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QUANTITATIVE DETERMINATION OF ALPROSTADIL (PGE_1) IN BULK DRUG AND PHARMACEUTICAL FORMULATIONS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

An adsorption high-performance liquid chromatographic method suitable for the quantitative determination of the purity of alprostadil (prostaglandin E_1) bulk drug and of the concentration of alprostadil in Prostin V.R. Pediatric® Sterile Solution is described. A variety of isomers and related compounds including 8-iso-PGE_1, 11-*epi*-PGE_1, 15-*epi*-PGE_1, PGE_2, 5,6-*trans*-PGE_2, and 8-iso-PGE_2 are separated from alprostadil and are quantifiable. The 2-naphthacyl ester derivatives are employed and derivatization conditions providing maximum response are described.

INTRODUCTION

Alprostadil, prostaglandin E_1, is the active ingredient in Prostin V.R. Pediatric® Sterile Solution. This product was recently approved by the United States food and Drug Administration for use in maintaining the patency of the ductus arteriosus in neontal infants until corrective surgery can be performed. A variety of methods have been employed for the determination of prostaglandins. A 1972 review by Oesterling *et al.*[1] discussed spectral, thin-layer, and gas chromatographic methods as well as radioimmuno, enzymatic and bioassays. A later review by Morozowich, *et al.* discussed gas chromatographic, gas chromatography–mass spectrometry and liquid chromatographic procedures[2].

Most prostaglandins possess minimal molar absorptivity in the ultraviolet or visible regions, therefore the majority of the high-performance liquid chromatographic (HPLC) methods involve derivatization. Generally, the carboxylic acid group common to almost all prostaglandins is derivatized. Several derivatizing reagents have been employed and their use has been reviewed by Morozowich and Cho[3]. The most frequently formed UV derivatives are phenacyl esters[4] and 2-naphtacyl esters[5]. The 4-bromomethyl-7-methoxycoumarin[6] and panacyl bromide [*p*-(9-anthryloxy)phenacyl bromide][7] reagents have been used to produce fluorescent derivatives of various prostaglandins.

This report describes assay methods suitable for determining the purity of bulk drug alprostadil, the levels of foreign prostaglandins in bulk drug and in sterile solution, and the concentration of alprostadil in the sterile solution. The 2-naphthacyl ester de-

rivative was employed. Because the naphthacyl esters are less polar than most of the other derivatives it was anticipated that superior separation of alprostadil from structurally similar compounds and isomers would be obtained. Since good sensitivity and adequate resolution of alprostadil from these compounds was obtaines, other derivatives were not evaluated.

EXPERIMENTAL

HPLC conditions

A modular HPLC system consisting of an Altex Model 110 pump, a loop injection valve, a Tracor Model 770 autosampler or an in-house designed and built autosampler, and an LDC UV Monitor III Model 1203 fixed-wavelength (254 nm) detector was used. A Waters Assoc. μPorasil column (30 cm × 3.9 mm I.D., 10 μm) was employed. The mobile phase consisted of 995 ml of methylene chloride, 5 ml of 1,3-butanediol and 0.5 ml of water. Flow-rates were 1.5 ml/min for the determination of alprostadil and 1.0 ml/min for foreign prostaglandins. Injection volume was 10 μl. Mobile phases were filtered and degassed before use. Hexane was used as the void volume marker.

Reagents and chemicals

Samples of 8-iso-PGE_1, 8-iso-PGE_2, 5,6-*trans*-PGE_2, 11-*epi*-PGE_1, and 15-*epi*-PGE_1 were obtained from F.H. Lincoln, Experimental Science I, The Upjohn Company. Acetonitrole and methylene chloride were Burdick & Jackson distilled-in-glass solvents. N,N-Diisopropylethylamine (DIPEA) (D-12,580-6), α-bromoacetonaphthone (α-BAN) (10,512-0), and 1,3-butanediol (B-8,478-5) were obtained from Aldrich. DIPEA and 1,3-butanediol were used as received. DIPEA and α-BAN were used as solutions in acetonitrile of 10 μl/ml and 20 mg/ml respectively, except where noted. These solutions were prepared fresh daily.

Stotz and Hassing[8] reported than α-BAN could contain several impurities, which were not effectively removed by the generally recommended procedure of recrystallization from carbon tetrachloride with carbon treatment. Preparative HPLC was required to remove these impurities. Most of the work described in this report was performed with α-BAN recrystallized from carbon tetrachloride; however, the identity of all peaks was verified using chromatographically purified α-BAN. It is recommended that chromatographically purified α-BAN be used for determining foreign prostaglandins.

