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Reversed-phase high-performance liquid chromatography for simultaneous determination of prostaglandins E_2, A_2 and B_2

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Abstract

A simultaneous determination of prostaglandin E_2, A_2 and B_2 for stability studies of PGE_2 in solution has been developed by reversed-phase high-performance liquid chromatography using a 3 μm C_{18} column. The mobile phase consisted of 35% acetonitrile in 0.002 M phosphate buffer (pH 3.5) at a flow rate of 1.5 ml/min. Quantitative measurement was performed at 192 nm. The method has been applied to primary kinetic studies on the main degradation reaction profile for $PGE_2 \rightarrow PGA_2 \rightarrow PGB_2$ at 60°C in pH 2.0, 7.2, and 10.0 buffer solutions and confirmed the mole percent kinetics of PGE_2, PGA_2 and PGB_2 for over 150 h.

Key words: Prostaglandin E_2; Prostaglandin A_2; Prostaglandin B_2; Stability kinetics; HPLC

Prostaglandin E_2 (PGE_2) is known as a potential therapeutic agent in the treatment of many arteriosclerotic disease (Curtis-Prior, 1988), but is very unstable in aqueous solution (Hageman, 1986).

PGE_2 readily undergoes dehydration in acidic and alkaline aqueous solution to yield the unsaturated prostaglandin A_2 (PGA_2) which further isomerizes to prostaglandin B_2 (PGB_2) under alkaline conditions (Monkhouse et al., 1973; Stehle, 1982). However, the simultaneous separation of these three prostaglandins has not been fully achieved by HPLC, since PGE_2 is significantly more polar than PGA_2 and PGB_2 and because prostaglandins A_2 and B_2 are very similar structurally and have similar polarities (Inayama et al., 1980).

Current assays for the determination of prostaglandins A_2 and B_2 are highly dependent upon the individual assay of PGA_2 and PGB_2 before and after alkaline treatment to covert PGA_2 to PGB_2 using the molar absorptivity of the respective λ_{max} by UV-visible spectrophotometry (Monkhouse et al., 1973; Hirayama et al., 1986). High-performance liquid chromatographic (HPLC) studies including ion-exchange utilizing cyclodextrin complexation (Uekama et al., 1977) using different detection wavelengths and derivatizing HPLC for fluorescence detection (Salari et

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al., 1987) and UV detection (Fitzpatrick et al., 1977) have been applied for the simultaneous determination of PGA_2 and PGB_2. Recently, a simple isocratic separation method for PGE_1, PGA_1 and PGB_1 using reversed-phase HPLC was reported (Lee and DeLuca, 1991).

The instability of PGE_2 and difficulties in the simultaneous determination of degradation products have severely hampered kinetic studies on their stability and the development of new formulations (Eriksson et al., 1988; Siegenthaler, 1989; Watkinson et al., 1991) and have therefore proved to present a challenge to investigators for understanding such persistent stability problems and analysis.

The purpose of the present work was to develop a simple and simultaneous separation method for PGE_2, PGA_2 and PGB_2 for primary stability kinetic studies of PGE_2 in solution.

All prostaglandins (PGE_2, PGA_2, PGB_2, PGE_1, PGA_1 and PGB_1) were purchased from Sigma (St. Louis, U.S.A.). Acetonitrile and methanol were HPLC grade from Fisher (Pittsburgh, U.S.A.) and all other materials were of reagent grade.

An HPLC system consisting of a model 501 pump, U6K injector, 484 turnable UV detector (Waters, U.S.A.) and CR 601 integrator (Shimadzu, Japan) was operated at ambient temperature. An LC-18 column (3 μm, 4.6 mm $\times$ 15 cm; Supelco, U.S.A.) was used with a mobile phase of 35% acetonitrile in 0.002 M phosphate buffer (pH 3.5). The flow rate and injection volume were 1.5 ml/min and 5 μl, respectively. Stock solutions of each prostaglandin (PGE_2, PGA_2, PGB_2, PGE_1, PGA_1 and PGB_1) were prepared at a concentration of 5 mg/ml in methanol and stored in nitrogen gas-filled vials at 4°C. The mixed working solution of six prostaglandins was prepared by mixing equal volumes of the respective stock solution and diluted in methanol to make 20 μg/ml of each prostaglandins. Chromatograms were recorded at a fixed wavelength of 192 nm and quantitations of PGE_2, PGA_2 and PGB_2 were calculated from the chromatograms by measurement of the corresponding peak area.

