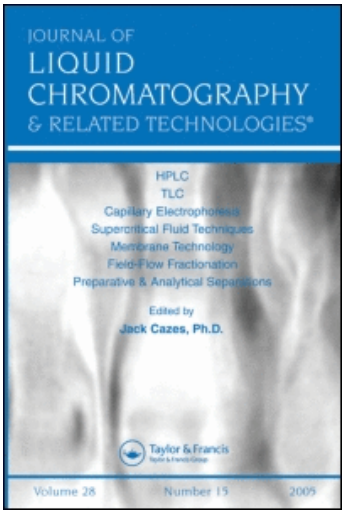

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Stability Indicating high Performance Liquid Chromatographic Procedure for the Analysis of Prostaglandin E_2 Raw Material and Tablets

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STABILITY INDICATING HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC PROCEDURE FOR THE ANALYSIS OF PROSTAGLANDIN E_2 RAW MATERIAL AND TABLETS

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ABSTRACT

A procedure is described for the quantitative analysis of prostaglandin E_2, its isomers and degradation products. The high performance liquid chromatographic method uses a chloroform/hexane mobile phase (70/30 v/v) mixture and a 250 x 4.6 mm, 5 micron cyano column, with testosterone as the internal standard. The time required for chromatography is approximately 15 minutes. The method gives a R.S.D. of 1.7% for the assay of the drug in tablets.

INTRODUCTION

Prostaglandin E_2 is a naturally occurring oxygenated arachidonic acid, first isolated from sheep seminal vesicle tissue. It is now obtained

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from total synthesis or by conversion of the PGA_2 methyl ester found in Plexaura homomalla, a soft coral (1).

The stability of prostaglandin E_2 in alcoholic solution has been studied. Roseman et al. (2) have reported a 20% degradation within 7 days at 25°C and pH 5.6. Monkhouse et al. (3) and Stehle et al. (4,5) studied the kinetic reaction rate for the conversion of PGE_2 to PGA_2 and PGB_2 as a function of pH and temperature. Epimerization of the hydroxyl group of $PGF_{2\alpha}$ under acidic conditions has been also reported (6).

Prostaglandins have been quantitatively assayed by TLC (7,8), gas chromatography coupled with an electron capture detector (9,10) or mass spectrometer (11-13), after derivatization, which increases detectability and stability. Unfortunately, the PGE series undergoes conversion to the PGB series under such conditions (9). Several HPLC procedures have been described including the reversed phase mode with UV detection, using end absorption at about 200 nm (14-17), and the forward mode with pre-column derivatization to allow detectability by either fluorescence (18-21) or UV at 254 mm (22-25). The procedure of Brown and Carpenter (23) was used in this study. However, complete resolution of the 5-trans isomers from PGE_2 was not achieved until Merritt and Bronson (26) overcame this difficulty using a silver-ion loaded cation exchange resin column.

This paper describes a forward phase HPLC procedure for the separation of prostaglandin E_2 isomers as well as the degradation products PGA_2 and PGB_2.

EXPERIMENTAL

Apparatus

A Spectra-physics (Santa Clara, CA) fully automated HPLC system comprising a model SP-8000 solvent delivery system, operated at 1.0 ml/min, an auto-sampler, an air-activated automatic loop injector (equipped with a 10 µl loop), a SP-8400 variable-wavelength detector (set at 254 nm) was used. Peak retention times, areas and heights were obtained with a model SP 4100 reporting integrator.

A 250 x 4.6 mm I.D., 5 μ, Ultrasphere-CN column (Beckman, Berkeley, CA) was used.

Materials

Prostaglandins A_2, B_2, E_2 and the isomers of E_2 were supplied by the Upjohn Company (Kalamazoo, MI). Testosterone was USP reference standard, α-bromo-2-acetonaphthone (Sigma Chemical Co., St-Louis, MO), N,N-diisopropyl ethylamine (Aldrich Chemical Co., Milwaukee, WI) was reagent grade, acetonitrile, methylene chloride, n-hexane, chloroform were HPLC grade (J.T. Baker Chemical Co., Phillipsburg, NJ) and the disposable extraction columns (cyano-bonded) (J.T. Baker Chemical Co., Phillipsburg, NJ) were conditioned with a 20% chloroform in n-hexane solution just before use.

Mobile Phase

Degassed chloroform and n-hexane were mixed using the solvent delivery system in a proportion of 70:30 v/v.

Derivatizing Reagents

α-Bromo-2-acetonaphthone solution in acetonitrile was prepared at a concentration of 20 mg per ml.

N,N-Diisopropyl ethylamine was diluted in acetonitrile (1 in 100).

