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DIRECT QUANTITATIVE THIN-LAYER CHROMATOGRAPHIC DETERMINATION OF PROSTAGLANDINS A_2, B_2, E_2 AND $F_{2\alpha}$

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SUMMARY

A direct method for the determination of prostaglandins (PG) A_2, B_2, E_2 and $F_{2\alpha}$ by thin-layer chromatography (TLC) is described. The TLC development is carried out on silica gel 60 F_{254} plates so that PGA_2, PGB_2 and PGE_2 can be directly determined together by chromatogram spectrodensitometry using the reflection method.

PGE_2 must be converted into PGB_2 so that it can be measured at $\lambda_{max.}$ 280 nm, which is carried out by spraying with potassium hydroxide solution.

PGA_2 can be measured both directly at $\lambda_{max.}$ 224 nm and after conversion into PGB_2 by spraying with potassium hydroxide solution.

$PGF_{2\alpha}$ is measured after spraying with methanolic sulphuric acid and subsequent heating at $\lambda_{max.}$ 530 nm.

The method of determination described here is characterized by a short analysis time and good accuracy, and it is therefore suitable for quality control and stability investigations with these prostaglandins. The coefficient of variation is $\pm 3.5\%$.

INTRODUCTION

Numerous reports on the separation, stability and quantitative determination of prostaglandins (PG) have appeared in recent years[1–35]. In some papers[11–14], the methods described for the determination of PGA_2, PGB_2 and PGE_2 involve the elution of the compounds from the adsorbent after thin-layer chromatographic (TLC) separation. PGA_2 and PGE_2 were converted into PGB_2 with alkali in the eluates and determined spectrophotometrically in this form at 278 nm[1–6]. $PGF_{2\alpha}$ was usually determined gas chromatographically[11,12,14–24]. In this work, a rapid TLC method for the determination of PGA_2, PGB_2, PGE_2 and $PGF_{2\alpha}$ was developed for the quantitative determination of PG on the TLC plate by spectrophotodensitometry.

EXPERIMENTAL

Compounds investigated

The prostaglandins investigated were PGA_2 [(5Z,13E,15S)-15-hydroxy-9-oxoprosta-5,10,13-trien-1-oic acid], PGB_2 [(5Z,13E,15S)-15-hydroxy-9-oxoprosta-

5,8(12),13-trien-1-oic acid], PGE_2 [(5Z,11α,13E,15S)-11,15-dihydroxy-9-oxoprosta-5,13-dien-1-oicacid], $PGF_{2\alpha}$[(5Z,9α,11α,13E,15S)-9,11,15-trihydroxyprosta-5,13-dien-1-oic acid] and a mixture of 1 mg each of PGA_2, B_2 and E_2.

Quantitative determination

Preparation of the solutions investigated. The compounds and the mixture to be investigated are dissolved in ethanol–water (7:3) so that the concentration of the PG in the solution is 1 mg/ml for PGA_2, PGB_2 and PGE_2 and 5 mg/ml for $PGF_{2\alpha}$.

Thin-layer chromatography. Commercial silica gel 60 F_{254} TLC plates (Merck, Darmstadt, G.F.R.), size 20 × 20 cm and with a layer 0.25 mm thick, were used. The layer was divided into strips 1.5 cm wide. The solutions were applied in the form of spots using micropipettes*. For plotting the calibration graphs, 1, 2, 3, 4 and 5 μg each of PGE_2, PGA_2 and PGB_2 and 5, 10, 15 and 20 μg of $PGF_{2\alpha}$ were applied to each spot. After saturation of the chambers, the following solvent systems were used.

(A) For PGA_2, PGB_2 and PGE_2, the systems used were (1) diethyl ether–methanol–chloroform (65:15:20), the distance travelled by the solvent front being 2 × 15 cm** and the development time *ca.* 60 min; (2) chloroform–methanol (95:5), the distance travelled by the solvent front being 3 × 20 cm** and the development time *ca.* 90 min.

(B) For $PGF_{2\alpha}$, the system used was chloroform–dioxan–water–glacial acetic acid (35:60:2:3), the distance travelled by the solvent front being 20 cm and the development time *ca.* 45 min.

