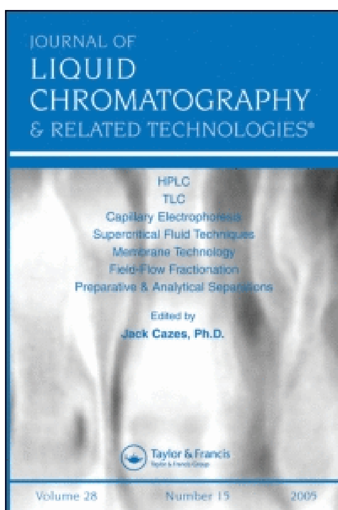

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Separation of Eicosanoids by Reverse-Phase Thin-Layer Chromatography

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SEPARATION OF EICOSANOIDS BY REVERSE-PHASE THIN-LAYER CHROMATOGRAPHY

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ABSTRACT

A method is described for separating eicosanoids including prostaglandins, their metabolites, and HETE. This was accomplished using commercially available reverse-phase thin-layer chromatographic plates and 0.0025M phosphoric acid-acetonitrile (52:48, v/v) containing 0.2M sodium chloride as a mobile phase. The use of reverse-phase thin-layer chromatography offers an alternative means of separating eicosanoids over traditional silica gel.

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INTRODUCTION

Silica gel thin-layer chromatography (TLC), since its introduction in 1956 by Stahl (1) has traditionally been used as both a preparative and analytical technique. Green and Samuelsson (2) first afforded silica gel TLC to separate prostaglandins. Since then, impregnation of the silica with silver (3) or iron (4) and numerous solvent systems (5-10) have been extended to separate the ever increasing number of arachidonic acid metabolites. Today, revisions are occurring to include the lipoxygenase products (11-13). The commercial availability in the late 1970's of n-alkyl bonded silica gel TLC plates has proven to be of much value in the analysis of substances with only minor structural

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differences (14,15). Although it is doubtful, due to the number of metabolites, that any one 20 x 20 cm TLC plate can effect complete separation of all eicosanoids in one dimension, reverse-phase TLC offers advantages over traditional silica TLC. This paper describes the first separation of eicosanoids by reverse-phase TLC using octadecyl bonded silica.

MATERIALS AND METHODS

All solvents were high performance liquid chromatography (HPLC) grade obtained from Fischer Scientific. Sodium chloride (NaCl) and phosphomolybdic acid were ACS grade (Fischer). Stock solutions of individual or mixtures of eicosanoids (Upjohn) were dissolved to a final concentration of 1 mg/ml in acetonitrile. A Drummond 10 ul Wiretrol was used to spot the TLC plates with 7.5-10 ul of each solution. Reverse-phase C_{18} plates (Whatman KC_{18}), 20 x 20 cm x 250 um, with a preadsorbant strip were used without prior activation. They were ascendingly developed in an unlined glass tank until the solvent had moved 0.5 cm from the top edge of the plate, dried, and developed again to the same distance. Development took approximately 75 minutes each time. The mobile phase consisted of 0.0025M phosphoric acid-acetonitrile (52:48, v/v). Addition of 0.2M NaCl to the mobile phase prevented disruption of the bonded layer by the water rich mobile phase as reported previously (16). Arachidonic acid and eicosanoids were visualized by spraying the plates with a saturated solution of phosphomolybdic acid in methanol.

RESULTS AND DISCUSSION

The resolution of arachidonic acid with its most common products by reverse-phase TLC is illustrated in Figure 1. As seen by the Rf values given in Table 1, there is separation of all the major biological metabolites. Although PGA_2 and PGB_2 comigrate; these are thought not to be formed enzymatically but by decomposition of PGE_2 in acid or alkaline environments respectively (12).

