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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY OF ANTI-INFLAMMATORY DRUGS INCORPORATED IN GEL OINTMENTS

SEPARATION AND STABILITY TESTING

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SUMMARY

High-performance liquid chromatographic determinations of gabexate mesilate (FOY), prostaglandin E_1 (PGE_1), PGE_1-α-cyclodextrin (PGE_1-CD), prednisolone (PD) and butyl flufenamate (BF) incorporated in gel ointment were investigated. The gel ointment is composed of a carboxy vinyl polymer (1.3%, w/w) and a large amount of an aqueous organic solvent. A methanol extraction system offered simultaneous advantages of the removal of the polymer and the recovery of active ingredients from the gel phase. The recoveries of the drugs were 100%. The stabilities of PGE_1, PGE_1-CD, FOY, PD and BF incorporated in gel ointment, stored at 5, 25 and 40°C for up to 90 days, were investigated.

INTRODUCTION

Carboxy vinyl polymers are used as vehicles in pharmaceutical and cosmetic systems[1–3], the gel component being a high-molecular-weight water-soluble acid polymer, forming a gel with high viscosity when neutralized by bases[4]. The resulting gel is a semi-solid system that consists of the polymer component at very low concentrations (usually 0.5–1.5%) and a large amount of fluids (water or water plus organic solvents). The drugs incorporated are usually dissolved in the fluid phase and it is therefore important to know their stabilities and solubilities under such circumstances.

The extemporaneous compounding of gel ointments with several anti-inflammatory drugs in our laboratory required a rapid and reliable method for examining content uniformity and the stability of each active ingredient in the preparation. This paper describes the separation and subsequent high-performance liquid chromatographic (HPLC) determination of gabexate mesilate (FOY), prostaglandin E_1 (PGE_1) and its α-cyclodextrin complex (PGE_1-CD), prednisolone (PD) and butyl

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flufenamate (BF) incorporated in a carboxy vinyl polymer gel. The method was applied to the investigation of the stabilities of these anti-inflammatory drugs.

EXPERIMENTAL

Materials

The carboxy vinyl polymer (HIVISWAKO-104) (Wako, Osaka, Japan) was used as received. FOY was a gift from Ono Pharmaceutical (Osaka, Japan). PGE_1 was obtained from Funakoshi Chemical Industries (Tokyo, Japan) and PGE_1-CD (20 μg potency per ampoule) from Ono Pharmaceutical, and were used as received. PD was obtained from Tokyo Kasei Kogyo (Tokyo, Japan). BF was a gift from Tokyo Tanabe Pharmaceutical (Tokyo, Japan). Distilled water, methanol and acetonitrile were of HPLC quality. Sodium 1-pentanesulphonate (LOW-UV PIC B5) was obtained from Waters Assoc. (Milford, MA, U.S.A.). All other reagents were of analytical-reagent grade.

Preparation of gel ointments

Aqueous and propylene glycol solutions of HIVISWAKO-104 were separately prepared at concentrations of 2 and 3% (w/w), respectively. The aqueous solution (45 g) and propylene glycol solution (15 g) were added to ethanol (20 g) and mixed, then an ethanol solution (10 g) of the drug to be incorporated was added. The whole solution was neutralized with 6 g of 5% ammonia solution, using a stirrer with a disc propeller (B-100, No. 3; Tokyo Rika Kikai, Tokyo, Japan). The total weight of the mixture was then adjusted to 100 g with distilled water. The amount of the drug incorporated was adjusted so as to give specified contents: FOY, 0.5–1.5; PGE_1, 0.008–0.016; PGE_1-CD, 0.008–0.16 (as potency); PD, 0.1–0.5; BF, 0.5–2.0% (w/w).

Extraction of active ingredients

A 0.1–0.3-g portion of gel ointment was accurately weighed and transferred into a 20-ml culture tube, then methanol (3 ml) was added with vigorous mixing, resulting in precipitation of the polymer. The methanol fraction was passed through a Millipore filter (0.2 μm pore size) (Millipore, Bedford, MA, U.S.A.), 2 ml of which were evaporated to dryness with a stream of nitrogen in the case of FOY and PGs. The residue was then dissolved in the mobile phase prepared for each drug and 10 and 100 μl, respectively, of the solutions were injected into the chromatograph. For PD and BF, 10 μl of each methanol fraction were passed through a Millipore filter (0.2 μm pore size) and injected into the chromatograph.

