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DETERMINATION OF EPOPROSTENOL SODIUM (PROSTAGLANDIN I_2 SODIUM) AND RELATED PROSTAGLANDINS IN EPOPROSTENOL SODIUM BULK DRUG BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY*

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SUMMARY

Two complementary high-performance liquid chromatographic assays for the quantitation of epoprostenol sodium (PGI_2) and seven potential impurities in epoprostenol sodium bulk drug are described. A reversed-phase assay using a mobile phase of acetonitrile–borate buffer (pH 9.5), an octadecylsilane column and ultraviolet detection at 214 nm is employed for the quantitation of PGI_2 and its isomer, $\Delta 6$-PGI_1. An adsorption system employing a dimethylsulfoxide–methylene chloride mobile phase is used to separate and quantitate the other compounds as their *p*-bromophenacyl derivatives. In this case, the effluent is monitored at 254 nm, and impurity levels are quantitated by area percent assuming equal response for all compounds. Chromatographic specificity and performance characteristics for both procedures are described.

INTRODUCTION

Epoprostenol, prostaglandin I_2, is a naturally occurring prostaglandin which has the property of inhibiting platelet aggregation at extremely low concentrations. The sodium salt in a freeze-dried powder was recently granted marketing approval in Great Britain for use in cardiopulmonary bypass surgery and charcoal hemo perfusion. It is marketed under the trade name Cyclo-Prostin® Sterile Powder by The Upjohn Company.

The analytical characterization of drug substances requires quantitation of both the drug substance and minor components present in the material. In an ideal situation this could be accomplished in a single chromatographic analysis. In the case of epoprostenol sodium, however, this is not the case. In this paper two complementary methods are described which provide characterization of epoprostenol sodium both with respect to its purity and the level of minor components.

Skrinska and Thomas[1] recently surveyed high-performance liquid chromato-

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graphic (HPLC) methods for epoprostenol sodium and described a reversed-phase procedure using a styrene–divinyl benzene column with highly alkaline mobile phases. These workers did not apply their procedure to the quantitation of process impurities.

A reversed-phase procedure using an aqueous based internal standard solution for dissolution of the sample was desired, since an assay would also be required for a freeze-dried formulation of epoprostenol containing mannitol, sodium chloride and glycine. However, the chemistry of epoprostenol placed severe constraints on reversed-phase HPLC conditions. The vinyl ether group present in epoprostenol has been identified as the cause of the extreme hydrolytic lability of the molecule[2]. At physiological pH, in aqueous solution epoprostenol is hydrolyzed to 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) with a half-life of 3 to 4 min[2]. At alkaline pH the hydrolysis becomes insignificant with respect to the chromatographic time frame. Wynalda *et al.*[3] reported that epoprostenol and 6-keto-$PGF_{1\alpha}$ interact with methanol as their hemi-ketals to form the corresponding methyl ketal. Prostaglandins are essentially transparent to ultraviolet (UV) radiation exhibiting only end-absorption; thus low-wavelength detection was mandated. The restrictions described above dictated that for a reversed-phase HPLC assay a mobile phase at a high pH with aprotic, UV transparent modifiers must be used. The reversed-phase system ultimately selected was a modification of that reported by Wynalda *et al.*[3]. This system provided rapid, accurate measurement of epoprostenol in bulk drug and formulations.

The use of low-wavelength UV detection was found less than satisfactory for the quantitation of process impurities and degradation products. As noted above, these compounds have essentially no UV absorptivity except end-absorption. At any given wavelength the molar absorptivities of some of the compounds were significantly different from epoprostenol[4]. If they were to be quantitated in this system, then accurately characterized standards of each compound would be required. These were not available. Further, the principal hydrolysis product, 6-keto-$PGF_{1\alpha}$, eluted near the solvent front and was difficult to quantitate.

A procedure permitting the assumption of equimolar response for each compound would avoid these problems. Zoutendam *et al.*[5] recently reviewed the use of derivatizing agents which have been useful for prostaglandins. The *p*-bromophenacyl derivative was selected for PGI_2. Since the molecular weights of the compounds of interest were similar, the assumption of equal response using 254-nm detection was valid. An adsorption HPLC system was developed which provided a means to estimate the level of the potential impurities and degradation products of epoprostenol sodium bulk drug.

