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Note

A rapid, sensitive method for detecting different arachidonic acid metabolites by thin-layer chromatography: the use of autoradiography

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Thin-layer chromatography (TLC) is widely used for the separation and identification of a number of arachidonic acid metabolites. There are descriptions of many TLC methods by which a variety of prostanoids can be separated [1—8]. Various spray reagents have been used for the detection of prostaglandins (PGs) following TLC separation. However, they require micro- or nanogram quantities of the compound to be detected [1, 4, 9—12].

Instead of spray reagents, direct scanning and autoradiography of labelled prostaglandins on TLC plates have been used, but these techniques have low sensitivity [13] or are time-consuming [14]. Thus large quantities of the isotope are necessary, especially with ^{3}H-labelled prostaglandins. For the detection of different arachidonic acid metabolites on TLC we have developed a rapid, sensitive autoradiographic method, in which 24 h is enough for visualization of a spot with an activity of 2400 dpm.

MATERIALS AND METHODS

LKC_{18} preadsorbent reversed-phase TLC plates were purchased from Whatman (Maidstone, U.K.), and were used without activation or a washing procedure. [^{3}H]PGE_2 (specific activity 160 Ci/mmol), 6-keto-[^{3}H]$PGF_{1\alpha}$ (specific activity 150 Ci/mmol) and [^{3}H]$PGF_{2\alpha}$ (specific activity 16.2 Ci/mmol) were from The Radiochemical Centre (Amersham, U.K.), and [^{3}H]-TXB_2 (thromboxane B_2, specific acitivity 100 Ci/mmol) was from New England Nuclear (Boston, MA, U.S.A.). Diethyl ether was from E. Merck (Darmstadt, F.R.G.), acetonitrile and chloroform were from Rathburn Chemicals (Walkerburn, U.K.). 2,5-Diphenyloxazole (PPO) was from Packard, (Downers

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Grove, IL, U.S.A.), and the film Kodak X-OMAT AR from Eastman-Kodak (Rochester, NY, U.S.A.).

The four arachidonic acid metabolites (activity of 8000 dpm) and their mixture were applied in 30 μl of chloroform onto the preadsorbent area with a micro-syringe using a brush-like downward stroke starting 3 mm below the reversed-phase/preadsorbent interface. The samples were dried under nitrogen and the plate was put in the rectangular chamber. The mobile phase was water—acetonitrile (70:30, v/v), and its pH was adjusted to 3.5 with 17 m*M* orthophosphoric acid phosphate buffer. The solvent system was 0.5 *M* in respect to sodium chloride. The amount of the mobile phase was chosen so that the solvent only wet the lowest 3 mm of the plate. For good saturation,

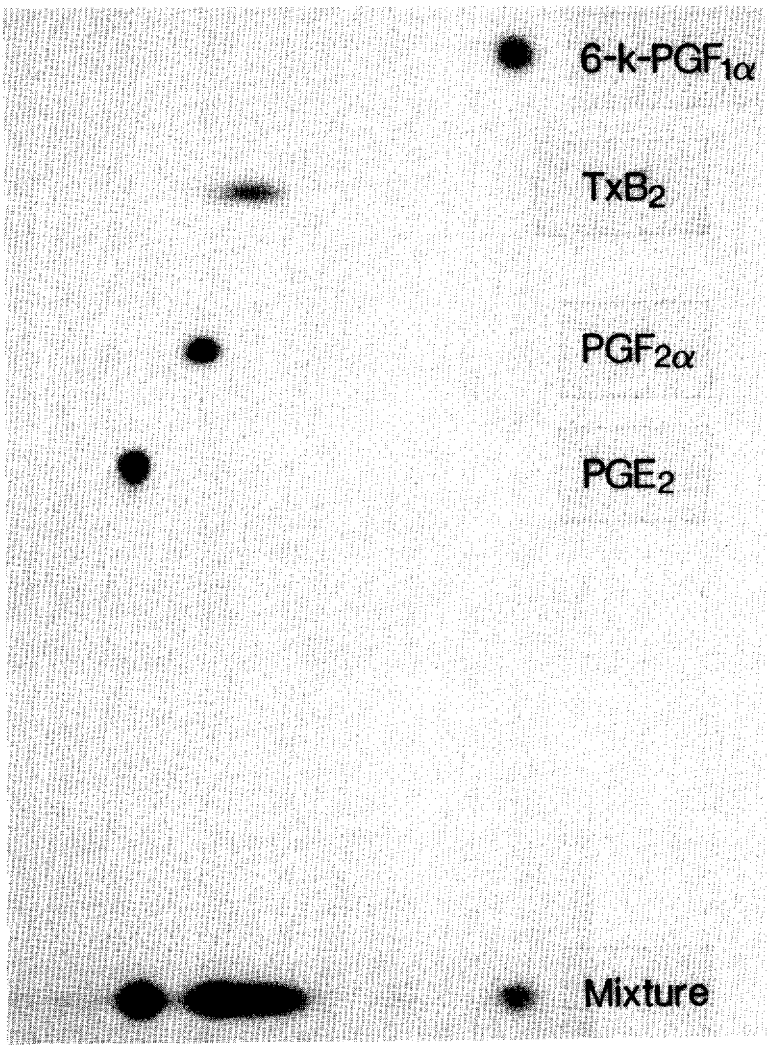

Fig. 1. The autoradiogram of 6-keto-[^{3}H]PGF$_{1\alpha}$, [^{3}H]PGF$_{2\alpha}$, [^{3}H]TXB$_2$ and [^{3}H]PGE$_2$. The radioactivity of each compound by application was 8000 dpm. The film was exposed for 24 h.