Chromatographic system

A Shimadzu LC-4A HPLC system (Shimadzu, Kyoto, Japan) was used, consisting of a SIL-1A LC injector, an SPD-2AS spectrophotometric detector and a C-R1A chromatopac data processor. A reversed-phase μBondapak C_{18} (10 μm) column (30 cm × 3.9 mm I.D.) (Waters Assoc.) was used. The following chromatographic conditions were used for respective drugs: for FOY, mobile phase methanol–water (7:3, v/v) containing 0.005 *M* sodium 1-pentanesulphonate, detection wavelength 235 nm, sensitivity 0.16 a.u.f.s., flow-rate 1.0 ml/min; for PGs, 0.02 *M* monobasic potassium phosphate (pH 4.9)–acetonitrile (3:2, v/v), 214 nm, 0.005

a.u.f.s., 1.5 ml/min; for PD, methanol–water (6:4, v/v), 254 nm, 0.08 a.u.f.s., 1.0 ml/min; and for BF, methanol, 280 nm, 0.04 a.u.f.s., 1.0 ml/min. All analyses were performed at ambient temperature.

Removal of polymer

A 1-g amount of gel vehicle was accurately weighed and transferred into a 20-ml culture tube, to which methanol (3, 5 and 10 ml) was added and the mixture was shaken on a vortex mixer. Then, 2 ml of the methanol fraction were withdrawn into a 10-ml beaker, evaporated to dryness (70°C, 5 h) and the residue was weighed.

Calibration

Chromatograms were quantitated by reference to the peak area. A regression line of peak area *vs.* concentration was constructed by analysing known amounts of the drug added to each mobile phase. The relationship between the concentration (ordinate) and the peak area (abscissa) was linear for all drugs, giving A (μg/g of ointment) = $(0.00024x + 11.6) \times 3.0/w$ (weight of ointment sampled) for FOY, $A = (0.00038x + 1.68) \times 1.5/w$ for PGs, $A = (0.00023x - 0.15) \times 3.0/w$ for PD and $A = (0.00020x + 0.94) \times 3.0/w$ for BF.

Stability studies

Gel ointments of FOY (1.0%, w/w), PGE_1 (0.012%), PGE_1-CD (0.012% potency of PGE_1), PD (0.3%) and BF (1.25%) were prepared as mentioned above. They were packed in hermetic glass vials and stored at 5, 25 and 40°C for up to 3 months. The stability of FOY ointment containing $FeCl_3$ (0.1%) was also examined after storage under the same conditions.

RESULTS AND DISCUSSION

Recovery of active ingredients from gel ointments

Carboxy vinyl polymer gel ointment is a semi-solid preparation and consists of the polymer (1.3%, w/w) and a large amount of an aqueous organic solvent. Although the concentration of the polymer is very low, it is desirable to remove it as far as possible from the samples prior to HPLC assay, otherwise the reliability of the chromatographic column may deteriorate.

The polymer component exists in the form of an ammonium salt in the gel ointment, and it was observed that the polymer was precipitated on addition of methanol. However, it is unclear whether complete precipitation of the polymer occurs. Therefore, the transfer of the polymer into the methanol phase was examined by measuring the residual weight in the methanol fraction, in which the volume of the methanol phase was varied from 3 to 10 ml with 1 g of the gel vehicle. The results indicated that the transfer into the methanol phase was negligible, irrespective of the volume of methanol added (data not shown).

