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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY FOR PROSTAGLANDIN E_1 IN VARIOUS OINTMENT VEHICLES

SEPARATION AND STABILITY TESTING

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SUMMARY

A high-performance liquid chromatographic method for the determination of prostaglandin E_1 (PGE_1) incorporated in white petrolatum, white ointment, hydrophilic petrolatum and Plastibase was investigated. The prostaglandin was separated from the oleaginous vehicles with *n*-hexane–aqueous acetonitrile. Most of white petrolatum, which is a principal component in the vehicles, remained in the *n*-hexane layer, and the recovery of the drug from any vehicle attained 100%. The method was applied to stability studies of PGE_1 in white petrolatum and macrogol ointment. In which pure PGE_1 and PGE_1–α-cyclodextrin complex (PGE_1–CD) were incorporated. The drug remained intact for up to 6 months when stored at 5°C. At 25 and 40°C, pure PGE_1 was more stable than PGE_1–CD and both PGE_1 species were more stable in white petrolatum than in macrogol ointment.

INTRODUCTION

The biological significance of prostaglandins (PGs) has been widely recognized and their pharmacological actions cover a wide range of therapeutic applications[1], and the quantitative determination of PGs is therefore of great interest. Extensive efforts have been made to determine micro-amounts of these agents in biological fluids, and methods used include thin-layer chromatography[2], gas–liquid chromatography–mass spectrometry[3], high-performance liquid chromatography (HPLC)[4–6] and biochemical methods such as radioimmunoassay[7] and a smooth muscle response[8].

In the small-scale compounding of semi-solid PG preparations that are prescribed in some cases in topical applications such as pressure sores and psoriasis vulgaris, the content uniformity and stability of the ingredient in the preparations must be controlled in order to ensure the correct dose. Semi-solid systems are so

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chemically complex that there is a problem in separating the ingredient from the vehicles, often requiring special analytical techniques to obtain a given ingredient as pure and concentrated as possible[9].

This study explores the separation and subsequent HPLC determination of PGE_1, selected as a model PG, incorporated in white petrolatum, white ointment, hydrophilic petrolatum and Plastibase. The method was then applied to the investigation of the stabilities of PGE_1 and its α-cyclodextrin complex incorporated in white petrolatum and macrogol ointment.

EXPERIMENTAL

Materials

PGE_1–α-cyclodextrin lyophilized (PGE_1–CD, 20 μg potency per ampoule, Prostangin; Ono Pharmaceutical, Osaka, Japan) was used as received. PGE_1 was obtained from Funakoshi Chemical Industries (Tokyo, Japan). White petrolatum (WP) J.P., white ointment (WO) J.P., hydrophilic petrolatum (HP) J.P., macrogol ointment (MO) J.P. and hydrophilic ointment (HO) J.P. (J.P. = Japanese Pharmacopoeia) were obtained from Maruishi Pharmaceutical (Osaka, Japan). Plastibase (PB) was obtained from Taisho Pharmaceutical (Tokyo, Japan). Distilled water, *n*-hexane, ethanol and acetonitrile were of HPLC quality, and potassium phosphate monobasic was of reagent grade (Wako, Osaka, Japan).

Preparation of ointments

Ointments were prepared by the incorporation method. Each ointment base (15–30 g) was first accurately weighed into a mortar, to which pure PGE_1 was gradually added so as to give specified contents (40, 80, 120 and 160 μg/g of vehicle) and mixed with a pestle.

Extraction of PGE_1 from WP ointment

A 0.25-g portion of the PGE_1–WP ointment (120 μg/g) was accurately weighed and transferred into a 20-ml centrifugal tube, to which *n*-hexane (2–5 ml) was added and shaken on a vortex mixer, giving a turbid solution. Then 2 ml of aqueous acetonitrile (40%, the same as the mobile phase) were added as an extraction phase for the prostaglandin and shaken vigorously for 10 min. The resulting emulsion was centrifuged at 3000 rpm for 5 min, 2 ml of the upper turbid (*n*-hexane) layer was transferred into a small flask and was evaporated to dryness in an oven (2 h, 60°C), and the residue was weighed. Under these drying conditions, no weight change of WP itself was observed. The *n*-hexane layer was replaced with fresh *n*-hexane three times (3 ml each). The fraction of WP remaining in the *n*-hexane phase was calculated by reference to the total amount of WP sampled above. On the other hand, after the upper layer had been aspirated out, 100 μl of the lower transparent layer were injected into the chromatograph and the recovery of PGE_1 was quantitated by reference to a calibration graph for the prostaglandin.