Studies on stability kinetics were performed with a PGE_2 solution of 50 μg/ml at different pH values prepared by placing 0.1 ml of 0.5 mg/ml PGE_2 methanol solution and 0.9 ml of the respective buffer solution into screw-capped vials. Buffers used were 0.05 M HCl/KCl (pH 2.0), 0.002 M NaH_2PO_4/NaOH (pH 7.2) and 0.002 M $NaHCO_3$/NaOH (pH 10.0). At specific time intervals in a 60°C water bath, each vial was removed, cooled to room temperature, 5 μl were injected into HPLC without any modification and then the vial was returned to the water bath.

A cosiderable body of data on the stability kinetics of PGE_2 have been monitored spectrophotometrically by measuring the PGE_2 concentration only and/or the decreased and increased absorbance of PGA_2 and of PGB_2 using their molar absorptivity at the respective maximum wavelengths before and after alkali treatment (Monkhouse et al., 1973; Cho et al., 1977; Stehle, 1982) and recently HPLC has been adopted (Hirayama et al., 1986). Nevertheless, both methods involve problems as mentioned above.

A simultaneous separation of the six prostaglandins, i.e., PGE_1, PGE_2, PGA_1, PGA_2, PGB_1 and PGB_2, was achieved using a 3 μm C_{18} column under the conditions described above and the HPLC chromatogram measured at a fixed wavelength of 192 nm is shown in Fig. 1. The chromatographic system developed here is based on the system described by Lee and DeLuca (1991) for PGE_1, PGA_1 and PGB_1. The standard calibration curves were constructed using the mixed standard solution of PGE_2, PGA_2, PGB_2. The correlation of peak area ratio with the concentration of PGE_2, PGA_2 and PGB_2 was linear in the range 1–60 μg/ml. The correlation coefficients were better than 0.999.

With the method developed, the changes in mole percent of PGE_2, PGA_2 and PGB_2 with time were plotted for the main degradation reaction profiles of PGE_2 at 60°C in different pH 2.0, 7.2 and 10.0 buffers, respectively, as shown in Fig. 2. Fig. 3 shows the changes in total mole percent of PGE_2, PGA_2 and PGB_2 with time in the same buffers.

At pH 2.0, the main degradation product was only PGA_2, the conversion ratio from PGE_2 to

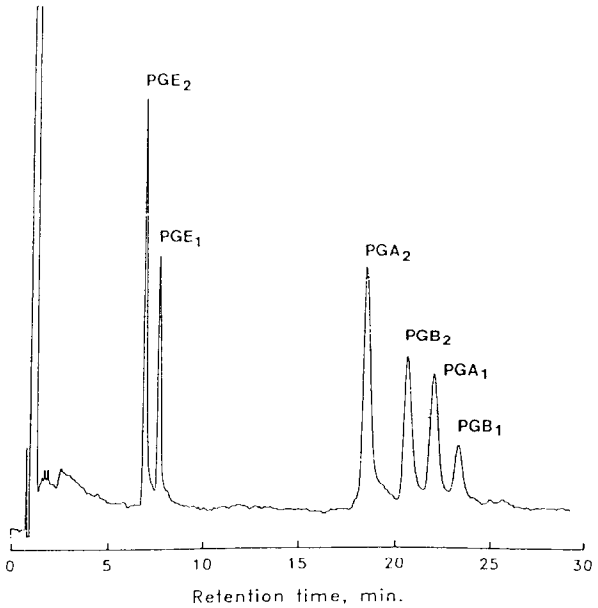

Fig. 1. HPLC chromatograms of prostaglandins measured at 192 nm. Concentrations: 100 ng each of PGE_1, PGE_2, PGA_1, PGA_2, PGB_1 and PGB_2 in 5 μl; attenuation, 3.