Simultaneously, the extent of extraction of the methanol system for the various drugs was examined. Fig. 1 shows typical chromatograms for FOY, PGE_1 (or PGE_1-CD)[5], PD and BF extracted from gel ointments, giving retention times of 5.7, 5.2, 7.2 and 4.3 min, respectively. The peaks of FOY, PGE_1 and PD were not subject to interference from their degradation products. In the chromatogram of PGE_1, un-

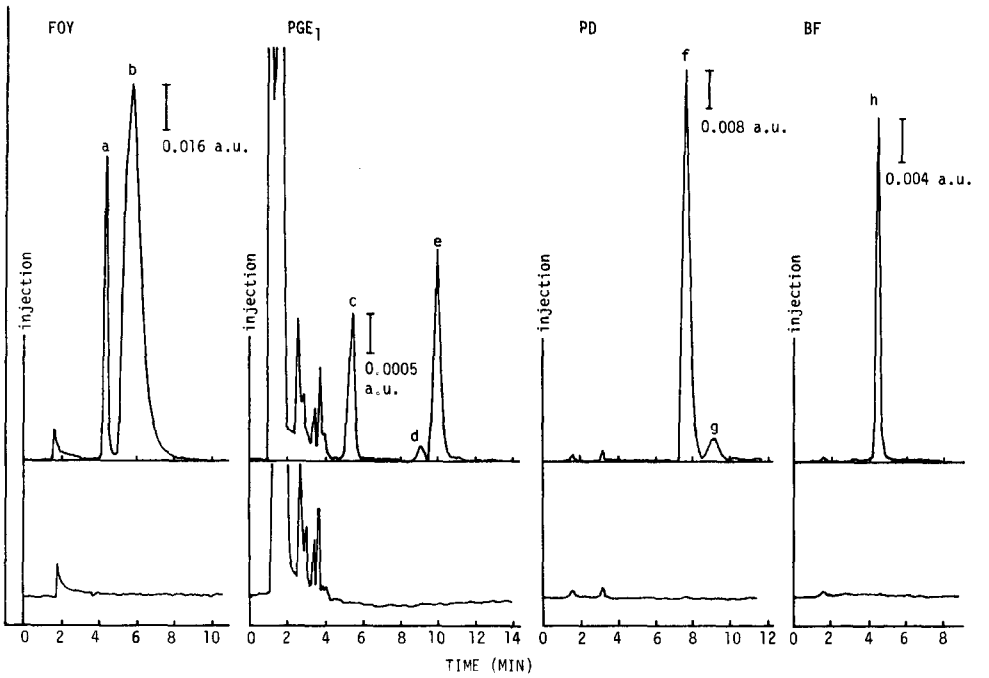

Fig. 1. Chromatograms of FOY, PGE_1, PD and BF extracted from gel ointments. Above: spiked extraction solution. FOY and PGE_1 were decomposed by dilute HCl and PD by increasing temperature in aqueous solution, and then incorporated in the gel ointment. Below: blank. (a) Ethyl *p*-hydroxybenzoate; (b) FOY; (c) PGE_1; (d) PGA_1; (e) PGB_1; (f) PD; (g) 11-β-hydroxy-1,4-androsten-3,17-dione; (h) BF.

TABLE I

RECOVERY OF FOY, PGE_1, PGE_1-CD, PD AND BF FROM GEL VEHICLE

Drug	*Amount incorporated (%)*	*Recovery ± S.D. (n = 6) (%)*
FOY	0.5	99.8 ± 1.6
	1.0	101.3 ± 2.1
	1.5	100.5 ± 1.9
PGE_1	0.016	97.3 ± 2.2
	0.012	99.1 ± 1.3
	0.008	96.4 ± 1.8
PGE_1-CD	0.016	97.5 ± 1.3
	0.012	98.6 ± 2.0
	0.008	98.1 ± 1.5
PD	0.5	101.2 ± 1.3
	0.25	99.7 ± 2.1
	0.1	100.3 ± 1.5
BF	2.0	99.6 ± 2.2
	1.0	100.4 ± 1.9
	0.5	101.3 ± 2.0

identified peaks with short retention times are probably due to traces of components extracted from the polymer vehicle. They seem to have been detected because a very high sensitivity at a short detection wavelength was used for the prostaglandin.

The recovery of the drugs by the methanol extraction system was investigated in the concentration ranges specified earlier, as shown in Table I. Quantitative extraction was achieved for various contents of the drugs incorporated. There was no difference between pure PGE_1 and PGE_1-CD ointments with respect to the recovery of the prostaglandin from the gel phase. Hence the methanol extraction offered the simultaneous advantages of removal of the polymer and the recovery of active ingredients.