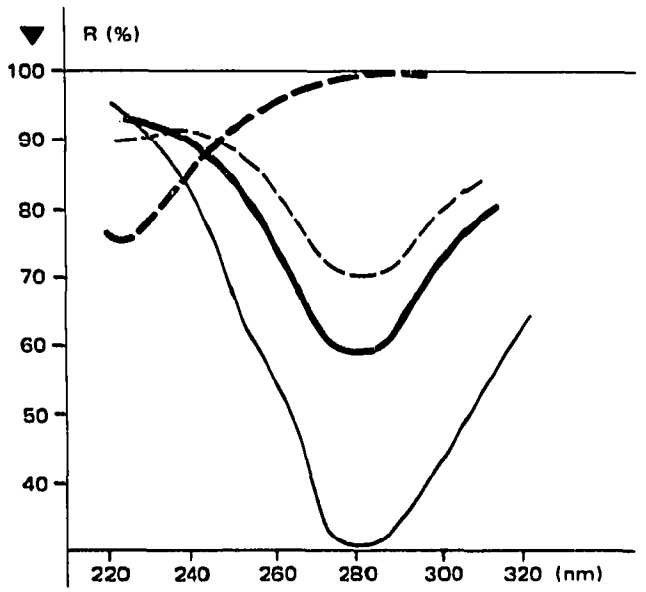


Fig. 1. Remission spectra: ▬ ▬ ▬, PGA_2 before spraying; ▬▬, PGA_2 after spraying; - - - PGE_2 after spraying; ———, PGB_2 before spraying.

* Automatic capillary dose pipettes, 1, 2 and 5 μl, supplied by Dr. Barrollier.

** Between each development, the plates were dried for 5 min in a stream of warm air.

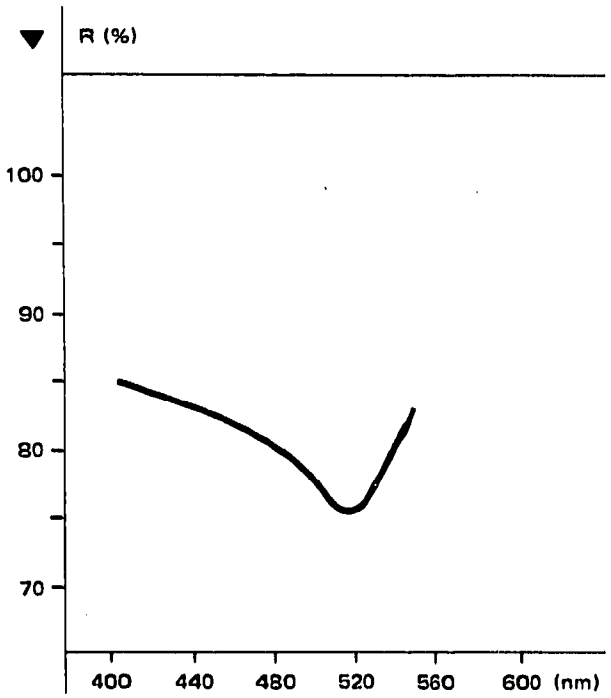


Fig. 2. Remission spectrum of $PGF_{2\alpha}$ after spraying.

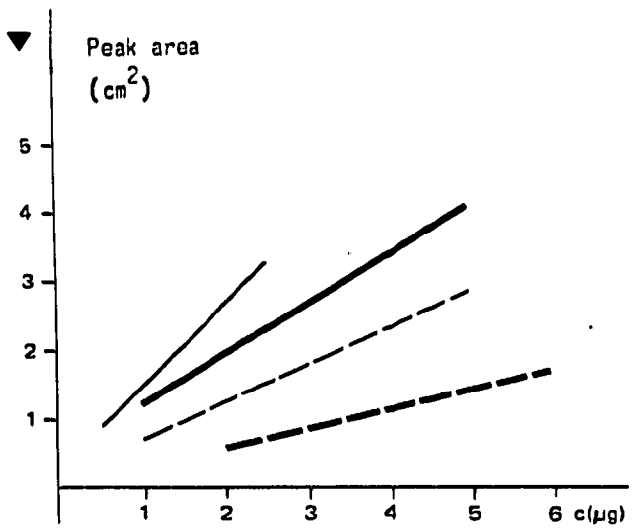


Fig. 3. Calibration lines: — — —, PGE_2 after spraying; ▬▬, PGA_2 after spraying; ———, PGB_2 before spraying; ▬ ▬ ▬, PGA_2 before spraying.

