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International Journal of Pharmaceutics 115 (1995) 113–117

international journal of pharmaceutics

Note

Stability study of prostaglandin E_1 (PGE_1) in physiological solutions by liquid chromatography (HPLC)

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Received 30 May 1994; accepted 26 July 1994

Abstract

The stability of prostaglandin E_1 (PGE_1) in physiological solution for the treatment of erectile dysfunctions was investigated by liquid chromatography (HPLC). Two different HPLC procedures were used; the first included a pre-chromatographic derivatization of PGE_1 with 2-bromoacetyl-6-methoxynaphthalene followed by fluorescence detection ($\lambda_{exc} = 300$ nm; $\lambda_{em} = 460$ nm), and the other was based on direct UV detection (205 nm) of the underivatized drug. The results showed that PGE_1 physiological solutions can be conveniently stored at 2–8°C; under these conditions about 85% of the initial concentration remained at 90 days, while at ambient temperature rapid degradation of the drug was observed.

Keywords: Prostaglandin E_1; Physiological solution; Stability; HPLC

Prostaglandin E_1 (PGE_1; Alprostadil) is today considered one of the drugs of choice, alone or associated with other drugs (vasoactive cocktails), for the diagnosis and treatment of erectile dysfunction (Lee et al., 1989; Earle et al., 1990). The papers concerning the cavernous use of PGE_1 are very encouraging regarding its safety (very few systemic and local side effects) and ease of use. In our University Andrological Center more than 500 patients have been treated with PGE_1 intracavernous injections for diagnostic or therapeutic goals.

PGE_1 was available only in 500 μg /ml ethanol solution vials (Prostin VR); therefore, for intracavernous applications each Prostin VR solution was subjected to aqueous dilution to the desired concentration. Therapeutical PGE_1 doses vary from 5 to 50 μg/ml on the basis of some pharmacostimulation tests to assess the 'right' dose to use for each patient. In our clinical experimentation, Prostin VR, diluted 1:24 in physiological solution using an aseptic technique in sterile vials and stored at 8°C, showed decreasing activity after some days or months, as observed when the same PGE_1 dose was repeated on the same patient after different storage periods. PGE_1, having a β-hydroxycyclopentanone moiety, is known to undergo dehydration in acidic or basic aqueous solutions to give prostaglandin A_1 (PGA_1), which further isomerizes to prostaglandin B_1 (PGB_1) in alkaline medium (Stehle and Oesterling, 1977).

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SSDI 0378-5173(94)00241-X

Detailed studies on the stability of PGE_1 in aqueous solutions (Stehle and Oesterling, 1977; Lee and DeLuca, 1991) and in lipid emulsion (Teagarden et al., 1989) have been performed, but only preliminary information has been reported on the stability of PGE_1 in physiological solution (Fraccaro et al., 1992). Thus, it was considered necessary to gain further information on the chemical stability of PGE_1 in physiological solution in order to verify the clinical indications and to suggest the most appropriate conditions for the storage of PGE_1 physiological solutions.

Among the various analytical methods developed for the determination of prostaglandins, high-performance liquid chromatography (HPLC) was chosen for its advantages such as selectivity and feasibility (Papadoyannis, 1990). Prostaglandins do not show strong absorption in the UV-visible region or native fluorescence; therefore, to enhance the sensitivity of the HPLC method prechromatographic derivatizations of the side chain carboxylic group to give UV-absorbing (Papadoyannis, 1990; USP XXII, 1990) or fluorescing (Papadoyannis, 1990; Toyo'oka et al., 1992; Wolf and Korf, 1992) derivatives are widely applied. The derivatization technique, however, may not be suitable for stability studies of PGE_1, since the derivatization conditions could accelerate the degradation reactions (dehydration and internal rearrangement). Thus, when the analysis does not requires high sensitivity, direct low-wavelength (200–215 nm) UV detection can be also used (Teagarden, 1988; Lee and DeLuca, 1991).

In the present study, both the described approaches have been followed and compared. Thus, derivatization of PGE_1 with 2-bromoacetyl-6-methoxynaphthalene, followed by HPLC analysis (fluorescence detection), was applied to the evaluation of the stability of PGE_1 in physiological solutions (10 μg/ml) and the results were then compared with those obtained by direct HPLC determination with UV detection at 205 nm.

