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Note

High-performance liquid chromatographic assay for prostacyclin

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Prostacyclin, (5-*Z*)-9-deoxy-6,9α-epoxy-Δ5-prostaglandin $F_{1\alpha}$, a recently discovered metabolite of arachidonic acid, is produced by the blood vessels, heart, and lungs[1–3]. Prostacyclin is a potent vasodilator and it inhibits platelet aggregation; consequently, it is considered a potentially important regulator of blood pressure and endothelial integrity[4]. These properties make it an interesting candidate for therapeutic use in cardiovascular, hematological or thrombotic disorders[4]. The investigation of the chemical purity and stability of prostacyclin sodium salt (PGI_2·Na) prepared by total organic synthesis[5] is necessary to complement other analytical methods which are less suitable for measuring impurities and decomposition products[6]. This report describes a reversed-phase high-performance liquid chromatographic (HPLC) method for determining the purity and stability of PGI_2·Na preparations possibly containing four other structurally related compounds. Included are data on PGI_2·Na stability under various condition, and results on the accuracy and precision of the method. The rationale for the use of certain chromatographic conditions and limitations imposed by the chemical nature of the vinyl ether structure of PGI_2·Na are discussed, in terms of the HPLC method of Hill and Coomber[7].

EXPERIMENTAL

Apparatus

Chromatography was performed with a Model 396 pulse damped piston pump (Laboratory Data Control, Riviera Beach, Fla., U.S.A.); a syringe-loaded Model 7120 loop injection valve, with an internal volume of 20 μl (Rheodyne, Berkeley, Calif., U.S.A.); a μBondapak® C_{18} column (Waters Assoc., Milford, Mass., U.S.A.); and a Model LC55 variable-wavelength spectrophotometric detector (Perkin-Elmer, Norwalk, Conn., U.S.A.) operated at 205 nm. Chromatograms were recorded on a Varian A-25 recorder, 1 mV, operated at 0.1 in. min^{-1}.

Reagents

Sterile, distilled water; boric acid and sodium borate (J. T. Baker, Phillipsburg, N.J., U.S.A.); and methanol and acetonitrile (Burdick & Jackson, Muskegon, Mich., U.S.A.) were used without purification. Two mobile phase systems are discussed in this report. Mobile phase 1 consisted of methanol–water (55:45, v/v), buffered at pH 8.9 with 0.04 *M* boric acid and 0.019 *M* sodium borate. Mobile phase 2 consisted of

acetonitrile–water (20:80, v/v), buffered at pH 9.3 with 0.009 *M* boric acid and 0.004 *M* sodium borate. Using mobile phase 1 the column was operated at 1500 p.s.i.g. at a flow-rate of 1.0 ml min^{-1}. Using mobile phase 2, the column was operated at 2000 p.s.i.g. at a flow-rate of 1.2 ml min^{-1}.

Reference standards of possible decomposition products or trace contaminants of the organic synthesis procedure were supplied by the Experimental Chemistry Laboratories of The Upjohn Company. A reference standard of $PGI_2 \cdot Na$ was verified by several complementary analytical techniques to be at least 99.8% (w/w) pure. For chromatographic analysis, accurately weighed samples were dissolved in mobile phase and a 20-μl full-loop injection was made at a recorder setting giving approximately 50% full scale deflection. Peak area measurements were used for all calculations. The injection reproducibility was verified at $\pm 1\%$.