Optimization of derivatization conditions

At least four samples were run at each set of conditions and the results averaged; 200 μl of α-BAN–acetonitrile solution and 100 μl of DIPEA–acetonitrile solution were used in all cases.

Alprostadil

Internal standard solution. Prepare a 0.4 mg/ml solution of methylprednisolone in methylene chloride.

Standard preparation. Accurately prepare a solution of *ca.* 0.5 mg/ml alprostadil reference standard in absolute ethanol. Transfer an accurately known volume equiv-

alent to *ca.* 1 mg to a suitable vial. Gently evaporate to dryness with a stream of nitrogen

Sample preparation: bulk drug. Proceed as described for the standard preparation.

Sample preparation: Prostin V.R. Pediatric® Sterile Solution. Transfer an accurately known volume of solution equivalent to *ca.* 1 mg of drug to a suitable vial and gently evaporate to dryness.

Procedure. Add 200 μl of α-BAN solution and swirl to wash down the sides. Add 100 μl of DIPEA solution and swirl again. Cap and heat at 45°C for 60 min. Every 15 min swirl each vial to ensure that all the alprostadil dissolves. Evaporate the acetonitrile solution to dryness under a stream of nitrogen. Add 10.0 ml of internal standard solution to each standard and sample. Inject 10 μl of each standard and sample into the chromatograph.

Foreign prostaglandins

Sample preparations: bulk drug. Weigh *ca.* 2.0 mg of sample. Place in a suitable vial, dissolve in 2 ml of absolute ethanol, and gently evaporate to dryness.

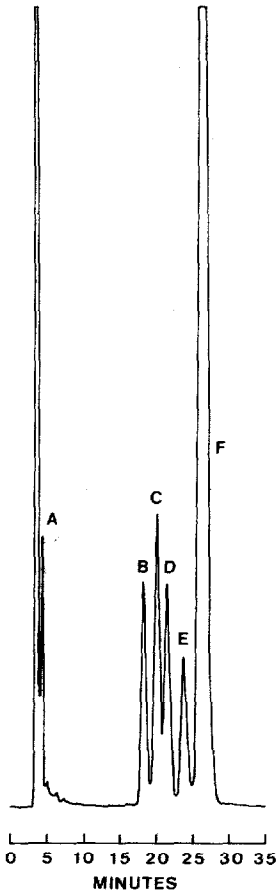
Sample preparation: sterile solution. Transfer an accurately known volume of sterile solution equivalent to 2 mg of alprostadil to a suitable vial and gently evaporate to dryness with a stream of nitrogen.

Procedure. Derivatize as described for the alprostadil assay. After derivatization evaporate the acetonitrile under a stream of nitrogen and add 10 ml of methylene chloride to each sample. Chromatograph 10 μl.

RESULTS AND DISCUSSION

The chromatographic system developed for alprostadil is based on the system described by Brown and Carpenter[5] for carboprost (15-*R*-15-methyl-prostaglandin $F_{2\alpha}$). These workers demonstrated that resolution of carboprost isomers was readily accomplished. The selectivity achieved in this system for the separation of alprostadil is demonstrated in Fig. 1, a chromatogram of alprostadil plus added related compounds as run in the Foreign prostaglandin method. A typical chromatogram of a sample preparation is illustrated in Fig. 2. The structures of the compounds are given in Fig. 3. The separation factors (relative to alprostadil) for each compound are shown parenthetically after the compound name. The major degradation products of alprostadil, prostaglandins A_1 and B_1 are significantly less polar than the analogs shown in Fig. 1 and elute in the solvent front in this system. A procedure for the measurement of PGA_1 and PGB_1 in alprostadil is described in the following paper[9].

The selectivity in this system is due to masking of the highly polar carboxyl group with the derivatization reagent, which permits the subtle polarity differences due to isomerization or unsaturation to influence the retention behavior of the compounds. In addition to masking the polarity of the carboxyl group, the use of the α-BAN derivative permits monitoring at 254 nm and the assumption of equimolar response for each of the foreign prostaglandins and alprostadil, since for each compound the absorbing chromophore is the same. This permits the estimation of the levels of the foreign prostaglandins on an area-percent basis. Using the conditions described, foreign prostaglandins in alprostadil were easily quantifiable at the 0.1% level. This corresponds to 2 ng injected on-column.