Detection of prostaglandins

PGE_2 and $PGF_{2\alpha}$ do not absorb UV light in the range available and are therefore not detected on a TLC plate under a UV lamp at 254 nm. For this reason, PGE_2 is converted into PGB_2, which is UV-active, using potassium hydroxide solution. $PGF_{2\alpha}$ is converted into a form that absorbs in the visible range by reaction with sulphuric acid. PGA_2 has a very low absorbance at 224 nm and becomes visible under a UV lamp at 254 nm only when amounts greater than 10 μg are present on the TLC plate. By reaction with potassium hydroxide solution, PGA_2 is also converted into PGB_2 and can therefore be detected in amounts less than 0.5 μg.

Reaction with potassium hydroxide on the TLC plate. The developed TLC plate is dried in a stream of warm air and then sprayed with 1% methanolic potassium hydroxide solution. The damp plate is heated in an oven for 15 min at 120°. Only when these conditions are strictly adhered to are PGE_2 and PGA_2 converted quantitatively into PGB_2.

Reaction with sulphuric acid on the TLC plate. The developed TLC plate is dried in a stream of warm air and then heated in an oven for 10 min at 120°. After cooling to room temperature, the TLC plate is sprayed with methanol–water–concentrated sulphuric acid (90:5:5), air-dried and heated for exactly 8 min in an oven at 110°, when $PGF_{2\alpha}$ appears as red spots on a white background.

Measurement and evaluation of reflection

The separated and sprayed or unsprayed PG spots are measured directly on the TLC plate with a PMQ II chromatogram spectrophotodensitometer by the reflec-

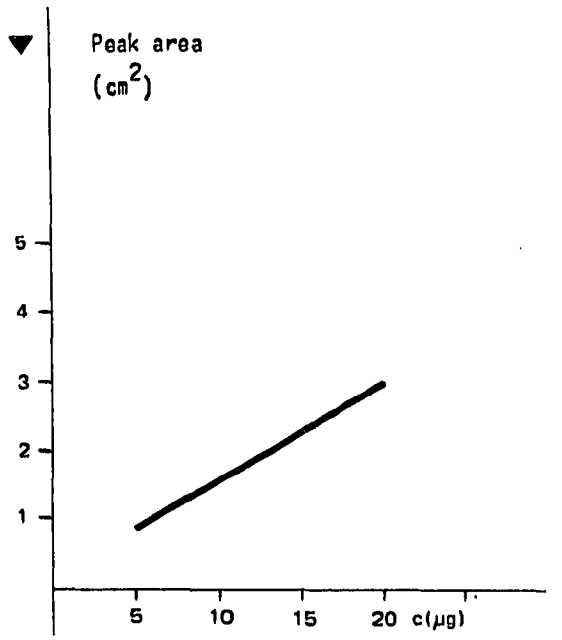


Fig. 4. Calibration line of $PGF_{2\alpha}$ after spraying.

tion method. The quantitative evaluation is carried out with reference to the degree of remission of a reference spot of known concentration according to the following equation:

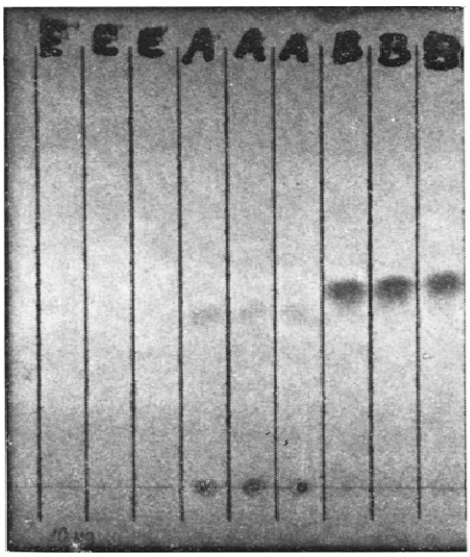
$$X = \frac{C_v \cdot R_p \cdot 100}{C_p \cdot R_v}$$

or

$$X = \frac{C_v \cdot F_p \cdot 100}{C_p \cdot F_v}$$

where

X = percentage of compound contained in the sample.
C_v = amount of reference compound applied (μg).
C_p = amount of test compound applied (μg) (calculated from the stated amount of the preparation).
R_v = relative degree of remission of the reference spot.
R_p = relative degree of remission of the test spot.
F_v = area of remission of the reference spot (cm²); area of remission = height × width, measured half way between the top and bottom.
F_p = area of remission of the test spot (cm²).
R = relative degree of remission (%) = $R_f/R_u \cdot 100$.
R_f = degree of remission of the compound spot.
R_u = degree of remission of the lower stratum of the plate.