EXPERIMENTAL

Apparatus

HPLC was performed on a modular HPLC system consisting of an Altex Model 110A pump (Altex Scientific, Berkley, CA, U.S.A.), a Rheodyne Model 7125 loop injector (Rheodyne, Cotati, CA, U.S.A.) and a LDC Model 1203 HPLC UV monitor (Laboratory Data Control, Riviera Beach, FL, U.S.A.). The detector was equipped with a 214-nm conversion kit for the reversed-phase assay. A saturator column of HC Pellosil (80 × 3 mm I.D.) (Whatman, Clifton, NJ, U.S.A.) was employed for the reversed-phase system. An in-house designed and built auto sampler was also used.

Reagents

Acetonitrile, dimethylsulfoxide, dimethylformamide and methylene chloride were distilled in glass (Burdick and Jackson, Muskegon, MI, U.S.A.). Boric acid, sodium borate and sodium hydroxide were ACS reagent grade. N,N-diisopropylethylamine (98%) and acetophenone (99%) were obtained from Aldrich (Milwaukee, WI, U.S.A.), and 2,4′-dibromoacetophenone from Eastman Kodak (Rochester, NY, U.S.A.). Glycine and sodium chloride were USP grade.

Samples of the prostaglandins were supplied by F. H. Lincoln, Experimental Science, The Upjohn Company, Kalamazoo, MI, U.S.A.

Adsorption chromatographic system

Column. 5-μm silica gel (25 cm × 4.6 mm I.D.) (DuPont Zorbax Sil or IBM columns were equivalent).

Mobile phase. 1000 ml methylene chloride, 15 ml dimethylsulfoxide and 1 ml water.

Derivitization solutions. 2,4′-Dibromoacetophenone: 30 mg/ml in dimethylformamide which had been dried over 4A molecular sieves, diisopropylethylamine: 15 mcl/ml in dry dimethylformamide. These solutions must be prepared fresh daily.

Procedure. Add 200 μl 2,4′-dibromoacetophenone solution and 100 μl of diisopropylethylamine solution to about 1 mg of sample. Swirl to mix and dissolve the drug, let stand 1 h at room temperature then add 10 ml methylene chloride. Filter a portion through a 10-μm Mitex Millipore filter. Chromatograph 20-μl aliquots.

Reversed-phase chromatographic system

Column. Waters μBondapak C_{18} (30 cm × 3.9 mm I.D.).

Mobile phase. 0.8 g boric acid, 0.8 g sodium borate, 790 ml double-distilled water and 210 ml acetonitrile, adjust the apparent pH to 9.5 with 1 *N* sodium hydroxide.

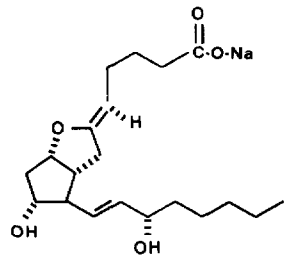
Internal standard solution. Dissolve about 77 mg (75 μl) of acetophenone in 500 ml of a solution of 940 mg glycine and 730 mg sodium chloride adjusted to pH 10.5 with 1 *N* sodium hydroxide.

Procedure. Dissolve an accurately weighed quantity of about 2 mg of sample or standard in 20.0 ml internal standard solution. Chromatograph 20-μl aliquots within 2 h of preparation.

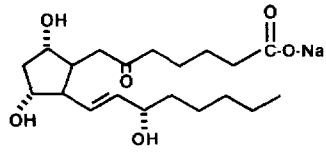
RESULTS AND DISCUSSION

The structures of epoprostenol sodium (I) and the other prostaglandins of interest are shown in Fig. 1. Hydrolysis of epoprostenol results in 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) (II)[6]. The 6β- and 6α-Δ4-PGI_1 isomers (III and IV respectively) are potential impurities from the synthesis[3]. Compounds V and VI 5(*E*)-PGI_2 and Δ6-PGI_1 result from isomerization either during the synthesis or on standing. The 6(9α)6(11α)-diepoxy-15α-hydroxy-(*E*)-prosta-13-enoic acid (diepoxy PGI_2) (VII) results from thermal degradation of epoprostenol.