RESULTS

$PGI_2 \cdot Na$, an exocyclic vinyl ether, hydrolyzes rapidly, in a pH dependent fashion, via a hemi-ketal intermediate into a stable end product: 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$)[5,6]. 6β-Δ4-Prostaglandin I_1 (6β-Δ4-PGI_1) and 6α-Δ4-PGI_1 are possible impurities from the synthetic procedure. The structures of these compounds are shown in Fig. 1. Their chemical nature, especially the potential lability of the parent compound, restricts the choice of chromatographic parameters. The vinyl ether moiety of $PGI_2 \cdot Na$ is best stabilized in solution by buffering under basic conditions (>pH 8.8). Thus, a reversed-phase HPLC system was indicated in spite of the fact that the separation of the 6α- and 6β-epimers of Δ4-PGI_1 might be difficult. Also, one would predict that PGI_2 or its principal decomposition product, 6-keto-$PGF_{1\alpha}$, should inter-

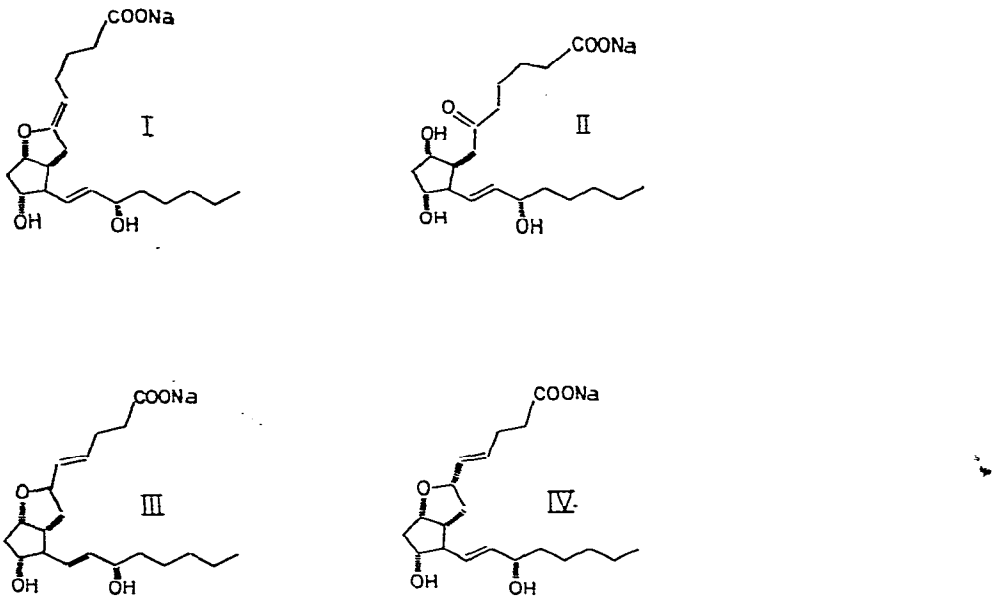


Fig. 1. Structures of prostacyclin (I) and its known decomposition products of contaminants: 6-keto-PS $F_{1\alpha}$ (II), 6β-Δ4-PS I_1 (III) and 6α-Δ4-PS I_1 (IV).

act, as their hemi-ketals, with alcohols (methanol or ethanol) to form methyl or ethyl ketals. These products might, under certain conditions, appear as distinct peaks and complicate the assay. Superficially, the methanol modified, mobile phase 1 adequately separated the compounds of interest (Fig. 2); however, initial quantitative experiments confirmed the predicted problem. Fig. 3 shows that pre-incubation with different proportions of methanol, immediately prior to injection, can distort the chromatographic profile of a pure reference standard of 6-keto-$PGF_{1\alpha}$. When exposed to methanol at concentrations equivalent to those of mobile phase 1, for intervals equivalent to the transit time through the column, the profile for a highly purified sample of 6-keto-$PGF_{1\alpha}$ still contains minor peaks which coincide in retention with 6α- or 6β-Δ4-PGI_1. As noted, the 6α- and 6β-epimers are not decomposition products, but are residual trace impurities from the synthetic procedure. Their concentration in any given sample ought to remain invariant. Thus, apparent increases in 6α- and 6β-Δ4-PGI_1 in several experiments using mobile phase 1 were attributable to the interaction between the hemi-ketal form of 6-keto-$PGF_{1\alpha}$ and methanol. Subsequent chro-