Stability studies

The stabilities of FOY, PGE_1, PGE_1-CD, PD and BF incorporated in the gel vehicle were investigated at different temperatures for up to 3 months. The apparent first-order rate constants (k_{app}) obtained from first-order plots of their degradation are presented in Table II, together with the periods during which more than 90% potency under the various storage conditions is maintained. For comparison, degradation data for FOY, PGE_1 and PD in aqueous solutions (25°C) are also given in Table II.

TABLE II

DEGRADATION OF ANTI-INFLAMMATORY DRUGS IN GEL OINTMENT

Drug	k_{app} *(day^{-1})**			
	5°C	*25°C*	*40°C*	*25°C (aq.)***
FOY	$1.0 \cdot 10^{-2}$ (10)***	$5.8 \cdot 10^{-2}$ (2)	n.d.§	$1.9 \cdot 10^{-1}$
FOY + 0.1% $FeCl_3$	$7.5 \cdot 10^{-3}$ (14)	$3.9 \cdot 10^{-2}$ (3)	n.d.	
PGE_1	$8.1 \cdot 10^{-4}$ (129)	$4.0 \cdot 10^{-3}$ (26)	$1.5 \cdot 10^{-2}$ (7)	$4.5 \cdot 10^{-2}$
PGE_1-CD	$2.3 \cdot 10^{-3}$ (46)	$6.1 \cdot 10^{-3}$ (17)	$1.5 \cdot 10^{-2}$ (7)	
PD	Stable	$2.2 \cdot 10^{-3}$ (48)	$1.2 \cdot 10^{-2}$ (9)	$3.0 \cdot 10^{-2}$
BF	Stable	Stable	Stable	

* The value of k_{app} was calculated from the data for 90 days' storage.

** Aqueous medium: pH 7.4 phosphate buffer for FOY and PGE_1 and distilled water for PD.

*** Values in parentheses indicate the period (days) during which more than 90% of the potency, estimated from the rate constant, was maintained.

§ n.d., not determined.

FOY, which is known to be hydrolysed to ε-guanidinocaproic acid and ethyl *p*-hydroxybenzoate[6], was easily degraded even at 5°C, probably owing to the large amount of water in the gel, resulting in no practical use. Kohbo[7] reported that FOY was stabilized by the addition of 0.1% of $FeCl_3$ to a macrogol ointment. The stability of the drug in the gel ointment containing $FeCl_3$ (0.1%, w/w) was slightly improved, but was still far from satisfactory for practical purposes.

With regard to the prostaglandin gel ointments, there were significant differences between PGE_1 and PGE_1-CD, as observed in a previous study[5] in which they were incoporated in white petrolatum and macrogol ointment. Different temperature

dependences of degradation were observed in the range 5–40°C. At 5°C, pure PGE_1 was much more stable than PGE_1-CD, its therapeutically effective content being maintained for about 4 months whereas PGE_1-CD would be effective for about 1.5 months. The prostaglandin, however, was more susceptible to degradation in the gel vehicle than in conventional ointments such as white petrolatum and macrogol ointment.

The stability of PD incorporated in various ointments has been studied[8] and the drug is susceptible to degradation especially with lauromacrogol, sorbitan sesquioleate and cetanol[9]. The PD content in gel ointment was well maintained for 3 months or longer when stored at 5°C. At higher temperatures, the degradation rates were comparable to the results with the ointment components mentioned above and the product was the same, 11-β-hydroxy-1,4-androsten-3,17-dione.

The BF content remained practically constant for at least 3 months or longer, even at 40°C.

These anti-inflammatory drugs may be inactivated faster in the gel vehicle than in conventional ointment bases. However, the advantage of the gel vehicle includes possibly improved drug delivery capabilities owing to the presence of an adequate amount of moisture and organic solvents that may act as promoting agents. Therefore, greater therapeutic effects may be achieved in the form of a lyogel, outweighing its disadvantages, if it is administered with careful instructions.

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