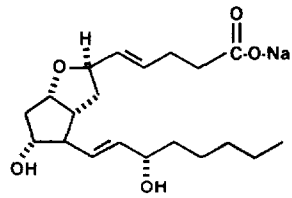
As noted above, the quantitation of impurities in epoprostenol was not satisfactory using the reversed-phase system and low-wavelength detection. Therefore, the derivatization-adsorption chromatographic procedure was investigated. A num-



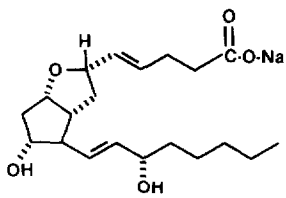
EPOPROSTENOL SODIUM
PGI_2-Na (I)



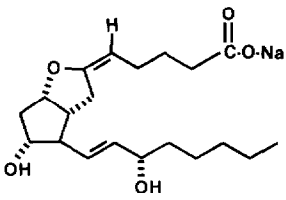
6-KETO-$PGF_{1\alpha}$-Na (II)



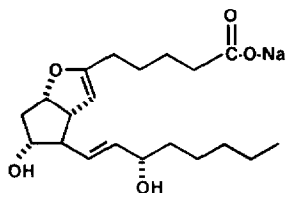
6β-Δ4-PGI_1-Na (III)



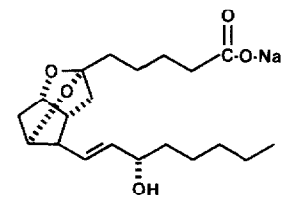
6α-Δ4-PGI_1-Na (IV)



5(E)-PGI_2-Na (V)



Δ6-PGI_1-Na (VI)



6,11-DIEPOXY (VII)

Fig. 1. Structures of epoprostenol and related prostaglandins.

TABLE I

OPTIMIZATION OF THE DERIVATIZATION OF EPOPROSTENOL SODIUM WITH RESPECT TO TIME AND TEMPERATURE

Response = area PGI_2 peak/sample weight.

Time (min)	*Response*	
	Room temperature	*45°C*
15	36.7	35.9
30	36.8	37.1
60	38.1	35.6
120	37.1	34.4

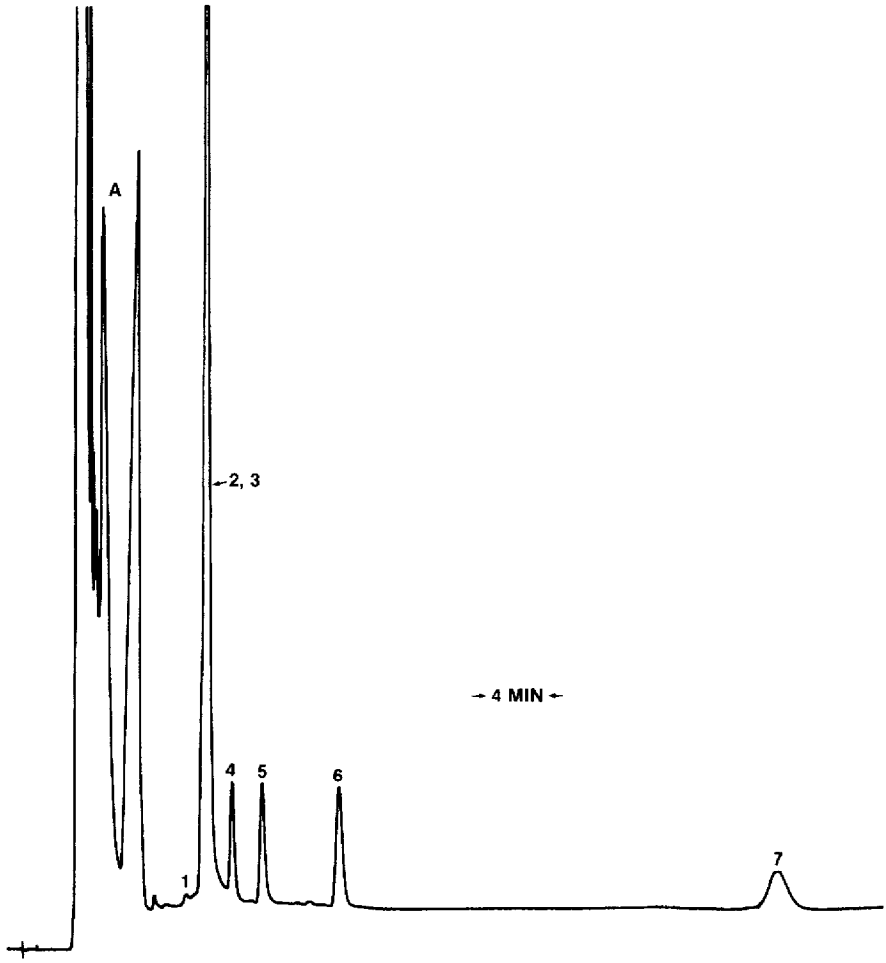


Fig. 2. Chromatogram of a synthetic mixture of epoprostenol and related compounds as their *p*-bromophenacyl derivatives in the adsorption system. Key: A = solvent front and reagent blank; 1 = 6,11-diepoxy analogue; 2 = Δ6-PGI_1; 3 = epoprostenol; 4 = 5(*E*)-PGI_2; 5 = 6α-Δ4-PGI_1; 6 = 6β-Δ4-PGI_1; 7 = 6-keto-$PGF_{1\alpha}$.