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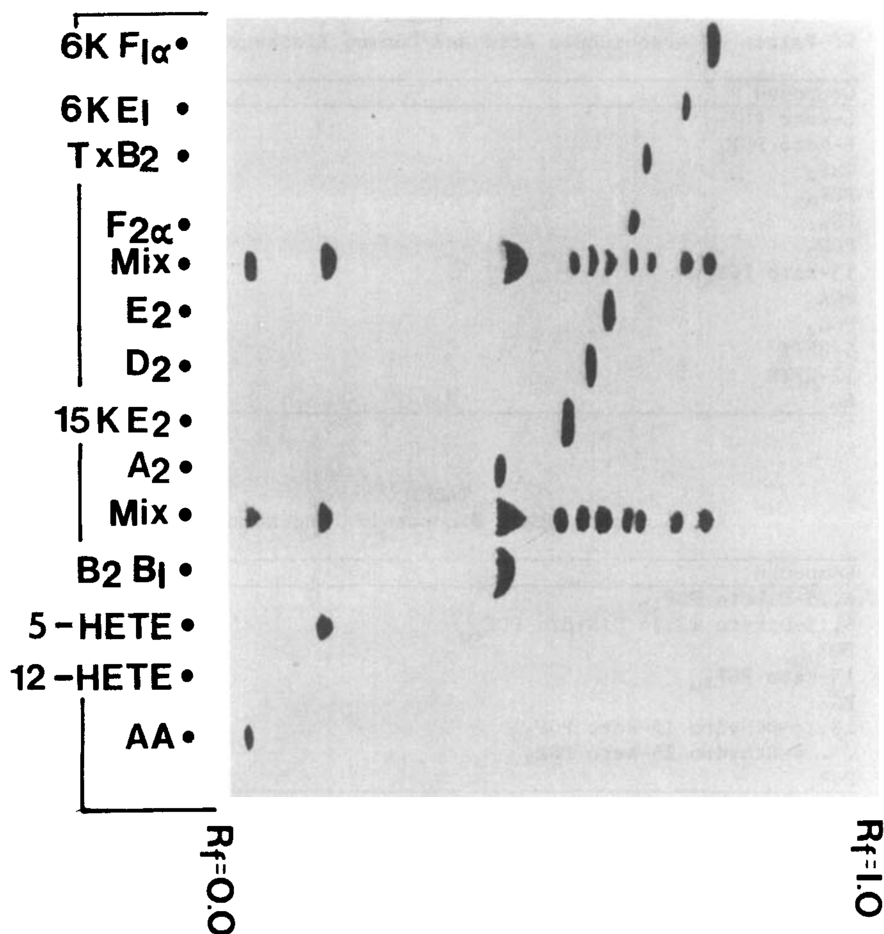

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FIGURE 1: Reverse-phase TLC plate illustrating the separation of arachidonic acid and major metabolites. Solvent system: 0.0025M phosphoric acid-acetonitrile (52:48, v/v) containing 0.2M NaCl.

Table 2 gives the Rf values of other eicosanoids and metabolites, mostly plasma metabolites.

Reverse-phase TLC also offers a degree of separation of prostaglandins which differ only in their degree of unsaturation as shown in Figure 2 and by the Rf values given in Tables 1 and 2. Separation of $PGF_{2\alpha}$ or PGE_2, with 2 alkene groups, from $PGF_{1\alpha}$ and PGE_1, respectively, containing a single carbon-carbon double bond,

TABLE 1
Rf Values of Arachidonic Acid and Common Eicosanoid Derivatives

Compound	Rf
6-keto $PGF_{1\alpha}$	.72
6-keto PGE_1	.68
TxB_2	.61
$PGF_{2\alpha}$	.59
PGE_2	.55
PGD_2	.53
15-keto PGE_2	.49
PGA_2	.43
PGB_2	.43
5-HETE	.16
12-HETE	.13
AA	.04

TABLE 2
Rf Values of Other Eicosanoids and Metabolites

Compound	Rf
6,15-Diketo $PGF_{1\alpha}$	.67
6,15-Diketo 13,14 Dihydro $PGF_{1\alpha}$	.65
$PGF_{1\alpha}$	.57
15-keto $PGF_{2\alpha}$	.54
PGE_1	.53
13,14-Dihydro 15-keto $PGF_{2\alpha}$	.50
13,14-Dihydro 15-keto PGE_2	.47
PGB_1	.43

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becomes important when TLC is used as a preparative step before radioimmunoassay. In the radioimmunoassay of prostaglandins, there is little cross-reactivity between prostaglandins having ring substituents, i.e. $PGF_{2\alpha}$ with PGE_2 or PGD_2, but a high cross-reactivity between prostaglandins having the same structure except for their number of double bonds (17). In contrast, in silica gel TLC, there is comigration of monoenoic and bisenoic prostaglandins, therefore iron or silver impregnation of the silica gel is used to overcome this (3,4).