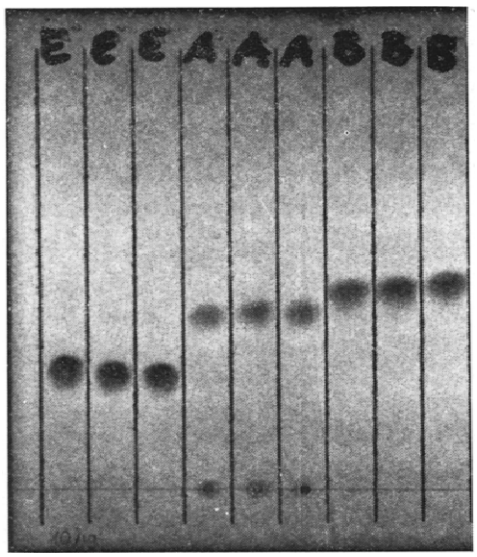


Fig. 5. Thin-layer chromatograms: unsprayed silica gel plate. E = PGE_2; A = PGA_2; B = PGB_2. Solvent: diethyl ether–methanol–chloroform (65:15:20). Amount applied: 10 μg per spot.

Fig. 6. Thin-layer chromatograms: silica gel plate sprayed with potassium hydroxide solution. E, A, B and conditions as in Fig. 5.

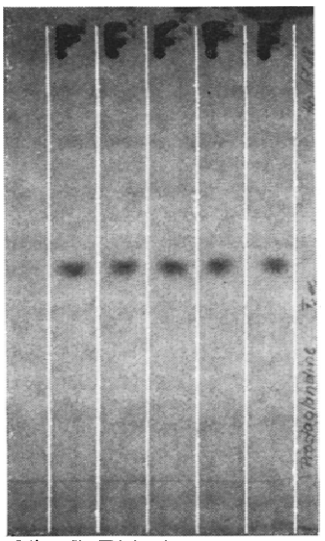


Fig. 7. Thin-layer chromatograms: silica gel plate sprayed with 5% methanolic sulphuric acid solution. F = $PGF_{2\alpha}$. Solvent: chloroform–dioxan–water–glacial acetic acid (35:60:2:3). Amount applied: 10 μg per spot.

TABLE I

REPRODUCIBILITY OF THE DIRECT QUANTITATIVE DETERMINATION CARRIED OUT WITH PURE PROSTAGLANDINS

Values from 11 experiments	*Plate**	*PGE_2*	*PGA_2*	*PGB_2*	*$PGF_{2\alpha}$*
Arithmetic mean (cm^2)	A	2.20	3.03		
	B		2.70	3.28	
	C				1.67
Standard deviation of single values (cm^2)	A	0.0675	0.046		
	B		0.134	0.104	
	C				0.032
Coefficient of variation (%)	A	3.0	1.5		
	B		5.0	3.17	
	C				1.9

* A, TLC plate sprayed with 1% methanolic KOH solution; B, unsprayed TLC plate; C, TLC plate sprayed with 5% methanolic H_2SO_4 solution.

TABLE II

QUANTITATIVE DETERMINATION OF PROSTAGLANDINS A_2, B_2 AND E_2 TOGETHER
Theoretical amounts present = 1 mg/ml in each case.

Values from 11 experiments	*Plate**	*PGA_2*	*PGB_2*	*PGE_2*
Arithmetic mean (mg)	A	1.05		0.983
	B	0.970	1.03	
Standard deviations of single values (mg)	A	0.0254		0.0335
	B	0.0467	0.0321	
Coefficient of variation (%)	A	2.32		3.40
	B	4.81	3.11	

* See Table I.