TABLE II

HPLC RESPONSE OF THE DERIVATIZED PROSTACYCLIN ANALOGUES AT 254 nm USING THE ADSORPTION SYSTEM

Response = total area/sample weight, RRT = relative retention time.

Compound	*RRT*	*Total area*	*Weight (mg)*	*Response*
PGI_2	1.0	76,600	1.025	74,700
Δ6-PGI_2	0.97	72,900	0.971	75,100
5(*E*)-PGI_2	1.12	49,200	0.717	68,600
6α-Δ4-PGI_1	1.24	98,400	1.415	69,500
6β-Δ4-PGI_1	1.63	78,700	1.025	79,000
6-keto-$PGF_{1\alpha}$	4.8	57,600	0.903	63,800

TABLE III

PRECISION OF THE METHODS FOR EPOPROSTENOL IMPURITIES

σ = Standard deviation, R.S.D. = relative standard deviation.

Parameter	*6,11-Diepoxy*	*5(E)-PGI$_2$*	*6α-Δ4-PGI$_1$*	*6β-Δ4-PGI$_1$*	*6-keto-PGF$_{1\alpha}$*	*Δ6-PGI$_1$*
n	4	6	6	6	6	6
$\bar{x}$	0.1	0.74	0.22	0.42	1.64	0.5
σ	0.07	0.12	0.04	0.04	0.32	0.18
R.S.D. (%)	–	17	15	9	19	35

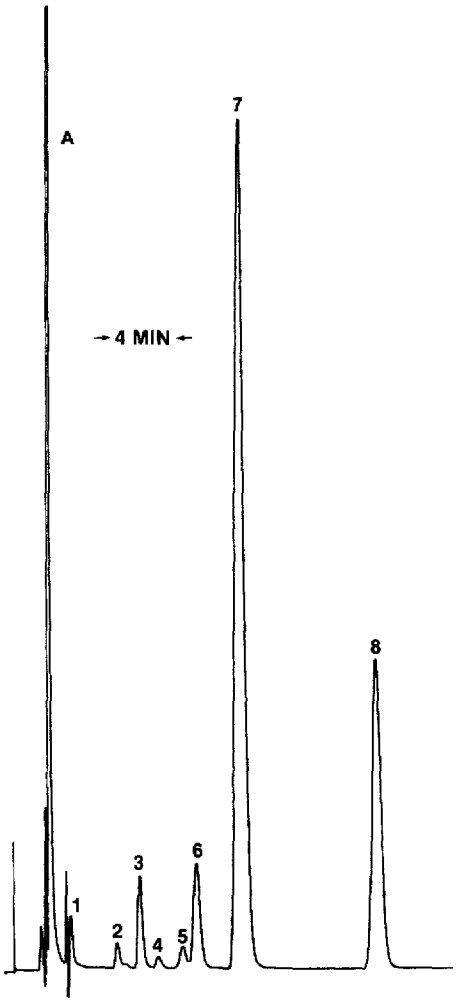


Fig. 3. Chromatogram of a synthetic mixture of epoprostenol and related prostaglandins in the reversed-phase system. Key: A = solvent front; 1 = 6-keto-PGF$_{1\alpha}$; 2 = 6β-Δ4-PGI$_1$; 3 = 5(*E*)-PGI$_2$; 4 = 6α-Δ4-PGI$_1$; 5 = 6,11-diepoxy analogue; 6 = Δ6-PGI$_1$; 7 = epoprostenol; 8 = acetophenone (internal standard).

ber of UV absorbing esterification agents were examined, 2,4'-dibromoacetophenone provided better reproducibility than either 2-bromo-4'-nitroacetophenone, or α-bromo-2'-acetonapthone. The former was selected for further work. The derivatization conditions with 2,4'-dibromoacetophenone were optimized with respect to time and temperature (see Table I). On the basis of this data, one hour at room temperature was selected for further study. The resolution of epoprostenol from the related compounds as the *p*-bromophenacyl derivatives using the adsorption HPLC system is shown in Fig. 2. This system does not resolve Δ6-PGI$_1$ from epoprostenol. Resolution of Δ6-PGI$_1$ and epoprostenol was not obtained using any of the other derivatizing reagents tested or other chromatographic systems examined including silver modified columns. The assumption of equal response was verified by determining the response of derivatived authentic samples of each of the compounds. These results are shown in Table II. Considering the purity of the samples and the levels anticipated in epoprostenol sodium the assumption of equal response is valid. Table III provides the results of a precision study by replicate analysis of a single lot of epoprostenol. Using conditions described in this paper we estimate the limit of detection to be about 0.1% for each of the impurities.