The mobile phase, a one phase solution, in this reverse-phase TLC separation of arachidonic acid and 19 eicosanoids gives separation of all compounds except PGE_1 with PGD_2 and PGB_1, PGB_2 and PGA_2. Reverse-phase TLC is used in complex mixtures because

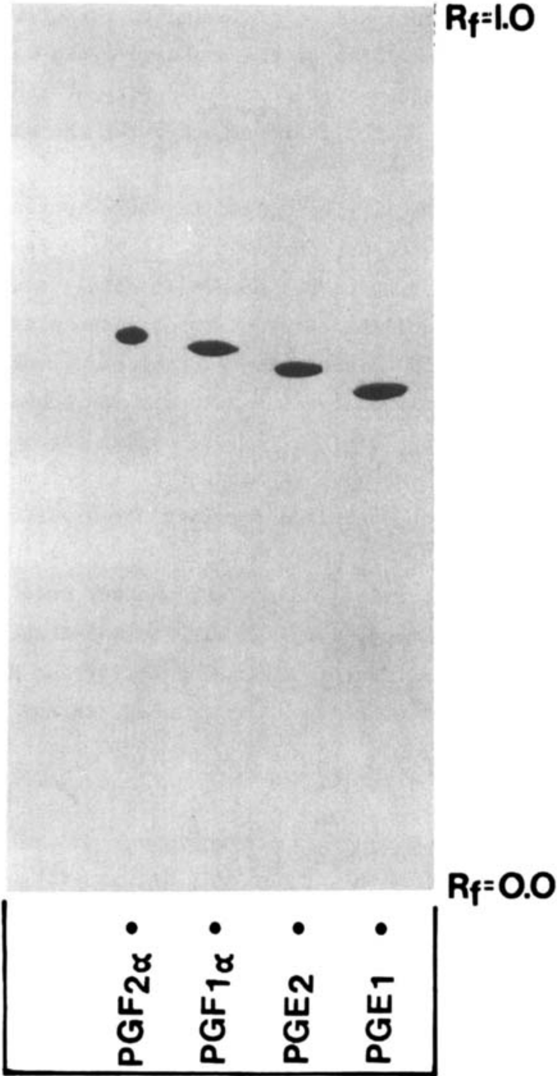

FIGURE 2: Migration of monoenoic versus bisenoic prostaglandins on reverse-phase TLC plate. Solvent system: 0.0025M phosphoric acid-acetonitrile (52:48, v/v) containing 0.2M NaCl.

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the mobile phase is a two solvent system that can easily be adjusted to achieve separation (16). Additionally, ion-pairing reagents or pH adjustments can be exploited to maximize separation. Ionic suppression of the carboxyl group was used in this mobile phase to increase the hydrophobicity of the eicosanoids, hence increasing their affinity for the stationary, C18 phase.

In contrast, the mobile phase used in normal phase TLC of eicosanoids can be the organic phase of a two phase system with the water content depending on the amount of acid, solvents, and temperature at which equilibration occurs or a one phase system with little or no water. Since water represents a weak solvent in reverse-phase TLC, little change in separation is seen in high humidity conditions; in sharp contrast to normal phase TLC (16). Therefore, activation of the plate by heating is not necessary in reverse-phase TLC; a step required for most normal TLC plates (12).

More importantly, reverse-phase TLC, whether used as a primary or secondary method, combined with normal-phase TLC, would further aid the confirmation of compound identity and help to avoid comigration of eicosanoids as experienced in the past with 6-keto $PGF_{1\alpha}$ and PGE_2 (13).

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