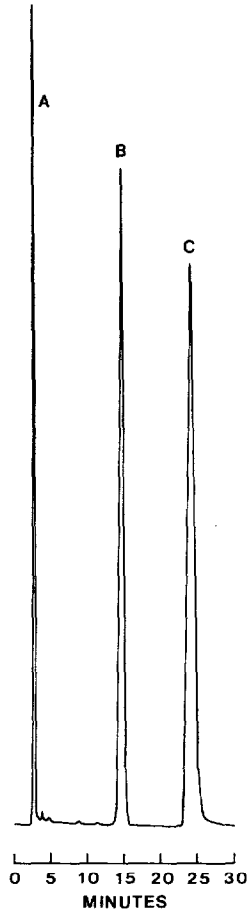


Fig. 1. Chromatograms of alprostadil with added foreign prostaglandins; flow-rate, 1.0 ml/min. Peaks: A = excess derivatizing reagent; B = 8-iso-PGE_2; C = 5,6-*trans*-PGE_2; D = PGE_2; E = 8-iso-PGE_1; F = PGE_1.

Fig. 2. Sample preparation of alprostadil; flow-rate = 1.5 ml/min. Peaks: A = excess derivatizing reagent; B = PGE_1; C = internal standard methylprednisolone (separation factor = 1.8).

Conformance of the detector response to Beer's law was demonstrated by determining the ratio of the peak height of alprostadil to the internal standard at various alprostadil concentrations from 40% to 200% of the recommended analytical concentration. The equation of the line produced in a plot of peak height ratio (PHR) *vs.* milligramms of alprostadil was PHR = 1.47 (mg alprostadil) – 0.071 with a correlation coefficient of 0.999.

The effect of varying the derivatization conditions such as the concentrations of α-BAN and DIPEA, the reaction time and the reaction temperature was investigated and optimal conditions selected. In all studies 200 μl of the α-BAN and 100 μl of DIPEA solutions in acetonitrile were used to derivatize 1 mg of alprostadil. The effect of the derivatization reaction temperature was evaluated by determining the PHR of alprostadil to internal standard at various times at room temperature (21–23°C) and 45°C using 20 mg/ml α-BAN and 10 μl/ml DIPEA. The results of this experiment are

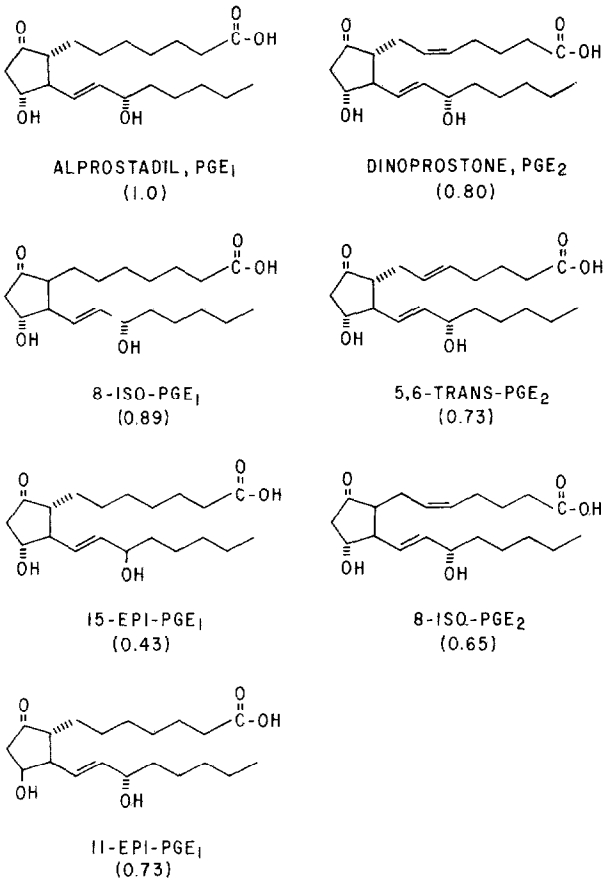


Fig. 3. Structures of alprostadil and related compounds. Separation factors in parentheses.

TABLE I

EFFECT OF REACTION TEMPERATURE ON THE PEAK HEIGHT RATIO (PHR) OF ALPROSTADIL TO INTERNAL STANDARD

Time (min)	*Room temperature**		*45°C*	
	PHR	*R.S.D. (%)*	*PHR*	*R.S.D. (%)*
15	1.40	1.9	1.63	0.7
30	1.43	2.6	1.60	1.9
45	1.44	7.3	1.62	3.9
60	1.43	1.5	1.62	2.0
90	1.45	4.0	1.63	0.7
120	1.60	0.4	1.63	0.5
240	1.62	0.2	1.62	0.4

* 21–23°C.

