Journal of Chromatography, 277 (1983) 287–291
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 1782

Note

High-performance liquid chromatography of prostacyclin

VICTOR SKRINSKA*

Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44106 (U.S.A.)

and

GEORGE THOMAS

Department of Chemistry, Cleveland State University, Cleveland, OH 44115 (U.S.A.)

(First received February 8th, 1983; revised manuscript received May 6th, 1983)

The vascular endothelium synthesizes prostacyclin (PGI_2) from metabolism of arachidonic acid [1–4]. It is a highly active substance with strong inhibitory action on platelet aggregation through elevation of cyclic AMP, and at higher concentrations prevents platelet adhesion [5–7]. In addition, PGI_2 relaxes vascular smooth muscle and induces hypotension [8–10]. These effects have stimulated studies concerning the role of PGI_2 in maintaining homeostasis and vascular tone as well as investigation of its use as a therapeutic agent in cardiovascular disease and thrombotic disorders [11–13].

Prostacyclin is unstable in acidic or neutral aqueous solutions and rapidly hydrolyzes to an inactive compound, 6-keto-prostaglandin $F_{1\alpha}$ (6-K-$PGF_{1\alpha}$) [14, 15]. Under alkaline conditions, pH > 10, the rate of hydrolysis of the vinyl ether moiety of PGI_2 is substantially reduced. A reliable method for separation of PGI_2 and 6-K-$PGF_{1\alpha}$ under alkaline conditions would be valuable for assaying purity of standard solutions and determining PGI_2 levels for in vivo and in vitro pharmacology studies. High-performance liquid chromatography (HPLC) procedures have been reported for the separation of PGI_2 and 6-K-$PGF_{1\alpha}$ in standard solutions and biological extracts [16–19]. These methods utilize reversed-phase octadecyl silane columns with mildly alkaline, pH ≈ 9, solvent systems which compromise the stability of the PGI_2 and the life of the silica-based column packing. In this report a procedure is described for HPLC separation of PGI_2 and 6-K-$PGF_{1\alpha}$ on a reversed-phase styrene–

0378-4347/83/$03.00 © 1983 Elsevier Science Publishers B.V.

divinylbenzene column with highly alkaline, pH > 11, solvent systems. Under these conditions there is no detectable hydrolysis of PGI_2, nor do the conditions cause deterioration of the column packing.

EXPERIMENTAL

Apparatus

A Varian Model 5020 liquid chromatograph (Varian, Palo Alto, CA, U.S.A.) was used for solvent delivery. Samples were injected with a manual 100-μl loop injector (Valco, Houston, TX, U.S.A.). Separations were performed on a 150 × 4.1 mm PRP-1 column (Hamilton, Reno, NV, U.S.A.) which contains a 10-μm particle size styrene–divinylbenzene copolymer. The effluent was continuously monitored with a Varichrome variable-wavelength detector (Varian).

Reagents

Acetonitrile and water were HPLC grade from Mallinckrodt (McGraw Park, IL, U.S.A.). Reagent grade sodium hydroxide and ammonium hydroxide (NH_3 29.8%) were also obtained from Mallinckrodt. Prostaglandins were a generous gift from Upjohn (Kalamazoo, MI, U.S.A.). The 6-keto-prostaglandin E_1, was a gift from Dr. P.K. Wong (New York Medical College, Valhalla, NY, U.S.A.). All solvents and reagents were used without further purification.

Procedure

Prostacyclin standard solutions were prepared in 0.01 *N* sodium hydroxide at a concentration of 1 mg/ml. Other prostaglandins were dissolved in acetonitrile or acetonitrile–water (90:10) at 1 mg/ml. Aliquots of standard solutions were evaporated to dryness and taken up in 0.01 *N* sodium hydroxide prior to analysis.

Chromatography of PGI_2 and 6-K-$PGF_{1\alpha}$ was studied with two alkaline mobile phases. Mobile phase I was a mixture of acetonitrile–0.01 *N* sodium hydroxide (21:79) with a measured pH of 12.3. Mobile phase II was a mixture of acetonitrile – 1% (v/v) ammonium hydroxide (17:83) with a pH of 11.0. A flow-rate of 1 ml/min was used for both mobile phases. Sample volumes of 20–100 μl were injected and the effluent continuously monitored at 206 nm. At the end of each day the column was washed with 40 ml of water followed by 20 ml of acetonitrile.