For the assay of PGE_1 in various semi-solid preparations, the *n*-hexane phase, whose volume was fixed at 3 ml, was replaced with fresh *n*-hexane three times to remove oleaginous components.

Chromatographic conditions

A Waters Assoc. Model 6000A HPLC apparatus equipped with a Waters Assoc. Model 441 UV detector and a reversed-phase column (μBondapak-C_{18}, 10 μm, 30 cm × 3.9 mm I.D.; Waters Assoc.) was used. The mobile phase was a mixture of 0.02 *M* monobasic potassium phosphate (pH 4.9) and acetonitrile (3:2, v/v), operated at a flow-rate of 1.5 ml/min. The column eluate was monitored at 214 nm with a sensitivity of 0.02 a.u.f.s. and a chart speed of 5 mm/min. Peak areas were obtained with a Shimadzu (Kyoto, Japan) C-R1A reporting integrator. All analyses were performed at room temperature.

Calibration

The concentration of PGE_1 was determined by reference to a regression line that was constructed using known concentrations of the drug ranging from 2.5 to 50 μg/ml in the mobile phase. The relationship between the concentration (ordinate) and the peak area (abscissa) was linear, giving an intercept of 0.2, a slope of 0.00018 and a correlation coefficient of 0.998. The amount of PGE_1 in the ointment (x) was calculated from the equation A (μg/g of ointment) = $(0.00018x + 0.2) \cdot 2.0/W$ (weight of ointment sampled).

Stability studies

Pure PGE_1 and PGE_1–CD, supplied as lyophilized powder, were incorporated in WP and MO ointments in the same manner (120 μg potency of PGE_1 per gram of vehicle). They were packaged in porcelain-white plastic jars and stored at 5, 25 and 40°C for up to 6 months. For the MO ointment, no extraction was carried out because it was readily miscible with the mobile phase.

RESULTS AND DISCUSSION

Recovery of PGE_1

Ointments are semi-solid preparations whose vehicle components are chemically very complex, and white beeswax, stearyl alcohol, cholesterol, white petrolatum, anhydrous lanolin, liquid paraffin, polyethylene and macrogol are employed in large amounts[10]. To avoid interferences from these components in the HPLC assay, it is necessary to remove the vehicle components as far as possible, which will also ensure the reliability of the chromatograph column. In the various ointment bases employed, WP is generally formulated to the greatest extent, 90–100% (w/w) for HP, WO and WP[10]. PB contains a large amount of liquid paraffin (95%, w/w)[11].

To extract PGE_1 from the oleaginous vehicles, the *n*-hexane–acetonitrile–water system was examined as a measure of the amount of WP remaining in the *n*-hexane layer and the magnitude of the transfer (recovery) of PGE_1 to the aqueous phase containing acetonitrile (40%, v/v).

Table I shows the fraction (wt.-%) of WP remaining in the *n*-hexane layer and the PGE_1 recovery, in which the volume of *n*-hexane was varied from 2 to 5 ml with an aqueous extraction phase of 2 ml. The amount of WP in the organic phase increased slightly with increasing volume, ranging from 91 to 95%.

The organic phase was replaced with fresh *n*-hexane three times (3 ml each), and by this means WP was almost eliminated from the extraction phase for PGE_1 (Table I).

TABLE I

FRACTION OF WP REMAINING IN *n*-HEXANE AND THE RECOVERY OF PGE_1 IN THE AQUEOUS ACETONITRILE PHASE

PGE_1 incorporated: 120 μg/g of WP. Volume of the aqueous acetonitrile phase: 2 ml.

n-Hexane (ml)	*No. of n-hexane replacements*	*WP remaining in n-hexane (n = 5) (%)*	*PGE_1 recovery (n = 6) (%)*
2	1	91.2 ± 2.1	101.2 ± 1.5
3	1	93.5 ± 2.2	99.1 ± 1.8
	2	4.3 ± 1.6	99.6 ± 1.9
	3	0	99.3 ± 1.5
4	1	95.3 ± 2.2	99.1 ± 1.5
5	1	94.6 ± 1.8	100.0 ± 1.4