Alprostadil (PGE_1) was obtained from Sigma (St. Louis, MO, U.S.A.); tetrahexylammonium bromide (THxABr), cortisone and pelargonic acid were from Fluka (Buchs, Switzerland); all other chemicals were from Farmitalia C. Erba (Milan, Italy). The reagent 2-bromoacetyl-6-methoxynaphthalene (Br-AMN) and the naphthacyl ester of pelargonic acid (the internal standard) were prepared as previously described (Gatti et al., 1992; Cavrini et al., 1993).

The reagent (Br-AMN) solution was prepared in acetone or acetonitrile (4.2 mg/ml) and was found to be stable for 2 weeks at 4°C. Tetrahexylammonium bromide (THxABr) solution (20 mM) was prepared in aqueous 100 mM phosphate buffer (pH 7.0). A stock PGE_1 solution was prepared in absolute ethanol and maintained at 4°C (Doehl and Greibrokk, 1990). Working standard solutions of PGE_1 were prepared in physiological solution-ethanol 9 : 1 (v/v) at the desired concentration (4.0–20 μg/ml).The solutions of TEA (1%), pelargonic acid naphthacyl ester (4.5 μg/ml) and cortisone (60 μg/ml) were prepared in acetonitrile.

The pre-column derivatization of PGE_1 was carried out as follows: 0.1 ml of PGE_1 solution, 0.1 ml of water, 0.15 ml of 20 mM tetrahexylammonium bromide solution (pH 7) and 0.1 ml of the reagent solution in acetone were subjected to ultrasonication for 3 min and allowed to stand at ambient temperature for 25 min. Then, 0.15 ml of the internal standard solution were added, the reaction mixture ultrasonicated for 1 min and a 50 μl volume of the resulting clear solution was injected into the chromatograph.

The HPLC analyses were performed using a Varian 2010 pump and a Varian 2070 fluorescence detector, operating at an emission wavelength of 460 nm with an excitation wavelength of 300 nm, connected to a personal computer IBM XT. The JCL 6000 chromatography data system was used. When UV detection was applied, a Jasco Uvidec 100 detector connected to a Varian 4270 integrator was used. Manual injections were carried out using a Rheodyne model 7125 injector with a 50 μl sample loop.

The HPLC separations of derivatized PGE_1 were performed at 35°C on a 5 μm Hypersil ODS stainless-steel column (250 × 4.6 mm i.d.) under isocratic conditions. A mobile phase consisting of the binary mixture A-B (70 : 30, v/v), where A = acetonitrile-methanol-tetrahydrofuran 40 : 40 : 20

(v/v) and B = water, at a flow rate of 1.2 ml/min was used.

Underivatized PGE_1 was chromatographed on a 10 μm C-18 Phenomenex Bondclone (300 × 3.9 mm i.d.) column using acetonitrile-0.02 M KH_2PO_4 42:58 (v/v) as the mobile phase at a flow rate of 1 ml/min

According to previous experience (Cavrini et al., 1993), the derivatization of PGE1 with Br-AMN was carried out in aqueous medium in the presence of tetrahexylammonium bromide; these conditions were also suggested by evidence of reduced acid-catalyzed dehydration of PGE_1 in the presence of cationic surfactants (Teagarden et al., 1989). The effects of temperature and ultrasonication on the reaction course were evaluated and, as a result, ambient temperature with initial (3 min) ultrasonication proved to be the most convenient conditions. Continuous ultrasonication and higher temperatures were found to be responsible for PGE_1 degradation. The presence of a high content of organic solvent (ethanol, methanol, acetone and acetonitrile) in the reaction mixture was found to prevent the formation of the PGE_1 ester. For comparison purposes, using Br-AMN, derivatization in acetonitrile according to the USP XXII method was also carried out. The derivatization in aqueous medium (room temperature for 25 min) proved to be advantageous over that in acetonitrile (45°C for 30 min), the latter conditions being responsible for greater degradation of the reagent.

Chromatographic separations were performed under isocratic conditions on a reversed-phase (Hypersil 5ODS) column, using the mobile phase described above. These conditions allowed adequate separations between derivatized PGE_1, the internal standard and the reagent degradation products to be achieved (Fig. 1).

When underivatized PGE_1 was analyzed, complete resolution between the analyte and internal standard was readily achieved (Fig. 2a) using a simple binary mixture as the mobile phase.