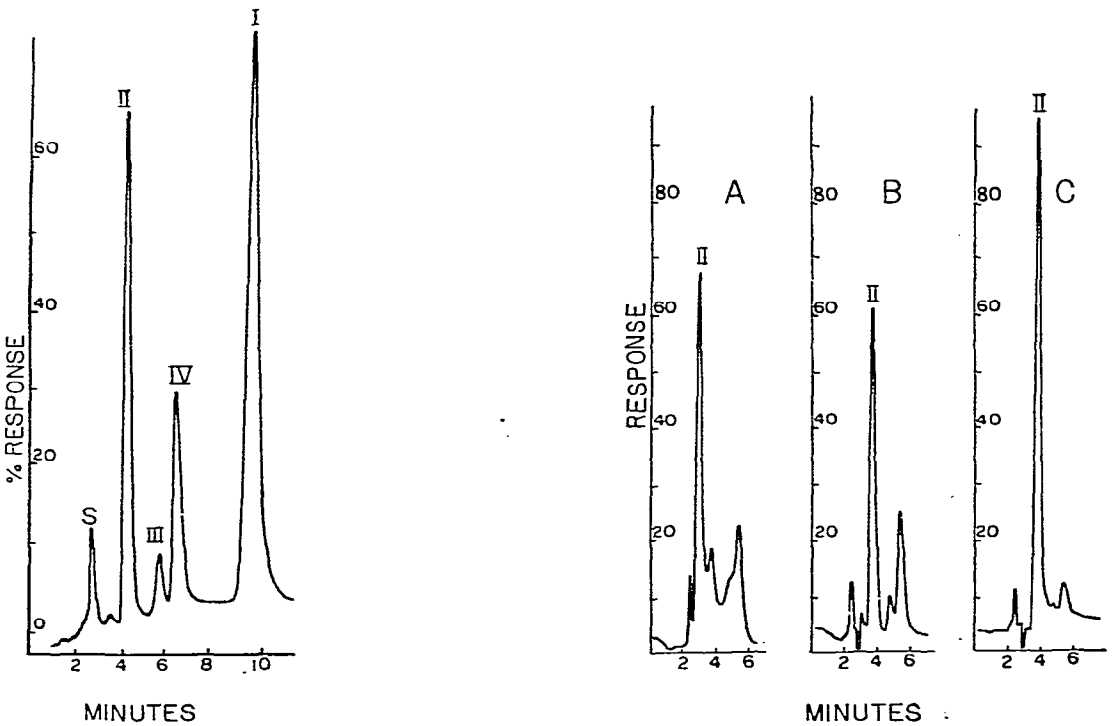
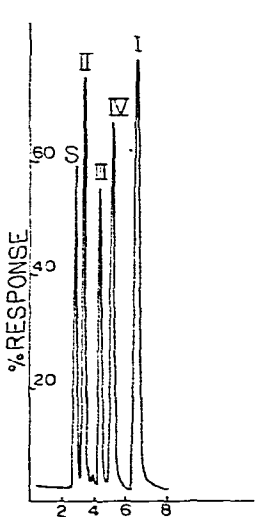


Fig. 2. Typical chromatogram of prostacyclin and its known decomposition products or contaminants. Chromatography was performed on a μBondapak C_{18} column using a mobile phase of methanol–water (55:45, v/v), buffered at pH 8.9 with 0.04 *M* boric acid and 0.019 sodium borate. The flow-rate was 1.0 ml min^{-1} at 1500 p.s.i.g. Compounds were detected with an LC55 variable-wavelength detector fixed at 205 nm and calibrated to register 0.10 a.u.f.s. S = solvent.

Fig. 3. Influence of methanol on 6-keto-PG $F_{1\alpha}$ chromatographic profile. A fixed concentration (1.5 ng/10.0 ml) of 6-keto-$PGF_{1\alpha}$ was incubated in different methanol–water compositions: A = 90:10, B = 50:50, C = 10:90. After 5 min incubation a sample was injected and chromatographed using the identical conditions of Fig. 2.