Internal standard solution:

A solution of testosterone in chloroform was prepared at a concentration of 100 μg/ml.

Standard Preparation:

A solution of PGE_2 in acetonitrile was prepared at a concentration of 100 μg/ml.

Sample Preparation

Raw materials:

A portion of about 10 mg, accurately weighed, of prostaglandin E_2 raw materials was dissolved in 100 ml of acetonitrile.

Tablets:

To an amount of powdered tablets equivalent to one tablet (500 µg of PGE_2), accurately weighed into a 15 ml centrifuge tube with a teflon lined screw cap, were added 3.0 ml of citrate buffer solution (0.5 M, pH 4.0) and 5.0 ml of methylene chloride. The tube was shaken for 30 minutes (vortex mixer) and then was centrifuged. The top layer (aqueous) was discarded.

Recovery study:

Sufficient inert materials (4.31 g of lactose, 0.51 g of corn starch, 0.2 g of povidone, 0.03 g of calcium stearate) to make a total weight of 5.0 g (after drying) were added to 10 ml of a solution of PGE_2 in methanol (1 mg/ml). This mixture was shaken to homogenize it, then dried at room temperature using reduced pressure.

A 250 mg portion of this synthetic preparation, equivalent to one tablet containing 500 µg of PGE_2, was treated as under 'Tablets' in 'Sample preparation'.

Derivatization:

One ml aliquots of standard preparation and sample preparation were transferred into separate 10 ml screw-capped test tubes, and were then evaporated to dryness with the aid of a gentle stream of nitrogen and low heat (<40°C). To each tube was added 160 µl of α-bromo-2'-acetonaphthone solution and 120 µl of N,N-diisopropyl ethylamine solution. The vials were then swirled to dissolve the residue, capped and heated at 30-35°C for 30 minutes, after which the solutions were evaporated to dryness.

Column purification:

The dried residue from the derivatization step was transferred to a conditioned 1 ml disposable extraction column, using 5 x 1 ml of 20% v/v chloroform in n-hexane solution. The column was then washed with a 15 ml portion of a 10% v/v chloroform in n-hexane solution. The prostaglandin derivatives were eluted using chloroform and collected in a 10 ml volumetric flask containing 1.0 ml of internal standard solution; the flask was made up to volume with chloroform passed through the column.

Procedure:

Aliquots (10 µl) of standard preparation and sample preparation were successively injected into the chromatograph. Peak areas and peak heights were measured. The quantity of PGE_2 in the sample preparation was calculated using the following formula:

$$C_u = C_s \times \frac{R_u}{R_s}$$

where:

C_u = concentration of PGE_2 in sample preparation (µg/ml)

C_s = concentration of PGE_2 in standard preparation (µg/ml)

R_u = peak height ratio of PGE_2 to internal standard from the sample preparation

R_s = peak height ratio of PGE_2 to internal standard from the standard preparation,

The relative abundance of any isomers and degradation products was calculated as follows:

$$\% \text{ of } X = \frac{A_x}{A_{E2}} \times 100$$

where:

% of X = relative abundance of isomer "x" in sample preparation

A_x = area of x in sample preparation

A_{E2} = area of PGE_2 in sample preparation

RESULTS AND DISCUSSION

Complete resolution was obtained between the derivatives of PGE_2 and its isomers, as well as from degradation products PGA_2 and PGB_2 (Fig. 1, Table 1). The separation of the derivatives of PGE_2 and its closely related 5-trans isomers was successfully achieved directly without the need of a silver-ion loaded column (26) which had been previously used to increase cis/trans - double bond differentiation. However, the two degradation products resulting from dehydration of PGE_2 (PGA_2 and PGB_2) were not separated from residual reagent (Fig. 2). In order to quantify these potential degradation products, the column purification procedure was developed. The choice of solvent for this cleaning procedure was governed by the necessity of removing all residual reagent without eluting

TABLE 1

Chromatographic Characteristics of Compounds of Interest.

	Retention time	K'	R^+
T_o :	2.62	-	-
Solvent :	2.80	-	1.16
PGA_2 and PGB_2 :	3.32	.27	2.38
Testosterone :	4.32	.65	7.21
15-epi PGE_2 :	7.78	1.97	1.25
Unknown* :	8.38	2.20	4.19
11-epi PGE_2 :	10.64	3.06	1.03
8-iso PGE_2 :	11.28	3.31	2.39
5-trans PGE_2 :	12.86	3.91	1.50
PGE_2 :	14.18	4.41	

* Found in 15-epi PGE_2 reference substance.

$^+$ Resolution factor from following peak.