The suitability of the HPLC method, involving pre-column derivatization and fluorescence detection (λ_{exc} = 300 nm; λ_{em} = 460 nm), was evaluated for the direct analysis of aqueous PGE_1 samples. Under the chromatographic conditions

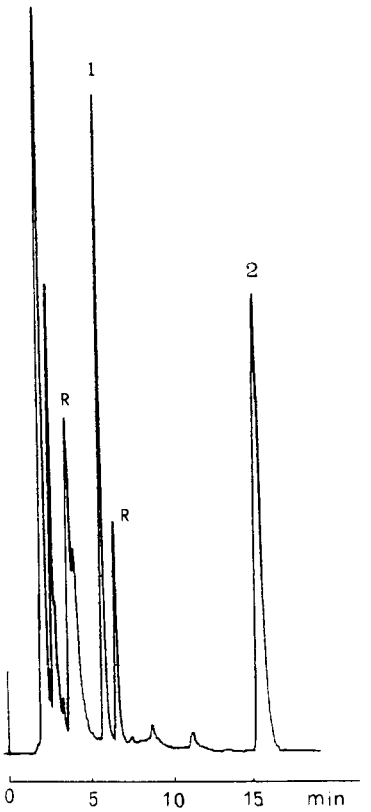

Fig. 1. Typical HPLC separation of PGE_1 (1) and pelargonic acid (2, the internal standard) derivatized with Br-AMN. R = reagent peak; column, Hypersil ODS (5 μm); mobile phase, mixture A-B (70:30, v/v), where A = acetonitrile-methanol-tetrahydrofuran (40:40:20, v/v) and B = water, at a flow rate of 1.2 ml/min. Fluorescence detection: λ_{em} = 460 nm; λ_{exc} = 300 nm.

of Fig. 1, a linear relationship was obtained between the peak height ratio of derivatized PGE_1 to internal standard (pelargonic acid naphthacyl ester) (Y) and the drug concentration (C; 11.28–33.85 nmol/ml) ($Y = 0.027C - 0.035$; $r = 0.9970$; $n = 6$). A similar, concordant calibration graph ($Y = 0.030C - 0.045$; $r = 0.998$; $n = 6$) was obtained when the PGE_1 derivatization was performed in acetonitrile. The first method was chosen for routine analysis, being more practical and rapid. The precision of the method was satisfactory, as indicated by the relative standard deviation (RSD = 2.4%) obtained from replicate (n = 8) analyses (derivatization and HPLC separation) of a single PGE_1 standard solution. When UV

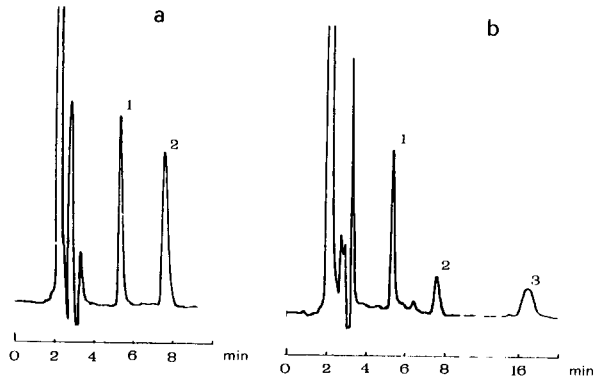

Fig. 2. HPLC chromatograms obtained from: (a) PGE_1 physiological solution (10 μg/ml), freshly prepared; (b) after 30 days storage at ambient temperature. Peaks: 1, cortisone (the internal standard); 2, PGE_1; 3, PGA_1; column, C-18 Phenomenex Bondclone; mobile phase, acetonitrile-0.02 M KH_2PO_4 (42:58, v/v) at a flow rate of 1 ml/min. UV detection at 205 nm.

detection was applied, PGE_1 standard solutions (4–20 μg/ml), containing a fixed concentration (0.1 mg/ml) of cortisone (internal standard) were directly analysed to obtain a linear relationship between the peak area ratio (analyte to internal standard) (Y) and drug concentration ($Y = 0.045C - 0.0116$; $r = 0.9996$; $n = 6$).

The proposed methods were applied to the evaluation of the stability of PGE_1 physiological solutions prepared from two different commercial PGE_1 preparation, Prostin VR and a lyophilized PGE_1 (Sigma) properly diluted in ethanol and physiological solution to the desired concentration (10 μg/ml) in ethanol-physiological solution (2:98, v/v). The samples were maintained under different conditions: (a) 2–8°C in the dark, (b) ambient temperature in the dark, and (c) ambient temperature in sunlight. Each PGE_1 sample was analysed by both HPLC methods, using derivatization-fluorescence detection and direct UV detection. The samples ($n = 2$) were analysed after 0, 15, 30, 90 and 120 days and triplicate analyses were performed for each sample. The results expressed as percentages of the initial concentration remaining for each condition are reported in Table 1 and illustrated in Fig. 3. As shown, only PGE_1 solutions stored at 2–8°C displayed good stability, their PGE_1 content being reduced to about 85% of the initial level after 3–4 months. The solutions maintained at ambient temperature decomposed rapidly after 15 days. A representative chromatogram obtained from a PGE_1 sample maintained at room temperature for 30 days is reported in Fig. 2b. The additional peak (no. 3) at longer retention time proved to be PGA_1; in fact,