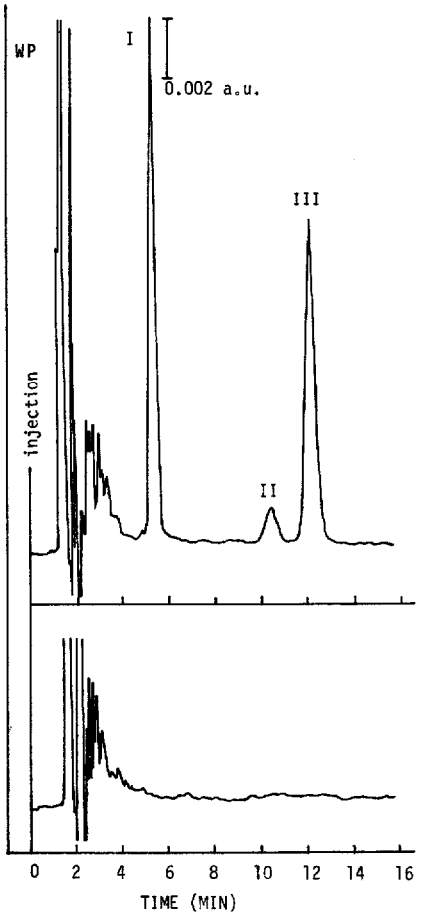

Fig. 1. Chromatograms of PGE_1(I), PGA_1(II) and PGB_1(III) extracted from WP ointment. Above: spiked extraction solution (40% acetonitrile). PGE_1 was arbitrily decomposed by dilute NaOH and incorporated in WP (120 μg/g as total PG). Below: blank.

Simultaneously, the amount of PGE_1 extracted into the aqueous acetonitrile phase was measured. Fig. 1 shows a typical chromatogram of the drug extracted from WP ointment, the *n*-hexane layer having been replaced three times. Although several unidentified peaks were observed with short retention times, the PGE_1 peak was hardly affected and was completely separated from its products, PGA_1 and PGB_1. The recovery of the drug was not affected by the volume or the number of times the organic layer was replaced and was assumed to be 100% under any conditions.

The extraction procedure for PGE_1 was extended to HP, WO and PB ointments that contained various components other than WP or liquid paraffin. Oleaginous components were repeatedly removed by *n*-hexane as described above. Fig. 2 shows chromatograms of PGE_1 extracted from these vehicles. As with the WP ointment, the separation of PGE_1 from unidentified peaks with short retention times was satisfactory.

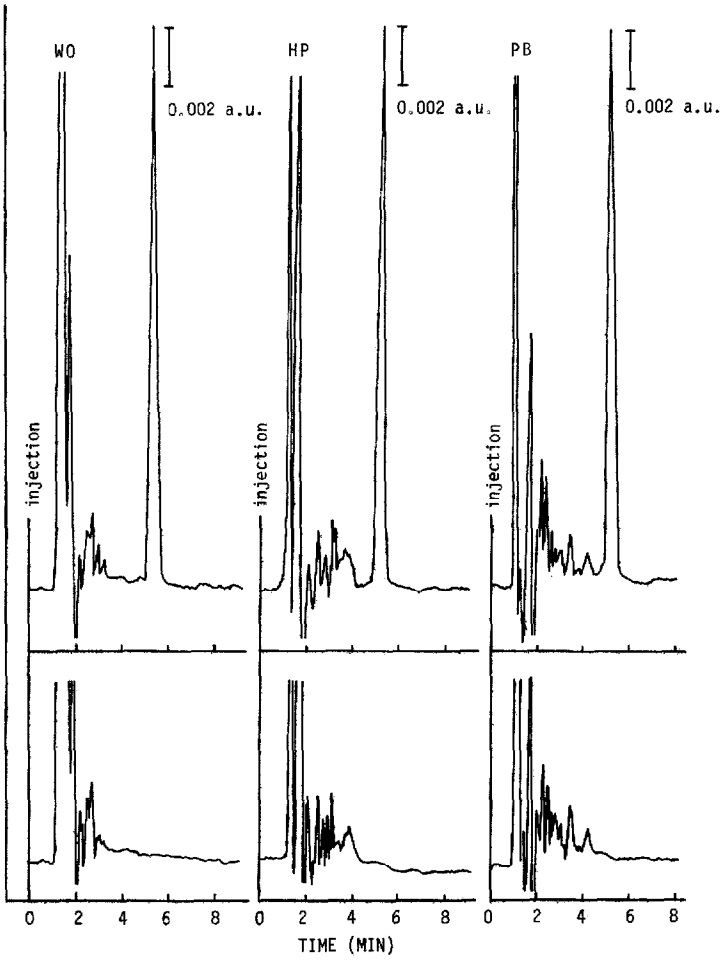

Fig. 2. Chromatograms of PGE_1 extracted from WO, HP and PB ointments (120 μg/g of vehicle). Above, spiked extraction solution (40% acetonitrile); below, blank.