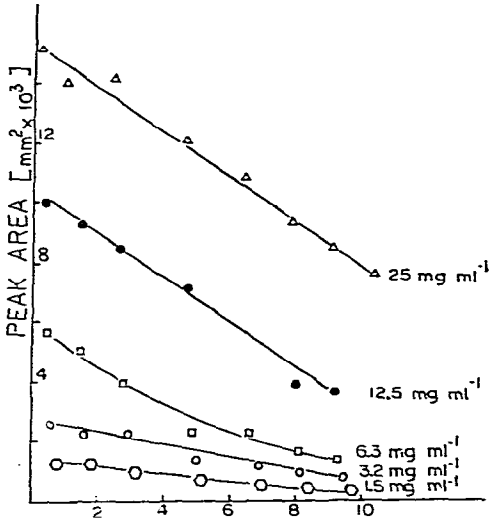


Fig. 4. Typical chromatogram of prostacyclin and its known decomposition products or contaminants. Chromatography was performed on a μBondapak C_{18} column using a mobile phase of acetonitrile–water (20:80, v/v), buffered at pH 9.3 with 0.009 *M* boric acid and 0.004 *M* sodium borate. The flow-rate was 1.2 ml min^{-1} at 2000 p.s.i.g. Compounds were detected with an LC55 variable-wavelength detector fixed at 205 nm and calibrated to register 0.10 a.u.f s.

Fig. 5. The concentration dependence of the stability of prostacyclin at pH 8.9, 23°.

matography using the aprotic organic modifier, acetonitrile, in mobile phase 2, also separated the compounds of interest (Fig. 4). Quantitative experiments with this system confirmed that the increases in 6α- and 6β-Δ14-PGI_1 were artifacts due to the methanol in mobile phase 1. Moreover, a previously unrecognized decomposition product, 6(9α),6(11α)-dioxido-15α-hydroxy-(*E*)-prosta-13-enoic acid[8,9] was resolved

TABLE I

HPLC MEASUREMENT OF PGI_2 STABILITY

Temperature (°C)	*pH*	*Half-life (h)*
0	8.9	21.0
23	8.9	4.4
23	9.3	10.3
23	7.4	0.033

TABLE II

QUANTITATIVE ACCURACY OF HPLC ASSAY

Mass found (mg/5.0 ml)	*Mass expected (mg/5.0 ml)*	*Error (%)*
0.301	0.307	2.0
0.544	0.565	3.8
2.074	2.095	1.1
3.572	3.959	9.8

from the other compounds using mobile phase 2. This impurity co-chromatographed with PGI_2 itself in mobile phase 1. Replicate analyses on several samples showed a precision of $\pm 3\%$. Analysis of several "blind" samples prepared for internal quality control purposes showed an accuracy of 4.2% (Table I). The pH and temperature dependence of the PGI_2 half-life (Table II) determined by HPLC agrees well with values obtained by a spectrophotometric method[6]. The half-life of PGI_2 also shows a concentration dependence (Fig. 5) which has practical implications for formulation purposes.

CONCLUSIONS

The measurement of $PGI_2 \cdot Na$ purity and stability by HPLC is not straightforward. The number of suspected impurities and decomposition products; the lability of the parent compound; and the generic interaction of hemi-ketals with alcoholic modifiers, all restrict the range of chromatographic parameters which are compatible with the assay requirements. HPLC in the reversed phase mode with microparticulate columns was sufficiently versatile to counter these restrictions. Karger and Giese[10] have reviewed this aspect of HPLC.

Our results confirm the need to integrate one's knowledge of the chemical nature of compounds being chromatographed with apparent chromatographic results. In the case of predictable chemical interactions one cannot rely exclusively on results from only one mobile phase. The HPLC method reported here is based on the original method of Hill *et al.*[7]. Our results show that acetonitrile should be used to eliminate systematic errors related to the use of methanol as the organic modifier in the chromatographic eluent. The method described reflects $PGI_2 \cdot Na$ purity with greater accuracy.

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