RESULTS AND DISCUSSION

Chromatography

Since the vinyl ether moiety of PGI_2 is subject to acid hydrolysis, chromatography should be performed with a highly alkaline solvent system to prevent hydrolysis. Bonded-phase silica HPLC columns degenerate rapidly when used with solvents with pH > 8 since alkaline solvents dissolve the silica and eventually strip the bonded phase from the packing. Styrene–divinylbenzene HPLC columns, however, can be used up to pH 13 without significant deterioration. The chromatography of PGI_2 and 6-K-$PGF_{1\alpha}$ was investigated on

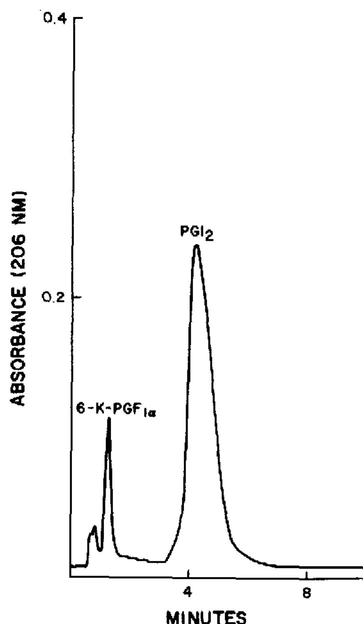

Fig. 1. HPLC of PGI_2 and 6-K-$PGF_{1\alpha}$ standards with mobile phase I.

TABLE I

CAPACITY FACTORS (k') FOR PROSTAGLANDINS

$k' = (V_r - V_0)/V_0$, where V_r is the elution volume and V_0 is the void volume.

Prostaglandin	Mobile phase I	Mobile phase II
PGI_2	6.4	8.5
6-K-$PGF_{1\alpha}$	1.6	1.3
6-K-PGE_1	1.6	1.3
PGA_2	8.1	9.5
PGB_2	10.9	11.3
PGD_2	3.7	4.2
PGE_2	3.0	3.5
$PGF_{2\alpha}$	2.4	3.5
TXB_2	2.0	2.7

a styrene–divinylbenzene reversed-phase HPLC column using two highly alkaline mobile phases. Fig. 1 is a chromatogram of 20 μg PGI_2 and 40 μg 6-K-$PGF_{1\alpha}$ injected in 20 μl of 0.01 N sodium hydroxide and eluted with mobile phase I. Chromatograms obtained with mobile phase II were essentially identical to those obtained with mobile phase I. The capacity factors, k', for PGI_2, 6-K-$PGF_{1\alpha}$ and other prostaglandins were determined by injecting 20 μg of each standard in 20 μl of 0.01 N sodium hydroxide and monitoring their elution at 206 nm. The values of k' for both mobile phases are listed in Table I.

Although mobile phase I had a significantly higher background absorbance than mobile phase II, the background was sufficiently low with both solvent systems to reliably monitor the effluent at 206 nm. Subsequent chromatography was run with mobile phase I primarily to avoid use of ammonium hydroxide because of its volatility.

Calibration

A standard calibration curve was prepared for PGI_2 by injection of 0.5—50 μg of standard in 100 μl of 0.01 *N* sodium hydroxide. The peak height response vs. amount injected was linear over the entire concentration range. The coefficient of variation for a 1-μg injection of PGI_2 was 7.3% (n = 7). Recovery from the chromatograph was determined by injecting 50 μg of PGI_2 and collecting the peak as it was eluted. The collected sample was diluted to 5.0 ml with 0.01 *N* sodium hydroxide and a 100-μl aliquot assayed by reinjection and quantitation using the calibration curve. The observed recovery was 99.1 ± 4.0% (n = 4). The quantitative recovery indicates that there is no detectable loss of PGI_2 due to either adsorption loss by the column or hydrolysis during the chromatography.

Incubation of [^{3}H]PGI_2 in plasma

Human and rabbit platelet poor plasma obtained from citrated blood were mixed with [^{3}H]PGI_2 (12 Ci/mmol, New England Nuclear, Boston, MA, U.S.A.), 4 ng/ml, and incubated at 37°C in a closed incubation system. Aliquots were withdrawn at 5-min intervals up to 25 min. The aliquots were immediately made alkaline with Na_2CO_3, 10 mg/ml, and the [^{3}H]PGI_2 extracted as previously described [16]. The extracted samples were run on the chromatograph to separate [^{3}H]PGI_2 from [^{3}H]6-K-$PGF_{1\alpha}$. Fig. 2 is a plot of the counts per min found for [^{3}H]PGI_2 vs. time of incubation with human and rabbit plasma. The data are normalized to the first sample withdrawn after mixing. The decrease in counts for [^{3}H]PGI_2 corresponded to an increase in counts for [^{3}H]6-K-$PGF_{1\alpha}$. The observed half-lives for [^{3}H]PGI_2 in human and rabbit plasma at 37°C were 11.2 min and 13.5 min, respectively.