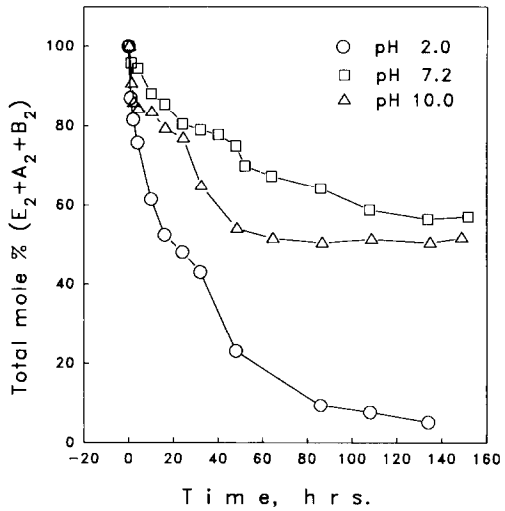

Fig. 3. Total mole percent of PGE_2, PGA_2 and PGB_2 as a function of time under different pH conditions and at 60°C. Initial concentration of PGE_2, 50 μg/ml.

PGA_2 was negligible in comparison with those under alkaline conditions and the total mole percent of PGE_2 and PGA_2 markedly decreased. Under alkaline conditions, the reaction rates of dehydration and isomerization became more rapid with increasing pH values, as described previously (Monkhouse et al., 1973; Hirayama et al., 1986). In contrast, the total mole percent of PGE_2, PGA_2 and PGB_2 under alkaline conditions was not maintained at approx. 100% of the initial PGE_2 during the experimental period as reported in previous papers (Monkhouse et al., 1973; Uekama et al., 1977; Stehle, 1982), but decreased to fall within the range of about 50–60% of the initial PGE_2 as shown in Fig. 3. Therefore, it is assumed that the major final degradation product of PGE_2 under alkaline conditions is PGB_2 with a conversion ratio of about 50% of the initial PGE_2, the remaining 50% of

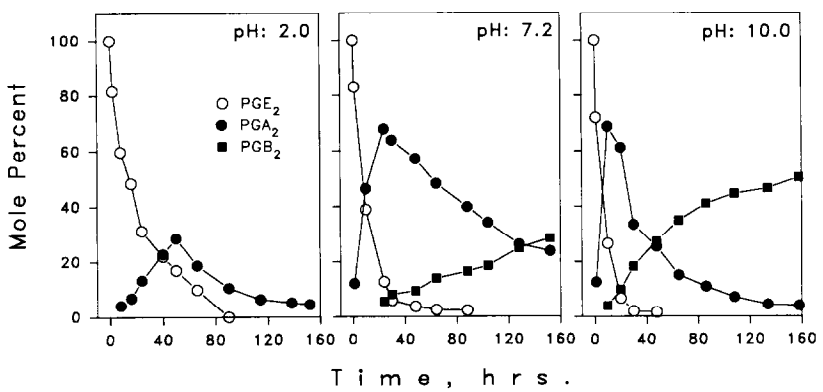

Fig. 2. Dehydration and rearrangement reaction profile of PGE_2 as a function of time under different pH conditions and at 60°C. Initial concentration of PGE_2, 50 μg/ml.

the PGE_2 being considered to degrade to yield unidentified products or analogs of prostaglandins except for PGA_2 and PGB_2.

Fig. 4 shows typical HPLC chromatograms of PGE_2 solution measured at 192 nm after 4 days at 60°C in acidic and alkaline buffer solutions. Besides the peaks of PGA_2 and PGB_2, the chromatograms show several typical peaks of unknown degradation products which remain to be determined.