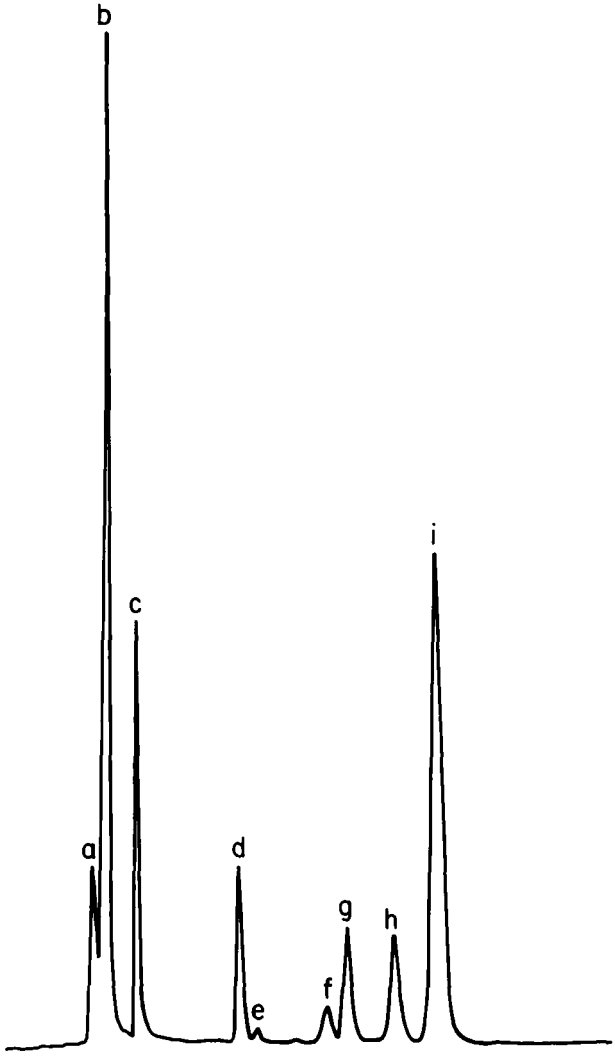

FIGURE 1: Chromatogram of prostaglandin mixture with internal standard. Peak: a = solvent; b = PGA_2 + PGB_2; c = testosterone (internal standard); d = 15 - epi - PGE_2; e = unknown; f = 11 - epi - PGE_2; g = 8 - iso- PGE_2; h = 5 - trans PGE_2; i = PGE_2.

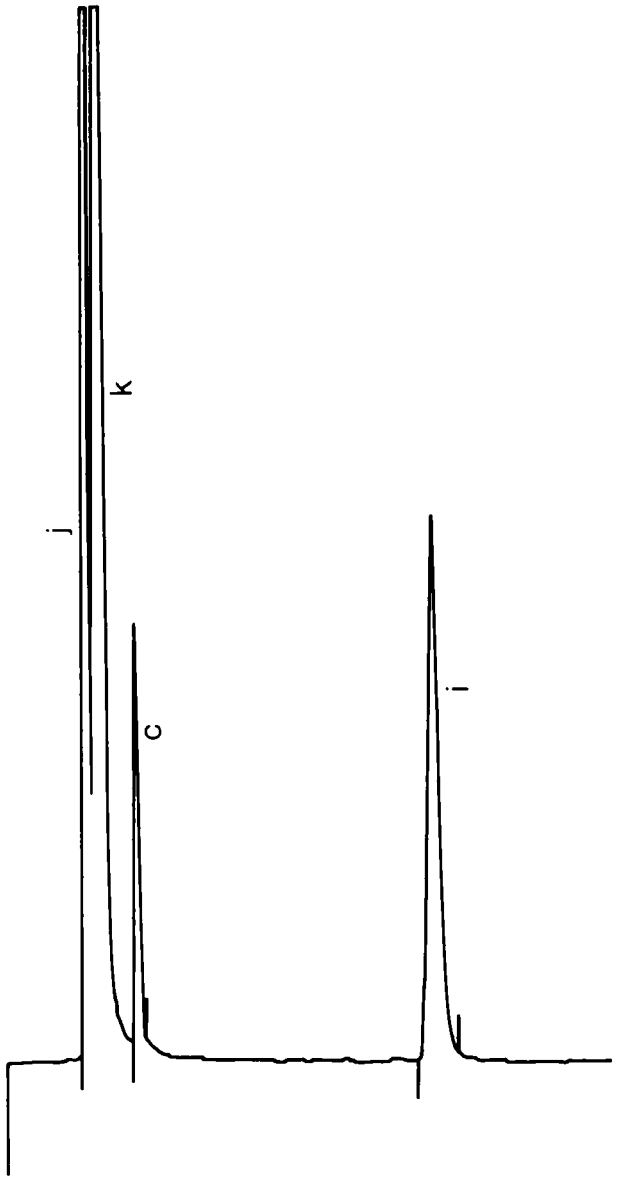

FIGURE 2: Analysis of a commercial formulation.