TABLE II

RECOVERY OF PGE_1 FROM VARIOUS OINTMENT VEHICLES

Vehicle	*PGE_1 content ($\mu g/g$)*	*Recovery ($n = 6$) (%)*
WP	160	98.5 ± 1.6
	120	99.3 ± 2.1
	80	100.0 ± 1.9
	40	99.9 ± 1.1
WO	160	100.3 ± 1.9
	120	99.2 ± 1.7
	80	99.3 ± 1.5
	40	99.5 ± 2.3
HP	160	100.0 ± 1.8
	120	99.0 ± 1.9
	80	98.9 ± 2.3
	40	98.0 ± 2.0
PB	160	101.1 ± 1.0
	120	99.1 ± 1.1
	80	98.9 ± 1.9
	40	99.0 ± 1.5

Table II shows the recovery of PGE_1 from various ointment bases; quantitative extraction was well established at various contents of the prostaglandin incorporated.

Stability studies

The stabilities of PGE_1 and PGE_1–CD incorporated in WP and MO ointments and stored at different temperatures were investigated for up to 6 months. WP and MO were selected as representatives of oleaginous and hydrophilic vehicles, respectively. There was no difference between pure PGE_1 and PGE_1–CD with respect to the recovery of the prostaglandin from WP. Either pure PGE_1 or PGE_1–CD incorporated in MO ointment was well separated from the ointment base (Fig. 3). Several series of determinations of different contents of the drug (40–60 $\mu g/g$) were as comparable and reproducible as other ointment vehicles. The results are shown in Tables III and IV.

At 5°C, PGE_1 remained essentially intact for at least 6 months or longer for either of the PGE_1 species, irrespective of the vehicle.

At 25°C, there were differences in the species of prostaglandin incorporated and the vehicles: the apparent first-order rate constants were approximately $7.7 \cdot 10^{-4}$ day^{-1} for PGE_1 and $1.2 \cdot 10^{-3}$ day^{-1} for PGE_1–CD in WP ointment and $1.4 \cdot 10^{-3}$ day^{-1} for PGE_1 and $1.8 \cdot 10^{-3}$ day^{-1} for PGE_1–CD in MO ointment. It is of interest that the pure form of PGE_1 was generally more stable than its cyclodextrin complex in either of the vehicles, and the WP base was superior to the MO base as far as stability is concerned. The same tendency was observed at 40°C. The reasons for these phenomena are unknown.

The results indicate that the therapeutically effective content in both ointments is well maintained for 6 months or longer when they are stored at 5°C. Further, the

TABLE III

STABILITY OF PURE PGE_1 IN WP AND MO OINTMENTS

Time (days)	*Remaining PGE_1 (%)**					
	WP			*MO*		
	5°C	*25°C*	*40°C*	*5°C*	*25°C*	*40°C*
40	103.1	100.5	100.2	100.3	95.1	93.3
90	100.2	97.3	91.1	95.8	90.4	82.1
120	96.9	92.3	82.2	93.9	87.2	70.6
180	99.7	85.6	72.5	100.1	70.8	61.0

* Each value indicates the mean of two locations in the vehicle.

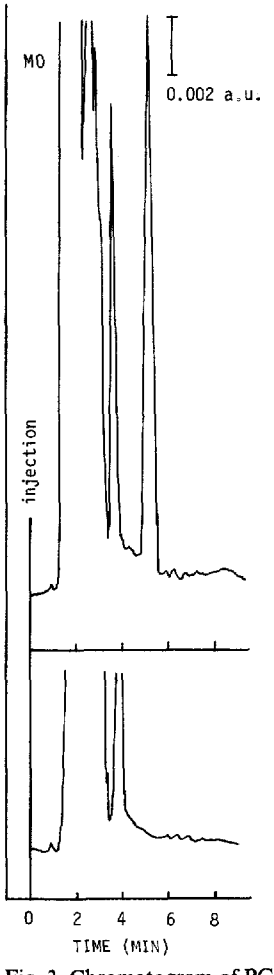

Fig. 3. Chromatogram of PGE_1 in MO ointment (120 μg/g of vehicle). Above, spiked sample diluted with aqueous acetonitrile (40%); below, blank.

TABLE IV

STABILITY OF PGE_1–CD IN WP AND MO OINTMENTS

Time (days)	*Remaining PGE_1 (%)**					
	WP			*MO*		
	5°C	*25°C*	*40°C*	*5°C*	*25°C*	*40°C*
45	98.2	95.4	91.3	97.4	93.2	88.4
95	104.8	92.2	81.4	92.7	83.5	80.0
125	100.3	85.6	73.5	85.4	82.1	68.2
180	101.2	78.3	64.0	92.3	68.5	55.1

* Each value indicates the mean of two locations in the vehicle.

content remained high enough for practical purposes for 3 months in the PGE_1–WP system and for 1 month in the PGE_1–MO system at room temperature. The PGE_1–CD ointment should remain effective with the proviso that it is used within 1 month when stored at room temperature.

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