Cho and Allen reported spontaneous conversion of the *p*-nitrophenacyl derivative of epoprostenol to 6-keto-PGF$_{1\alpha}$[6]. The authors found the *p*-bromophenacyl derivatives to be stable for at least 24 h before significant increase in the levels of 6-keto-PGF$_{1\alpha}$ were noted.

To quantitate epoprostenol sodium and Δ6-PGI$_1$ in the bulk drug, a modification of the reversed-phase system described by Wynalda *et al.*[3] was employed. This system, which also separates all the compounds of concern from epoprostenol, is illustrated in Fig. 3. The sample is dissolved in an internal standard solution and chromatographed. Epoprostenol purity is calculated *versus* an external reference preparation. The level of Δ6-PGI$_1$ is calculated by area percent using the sum of epoprostenol and Δ6-PGI$_1$ areas in the denominator.

The quantitative accuracy of the procedure for epoprostenol is illustrated in Table IV. The relative response of Δ6-PGI$_1$ and epoprostenol was determined on four different days. The ratio averaged was 1.03 ± 7%. For the levels of Δ6-PGI$_1$ expected, the assumption of equal response was valid. Precision of the assay for

TABLE IV

QUANTITATIVE PERFORMANCE OF THE REVERSED-PHASE ASSAY FOR EPOPROSTENOL

R.S.D. = relative standard deviation.

Mg added (per 5.0 ml)	*Mg found (per 5.0 ml)*	*% Recovery*
1.307	1.32	101.1
1.688	1.68	99.6
2.184	2.20	100.9
2.402	2.43	101.4
2.823	2.74	97.2
	Mean =	100.0
	R.S.D. =	1.7%

TABLE V

RESULTS (IN %) FOR VARIOUS EXPERIMENTAL LOTS OF EPOPROSTENOL SODIUM BULK DRUG

Lot	*Δ6-PGI$_1$*	*6,11-Ether*	*5(E)-PGI$_2$*	*6α-Δ4-PGI$_1$*	*6β-Δ4-PGI$_1$*	*6-keto-PGF$_{1\alpha}$*	*Total*
A	0.2	< 0.1	0.6	0.2	0.6	1.4	3.0
B	0.4	< 0.1	0.6	< 0.1	0.5	1.3	2.8
C	0.3	< 0.1	0.5	0.1	0.6	1.2	2.6
D	0.2	< 0.1	0.4	0.1	0.5	1.3	2.5
E	0.7	< 0.1	0.4	0.1	0.6	1.4	3.2
F	0.4	< 0.1	0.5	< 0.1	0.8	1.9	3.6
G	1.6	0.1	0.7	0.2	0.4	1.6	4.6
H	0.4	< 0.1	0.4	< 0.1	0.1	0.6	1.5
I	0.4	< 0.1	< 0.1	<0.1	0.1	0.9	1.4
J	0.6	0.1	< 0.1	0.3	0.4	< 0.1	1.4
K	0.1	0.1	0.3	< 0.1	1.1	< 0.1	1.6
L	0.1	0.3	0.2	0.3	0.8	< 0.1	1.7

Δ6-PGI$_1$ was measured by six replicate assays of a single lot; these results are shown in Table III.

The use of a mobile phase with a pH 9.5 was of concern to the authors since this was above the maximum recommended by the column manufacturer. We found, however, that using the presaturator column[7] prior to the injector protected the column. As an additional precaution, the mobile phase was never allowed to stand static on the column. The mobile phase was recycled overnight and the system rinsed with acetonitrile–water (50:50) prior to shut down over weekends. With these precautions the life of a column was in excess of two months of continuous use.

CONCLUSIONS

Two complementary HPLC procedures are described which provide accurate and precise characterization of the purity of epoprostenol sodium. The utility of quantitating low-level impurities by employing a UV absorbing derivative permitting an assumption of equal response for the potential impurities is illustrated. Table V provides data for twelve lots of epoprostenol sodium of different ages.

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