A) Before column purification, B) After column purification

Peak: a = solvent; b = PGA_2 + PGB_2; c = testosterone (internal standard); i = PGE_2; J and K = residual reagents.

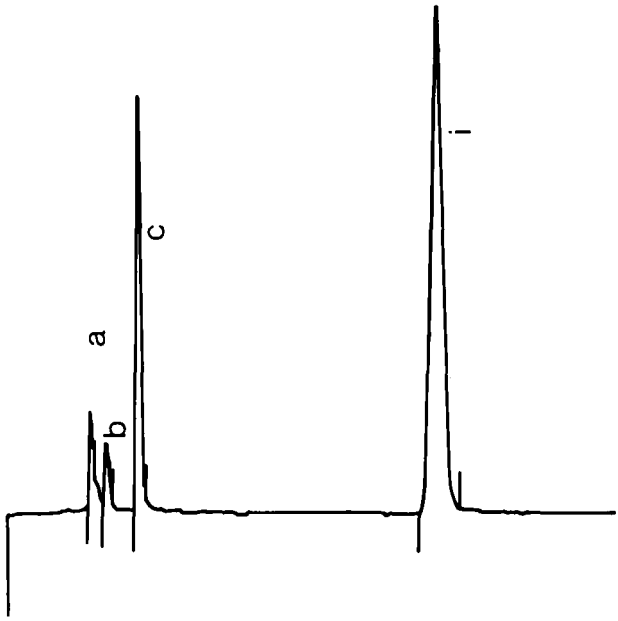

FIGURE 2B

any of the prostaglandin analogs, before subsequently effecting their total recovery from the column. It was found that the derivatizing reagent was completely eluted with 15 ml of 10% chloroform in n-hexane; unfortunately, PGE_2 was not completely transferred to the column using this solvent. Therefore, 5 x 1 ml of 20% chloroform in n-hexane were used to quantitatively transfer samples to the column. PGA_2 and PGB_2 were not eluted from the column before 15 ml of this latter solvent were eluted. All prostaglandins were quantitatively recovered within 10 ml of chloroform. Chromatograms of sample preparation before and after column purification are presented in Fig. 2.

Limits of detection of PGE_2, PGE_2-isomers and degradation products were found to be about 1 ng (i.e. 1% of PGE, in assay preparation).

Either peak height or peak area could be used to calculate PGE_2 content, as it was compared to a reference standard solution. Relative abundance of PGE_2 analogs and degradation products could be estimated by

TABLE 2

Standard curves for PGE_2 within concentration range of 20 to 120 ng injected.

Measurement mode	No. of Preparations	Curve y = mx + b	Correlation coefficient
Peak height ratio	6	0.0129 x -.007	.9996
Peak area ratio	6	0.0372 x -.089	.9997

TABLE 3

Assay of Commercial Tablets[a]

Prostaglandin	label claim %	RSD %
PGE_2	97.7	1.7
PGA_2 + PGB_2	2.2	3.5
5-trans PGE_2	≃ limits of detection (1%)	-
other isomers	not detected	

[a] Average of 8 determinations.

using the ratio of their peak area to that of PGE_2. Since the U.V. chromophore in each derivative is the naphthacyl group, all impurities should have similar absorptivity, except PGA_2 and PGB_2, where their intrinsic chromophore was found to add less than 10% when measured at 254 nm in mobile phase (70% chloroform in n-hexane).

Linearity of response versus concentration was studied on 6 preparations within a concentration range of 20 to 120 ng for both peak

height and peak area (Table 2). Each mode gave a linear standard curve in the concentration range studied. Their correlation coefficients were nearly ideal ($\geq$ .9996).

Completeness of extraction was assessed by a second extraction of the aqueous layer with 5 ml of methylene chloride. This second extract, when treated in the same way as the first, did not show any residual PGE_2.

The accuracy of the procedure was assessed by means of a synthetic mixture analysis. Its assay value was 100.2% as an average of 6 determinations and a relative standard deviation (RSD) of 0.4%, showing excellent reproducibility.

The quantitative analysis of one commercial tablet formulation is presented in Table 3. PGE_2 content is within regulatory limits (90-110%) and reproducibility is good (RSD 1.7%). Degradation products resulting from dehydration (PGA_2 + PGB_2) were found at a level of 2.2%, based on the PGE_2 content.

CONCLUSION

This HPLC procedure, using derivatization and column purification, is fast and accurate. It has been specially designed for single dosage form analysis.

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