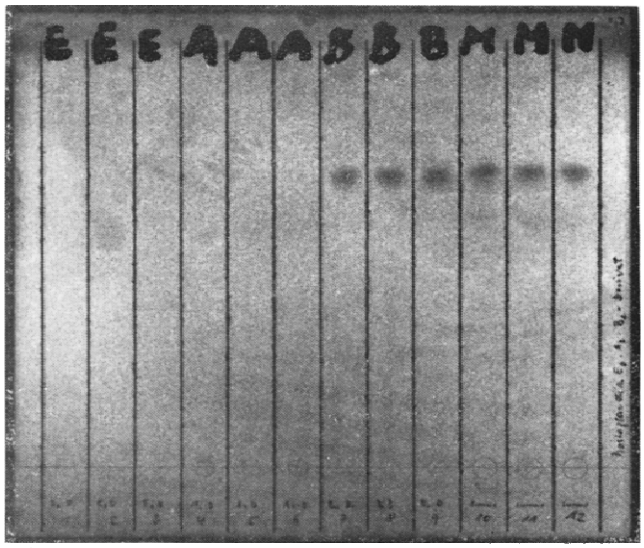


Fig. 8. Separation of PGA_2, PGB_2 and PGE_2 from a mixture; silica plate before spraying with potassium hydroxide solution. E, A and B = as in Fig. 5; M = mixture of 1 mg each of PGA_2, PGB_2 and PGE_2. Amount applied: 5 μg per spot.

RESULTS

Figs. 1–4 show the reflection spectra and the calibration lines of PGA_2 and PGB_2 measured before and PGA_2 and PGE_2 after treatment with potassium hydroxide solution, and of $PGF_{2\alpha}$ after treatment with sulphuric acid.

Figs. 5–7 show the thin-layer chromatograms of PGA_2, PGB_2, PGE_2 and $PGF_{2\alpha}$ before and after treatment with potassium hydroxide solution or sulphuric

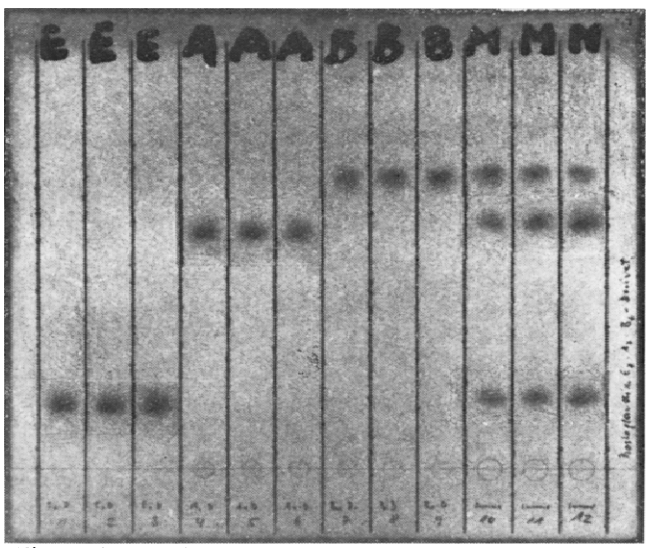


Fig. 9. Separation of PGA_2, PGB_2 and PGE_2 from a mixture; silica plate after spraying with potassium hydroxide solution. E, A, B and M as in Fig. 8. Amount applied: 5 μg per spot.

acid. It can be seen that PGA_2 and PGE_2 have been converted into PGB_2. The PGA_2 reference substance contains a slight impurity, which is separated well from PGA_2 and remains at the starting line on the TLC plate, as can be seen in Figs. 5, 6, 8 and 9. The impurity is visible both before and after spraying with potassium hydroxide solution with the same intensity under the UV lamp at 254 nm.

The determination of standard deviations and the coefficients of variation was carried out both on the pure substance and on the mixture. The results are summarized in Tables I and II.

The TLC separation of PGA_2 and PGB_2 together is difficult, as both compounds have the same hR_F values in numerous solvent systems. Figs. 8 and 9 show the separation of PGA_2, PGB_2 and PGE_2 from a mixture containing all three compounds, before and after treatment with potassium hydroxide solution. A suitable solvent is chloroform–methanol (95:5). The chromatograms were run three times over a distance of 20 cm.

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