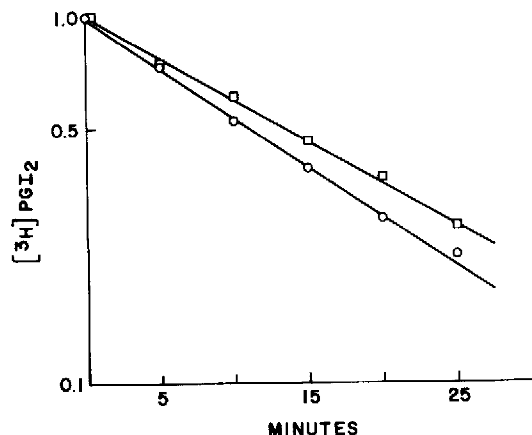

Fig. 2. Decrease in plasma concentration of [^{3}H]PGI_2 vs. time of incubation at 37°C with human (○) and rabbit (□) platelet poor plasma.

ACKNOWLEDGEMENT

This work is supported in part by Grant No. 79902 from The American Heart Association.

REFERENCES

1 S. Moncada, R.J. Gryglewski, S. Bunting and J.R. Vane, Nature (London), 263 (1976) 663.
2 R.J. Gryglewski, S. Bunting, S. Moncada, R.J. Flower and J.R. Vane, Prostaglandins, 12 (1976) 685.
3 S. Bunting, R.J. Gryglewski, S. Moncada and J.R. Vane, Prostaglandins, 12 (1976) 897.
4 R.A. Johnson, D.R. Morton, J.H. Kinner, R.R. Gorman, J.C. McGuire, F.F. Sun, N. Wittaker, S. Bunting, J. Salmon, S. Moncada and J.R. Vane, Prostaglandins, 12 (1976) 915.
5 E.A. Higgs, S. Moncada and J.R. Vane, Prostaglandins, 16 (1978) 17.
6 R.R. Gorman, S. Bunting and O.V. Miller, Prostaglandins, 13 (1977) 377.
7 J.E. Tateson, S. Moncada and J.R. Vane, Prostaglandins, 13 (1977) 389.
8 H.M. Waldman, I. Alter, P.A. Kot, J.C. Rose and P.M. Ramwell, J. Pharmacol. Exp. Ther., 204 (1978) 289.
9 D. Horii, T. Kanayama, M. Mori, M. Shibasaki and S. Ikegami, Eur. J. Pharmacol., 51 (1978) 313.
10 J. O'Grady, S. Warrington and M.J. Moti, Prostaglandins, 19 (1980) 319.
11 I.D. Walker, J.F. Davidson, A. Faichney, D.J. Wheatley and K.G. Davidson, Brit. J. Haematol., 49 (1981) 415.
12 R.M. Zusman, R.H. Rubin, A.E. Cato, D.M. Cochetto, J.W. Crow and N. Tolkoff-Rubin, N. Engl. J. Med., 304 (1981) 934.
13 P.M. Dowd, M.F.R. Martin, E.D. Cooke, S.A. Bowcock, R. Jones, P.A. Dieppe and J.D.T. Kirby, Brit. J. Dermatol., 106 (1982) 81.
14 M.J. Cho and M.A. Allen, Prostaglandins, 15 (1978) 943.
15 M.A. Wynalda and F.A. Fitzpatrick, Prostaglandins, 20 (1981) 853.
16 V. Skrinska and F.V. Lucas, Prostaglandins, 22 (1981) 365.
17 G.T. Hill, J. Chromatogr., 176 (1979) 407.
18 M.A. Wynalda, F.H. Lincoln and F.A. Fitzpatrick, J. Chromatogr., 176 (1979) 413.
19 R.S.P. Hsi, W.T. Stolle, J.P. McGrath and D.R. Morton, J. Labelled Compd. Radiopharm., 18 (1981) 1437.