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Note

Simple and rapid separation of certain prostaglandins by reversed-phase high-performance liquid chromatography

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Prostaglandins (PGs) are diversely oxygenated eicosanoic acids with various physiological, pathological and pharmacological significance based on their biochemical responses. The simultaneous and quantitative determination of PGs in biological fluids is an important problem. Various methods have been developed for the determination of micro-amounts of these active but deteriorating compounds in biological samples[1]. Thin-layer chromatography[2], fluorimetry[3], gas–liquid chromatography (GC)[4,5], mass spectrometry (MS)[6] and gas chromatography–mass spectrometry (GC–MS)[7–14] have been used, and biochemical methods such as radioimmunoassay (RIA)[15] and enzyme assay[4,16], with a few bioassays by human platelet aggregation[9] and a smooth muscle response[17], are also useful. Of the biological methods, RIA has been most frequently applied in clinical investigations in recent years, but it is time consuming and has some limitations. One of the problems is the specificity and affinity of the antibody used in the RIA, as it is extremely difficult to obtain the antibody with little cross-reactivity and relatively high affinity. In addition, procedures for avoiding interferences from substances other than PGs have to be incorporated in the assay, which may also lead to a loss or alteration of PGs.

GC–MS has been shown to be the most reliable technique for the quantification of PGs. However, there are difficulties in accurate quantitative analyses, *e.g.*, complex pre-treatments and derivatization may be necessary, accompanied by changes in the PGs, in electron impact MS combined with GC[7–13] and also in ammonia chemical ionization MS combined with GC[14].

A rapid and efficient method for the isolation and purification of PGs is often required in various biological and clinical studies, and also a highly accurate analysis of many closely related PGs may be necessary. High-performance liquid chromatography (HPLC) has been increasingly applied to give separations of a series of PGs in high yields for this purpose. Fitzpatrick[18] utilized HPLC for the separation and analysis of PGs by using their *p*-nitrophenacyl esters. The preparation of the appropriate derivatives should throw light on the problem of the poorer sensitivity in the

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detection of these compounds using a fixed wavelength of 254 nm, with the drawbacks of the long time required and the decrease in the precision of the analysis caused by the derivatization. HPLC methods using silicic acid columns[19,20] and conventional reversed-phase chromatography[4,5] have not proved adequate for PG analysis because of the poor resolution and the long retention times, with unsatisfactory sample recovery.

Recently, the usefulness of an HPLC method using a reversed-phase column in combination with ordinary adsorption chromatography has been reported for the isolation of several PGs from a biological matrix[21]. However, this method required the inconvenient use of liquid scintillation spectrometry, and retention times were excessively long (about 60 min).

We describe here a simple, rapid and convenient method for the separation of prostaglandins such as 6K-$PGF_{1\alpha}$, $PFE_{2\alpha}$, PGE_2, PGE_1, PGA_2 (or PGB_2) and PGA_1 (or PGB_1) using reversed-phase HPLC.

EXPERIMENTAL

Apparatus

HPLC was carried out using an ATTO Corp. (Tokyo, Japan) solvent delivery system (Model HSLC-013-4) and a syringe-loaded loop injection valve (Model 7120) with an internal volume of 100 μl (Rheodyne, CA, U.S.A.). A column (25 cm × 4.6 mm I.D.) packed with Nucleosil 5 C_{18} (Merck, Darmstadt, G.F.R.) was used for reversed-phase chromatography. An ATTO-LDC Spectromonitor III was used to measure the absorbance at 208 nm. Chromatograms were recorded on a Rikaken R-21 recorder with a 10-mV span set at a chart speed of 2.0 cm/min. The solvent system used for elution was water–acetonitrile–tetrahydrofuran (70:30:2).

Reagents and materials

Acetonitrile and tetrahydrofuran used for the chromatography were purchased from Kanto Chemical (Tokyo, Japan). Water used for the chromatography was prepared by glass distillation and filtration with a TM-2 membrane filter (0.45 μm) (Tokyo Roshi, Tokyo, Japan). Standard samples of PGs were kindly supplied by Ono Pharmaceutical Co. (Osaka, Japan),to whom our thanks are due. All other chemicals used were special-grade materials.

Procedure

A solution of each sample of prostaglandins 6K-$PG_{1\alpha}$, $PGF_{2\alpha}$, PGE_2, PGE_1, PGA_2, PGA_1, PGB_2 and PGB_1 as free acids in water–acetonitrile–tetrahydrofuran (70:30:2) was injected and eluted witht he same solvent system as above. The flow-rate was 1 ml/min under a pressure of 80 kg/cm^2 and the collected fractions were monitored with a UV detector at 208 nm (0.1–0.005 a.u.f.s.). Each compound separated was identified by GC–MS.

RESULTS AND DISCUSSION

The chromatographic peaks for 6K-$PGF_{1\alpha}$, $PGF_{2\alpha}$, PGE_2 and PGE_1 occur at 2 min, 3 min 30 sec, 4 min and 4 min 40 sec, respectively, under the conditions

described above. A peak for a mixture of PGA_2 and PGB_2 and a peak for a mixture of PGA_1 and PGB_1 appear at 7 min 20 sec and 9 min 30 sec, respectively (Fig. 1). The retention times of PGA_2, PGB_2, PGA_1 and PGB_1 could be different from each other when injected separately. However, good resolutions of PGA_2 and PGB_2 and of PGA_1 and PGB_1 were not achieved with a mixture of all four. The identification of each PG separated was confirmed by GC–MS as usual[11,12]. The UV monitoring wavelength of 208 nm provided the best compromise between maximum sensitivity for each PG, efficiency of the detector and interferences from the solvents. PGs at levels of a few nanograms were detected by using this wavelength at 0.005 a.u.f.s.

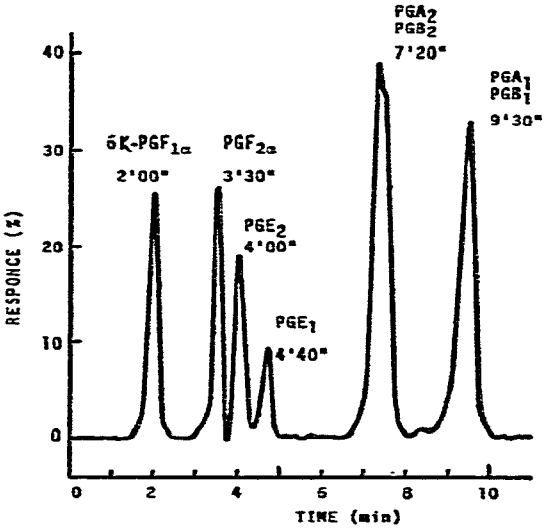

Fig. 1. Chromatogram produced by HPLC of prostaglandins (350 ng of each compound injected) as free acids on a reversed-phase column. Eluent, water–acetonitrile–tetrahydrofuran (70:30:2); pressure, 80 kg/cm²; flow-rate, 1 ml/min; column, Nucleosil 5 C_{18} (25 cm × 4.6 mm I.D.); detection, UV, 208 nm (0.05 a.u.f.s.).

The proposed HPLC method thus seems useful for the separation of a large number of prostaglandins, including thromboxane B_2 (equivalent to thromboxane A_2) and leukotrienes, under neutral and mild conditions, in place of an alkaline and inadequate method[22]. A further study on the separation of PGA_2, PGB_2, PGA_1 and PGB_1 is in progress.

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