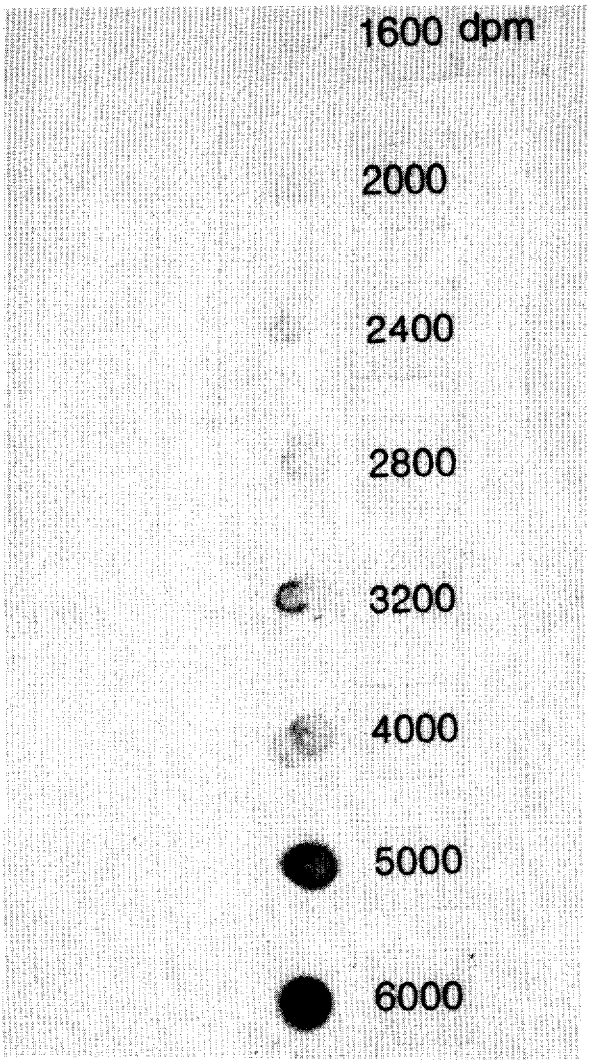

Fig. 2. The detection of spots with different activities of [^{3}H]PGE_2 in autoradiogram. The spot diameters were 10 mm. The figures represent the radioactivities applied. The film was exposed for 24 h.

TABLE I

R_F VALUES OF FOUR ARACHIDONIC ACID METABOLITES

Compound	R_F
PGE_2	0.13
$PGF_{2\alpha}$	0.19
TXB_2	0.21
6-keto-$PGF_{1\alpha}$	0.39

the walls of the chamber were lined with Whatman 3 MM filter paper. The mobile phase was used only once, and it was equilibrated 2 h before use. When the solvent front had migrated 15 cm from the application line, the plate was removed from the chamber and dried under a stream of air. Thereafter the autoradiography was performed. We also applied [^{3}H]PGE_2 samples of various activities directly onto the plate as spots, after which the autoradiography was performed. This was to test the real sensitivity of the method, independent of the different TLC techniques.

A solution of the scintillator (7% PPO in chloroform) was sprayed over the dry chromatogram (35 μl/cm^2), and the solvent was evaporated. All further operations were carried out in a dark room under proper lighting conditions. The dry chromatogram was placed in contact with Kodak X-OMAT AR film and kept between two glass plates. This package was wrapped in aluminium foil and stored at −70°C for 24 h. The film was developed in a Kodak RP X-OMAT M8 processor.

RESULTS AND DISCUSSION

A scintillator was added on the chromatogram to convert the very low energy of ^{3}H β-particles to light. The scintillation autoradiography together with the exposure of the film at a low temperature makes this method 100 times more efficient than the conventional one [15]. Fig. 1 shows the autoradiogram of four arachidonic acid metabolites. The mobilities of the compounds expressed as their R_F values are shown in Table I. The detection of spots with different activities of [^{3}H]PGE_2 are presented in Fig. 2. For clear visualization radioactivity of only 2400 dpm is needed when the scintillator has been dissolved in chloroform and sprayed onto the plate.

Tritium scintillator autoradiography has been used before [16—18], but we have applied it for the detection of different arachidonic acid metabolites. Since it is important to get a uniform distribution of the scintillator over the

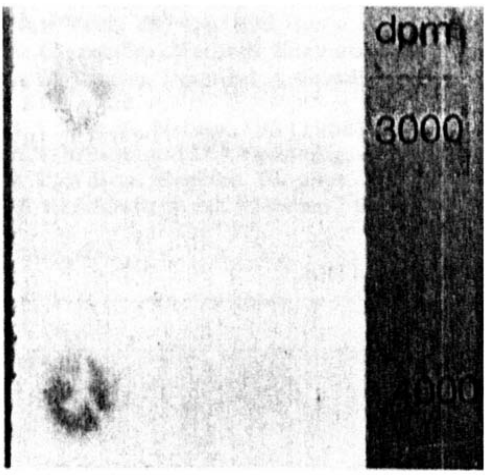

Fig. 3. Distribution of PPO on TLC plate when chloroform (on the left) or diethyl ether (on the right) was used. The figures represent the radioactivities applied.

desired chromatographic area [15, 19], a 7% solution of PPO in diethyl ether was used, and it was poured onto the chromatogram [8]. We have noted that dissolving the scintillator in chloroform and spraying the solution onto the chromatogram give a more uniform distribution of PPO and keep it on the desired area on the TLC plate (Fig. 3).

Thin-layer chromatography has been used for the identification of arachidonic acid metabolites, and nowadays, to an increasing extent, for their purification. The detection systems for prostaglandins on TLC have been so far insensitive and/or time-consuming. Here we present a rapid and sensitive alternative for analytical detection of the prostanoids.

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