The formation of epimers at C-8 and C-15 of PGE_2, PGA_2 and PGB_2, a highly conjugated 13,15-dehydration product of PGA_2 in acidic solution and 13-hydroxy diastereomers of PGA_2, in addition to the main degradation pathway of $PGE_2 \rightarrow PGA_2 \rightarrow PGB_2$, has been considered based on limited evidence (Monkhouse et al., 1973; Stehle, 1982). A better understanding of the HPLC chromatograms of Fig. 4 followed by identification of the unknown peaks appears to be essential for further studies of the complex degradation kinetics of PGE_2 in solution.

In conclusion, PGE_2 and its major degradation products, PGA_2 and PGB_2 including PGE_1, PGA_1 and PGB_1 were determined simultaneously without any modification and derivatization pro-

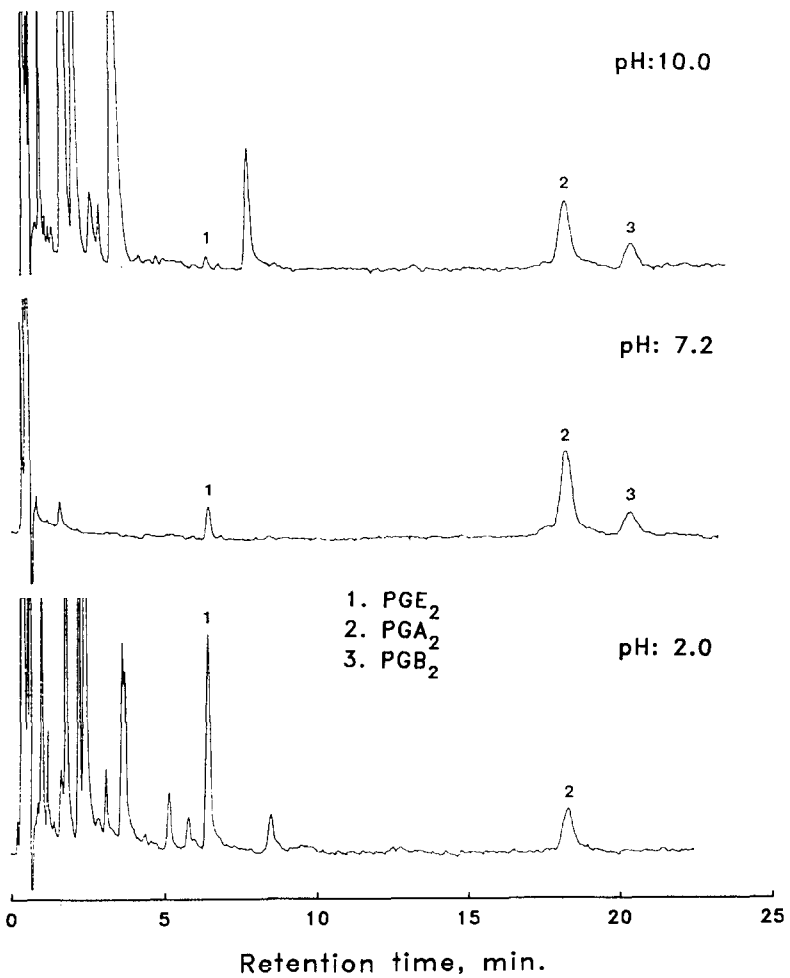

Fig. 4. Typical HPLC chromatograms measured after 6 days at 60°C in acidic and alkaline buffers measured at 192 nm. Initial concentration of PGE_2, 50 μg/ml; attenuation, 3.

cedures. With this method, primary kinetic studies of PGE_2 were performed at 60°C in solutions of different pH and it was confirmed that various degradation products resulted except for PGA_2 and PGB_2, which remain to be determined. Furthermore, it was demonstrated that the total mole percent of PGE_2, PGA_2 and PGB_2 at alkaline pH during the experimental time was not 100% as reported previously but approached the range of 50–60% of the initial PGE_2.

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