Table 1
Stability of PGE_1 in physiological solution

Sample [a]	Storage temperature	Time zero concentration (μg/ml)	% initial concentration at day				
			15	30	60	90	120
A 1	2–8°C	10	98.50 (1.25)	93.00 (1.10)	88.60 (0.93)	86.10 (1.16)	83.00 (1.30)
			98.00 (1.70)	92.40 (2.05)	89.50 (1.86)	87.43 (2.10)	85.40 (2.25)
2	r.t. [b]	10	94.55 (0.85)	56.40 (0.95)	38.65 (1.15)	25.60 (1.60)	11.50 (2.13)
			95.30 (1.65)	61.00 (1.84)	40.05 (2.10)	27.80 (2.65)	12.20 (2.95)
3	r.t. [c]	10	90.50 (0.95)	43.64 (1.24)	24.63 (1.52)	11.60 (1.84)	9.53 (1.63)
			89.85 (2.10)	45.00 (2.32)	26.43 (2.25)	12.80 (2.87)	10.45 (3.35)
B 1	2–8°C	9.7	95.00 (1.32)	91.15 (1.04)	86.00 (0.94)	82.14 (0.94)	80.00 (1.32)
			94.35 (2.15)	90.00 (2.42)	86.64 (2.14)	83.10 (2.25)	80.50 (2.30)
2	r.t. [b]	9.7	85.00 (0.84)	37.64 (1.34)	26.55 (1.67)	10.43 (2.12)	8.06 (2.36)
			83.15 (2.16)	35.00 (2.24)	–	–	–
3	r.t. [c]	9.7	83.00 (0.92)	32.45 (1.04)	23.00 (1.25)	8.50 (2.43)	6.70 (2.65)
			82.25 (1.90)	30.70 (2.54)	–	–	–

[a] Sample A was prepared from PGE_1 (Sigma); sample B was prepared from Prostin VR solution.
[b] Room temperature in the dark.
[c] Room temperature in sunlight.
The data, obtained by HPLC-UV and HPLC-fluorescence methods, are expressed as percentage (mean and RSD%) of the initial concentration ($n = 6$). –, not done.

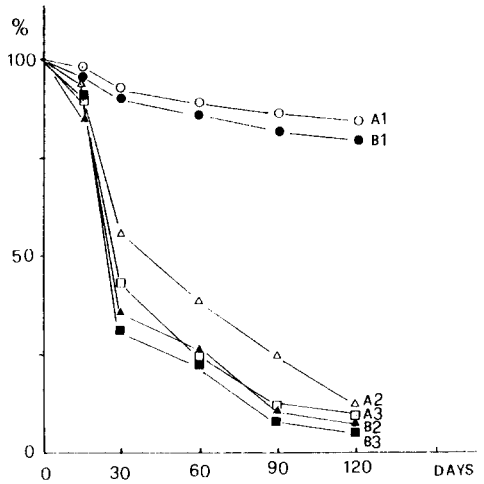

Fig. 3. Stability of PGE_1 in physiological solution. Mean percentage of the initial PGE_1 concentration found over a 4 month period. Conditions and data as in Table 1.

according to the PGA_1 spectrum (Lee and DeLuca, 1991), a stronger response was obtained when detection was set at 230 nm. Peaks were not observed using UV detection at 280 nm, the absorption maximum of PGB_1, indicating that PGA_1 was not appreciably converted into PGB_1 under the described conditions. Conversely, the expected intense peak at longer retention time due to PGB_1 was observed when a forcedly degraded PGE_1 sample at pH 10 was analysed with detection at 280 nm (Lee and DeLuca, 1991).

In summary, the data achieved with two different HPLC methods were concordant in suggesting that PGE_1 physiological solutions, for the treatment of erectile dysfunction, should be stored at 2–8°C. Under these conditions, the PGE_1 level was found to remain at about 85% of the initial content after 3 months, while at ambient temperature, with or without exposure to sunlight, the drug was subjected to serious degradation.

Acknowledgements

This work is part of the Dottorato di Ricerca of R. Gotti. Financial support from MURST and CNR is gratefully acknowledged.

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