TABLE II

EFFECT OF DIPEA CONCENTRATION ON THE PEAK HEIGT RATIO (PHR) OF ALPROSTADIL TO INTERNAL STANDARD

DIPEA (μg/ml)	*Room temperature**		*45°C*	
	PHR	*R.S.D. (%)*	*PHR*	*R.S.D. (%)*
5	1.23	2.7	1.66	0.9
10	1.57	1.4	1.70	1.0
15	1.66	0.7	1.66	0.6
20	1.66	1.9	—	—

* 21–23°C.

summarized in Table I. At room temperature the PHR increases with time, reaching a stable value only after 120 min. At 45°C the PHR is constant after only 15 min, indicating that the reaction has reached completion in this time period.

The effect of the DIPEA concentration on the derivatization of alprostadil was evaluated by preparing DIPEA solutions of 5, 10, 15 and 20 μl of the amine per milliliter of acetonitrile. This corresponds to molar ratios of DIPEA to alprostadil of 1:1, 2:1, 3:1 and 4:1, respectively. The α-BAN concentration was held constant at 20 mg/ml; derivatization time was 30 min. These results are summarized in Table II. The PHR shows a dependence on the concentration of the DIPEA when the reaction is carried out at room temperature; however, the use of a 2:1 or greater molar ratio of DIPEA to alprostadil is sufficient to push the reaction to completion. At 45°C, the extent of derivatization is independent of the amount of DIPEA used and the reaction goes to completion.

The effect of the α-BAN concentration was evaluated by preparing derivatizing solutions at concentrations of 10, 15, 20, 25 and 30 mg of α-BAN per milliliter of acetonitrile. Samples of alprostadil were derivatized with these solutions at room temperature and at 45°C for 1 h. DIPEA concentration was 10 μl/ml. The results are summarized in Table III. The PHRs are essentially constant for all concentrations of α-BAN at both room temperature and 45°C. The relative standard deviation (R.S.D.) of the 45°C samples are significantly smaller than those for the room temperature samples, indicating the superior reproducibility of performing the derivatization at 45°C.

On the basis of these studies the following derivatization conditions were selected

TABLE III

EFFECT OF α-BAN CONCENTRATION ON THE PEAK HEIGHT RATIO (PHR) OF ALPROSTADIL TO INTERNAL STANDARD

mg α-BAN per ml acetonitrile	*Room temperature**		*45°C*	
	PHR	*R.S.D. (%)*	*PHR*	*R.S.D. (%)*
10	1.13	3.8	1.20	0.4
15	1.14	1.9	1.20	0.5
20	1.15	0.4	1.19	0.5
25	1.18	1.0	1.20	0.5
30	1.14	4.4	1.19	0.2

* 21–23°C.

TABLE IV

ASSAY PRECISION FOR BULK DRUG

The different peak height ratios reflect the slightly different concentrations of alprostadil used on each of the 3 days.

	Normalized peak height ratio			
	Day 1	*Day 2*	*Day 3*	
Lot A	174.8	147.0	125.5	
	177.2	149.0	124.6	
	187.7	146.6	125.0	
	187.6	148.7	125.9	
	184.3	150.3	126.8	
			126.0	
Mean	182.3	148.3	125.6	Avg. R.S.D.
R.S.D.	3.3%	1.0%	0.6%	1.6%
Lot B	183.1	132.8	124.8	
	179.5	126.4	124.8	
	181.9	125.9	124.5	
	176.3	132.0	124.0	
	179.1	125.2	124.4	
			124.8	
Mean	180.0	128.5	124.6	Avg. R.S.D.
R.S.D.	1.5%	2.8%	0.3%	1.5%

as optimal: 200 μl of a 20 mg/ml solution of α-BAN, 100 μl of a 10 μl/ml solution of DIPEA, and 45°C for 60 min. Under these conditions the derivatization is complete in 15 min. The longer reaction time is recommended because samples derivatized for 60 min gave more reproducible results, *i.e.* the R.S.D. for replicate assays of a sample derivatized for 60 min was consistently lower than for replicate assays of a sample derivatized for only 15 min. At room temperature occasional random samples were observed to give unaccountably low results. In many of these samples a ring or partial ring of drug formed upon evaporating the alcoholic solutions to dryness prior to derivatization. This material was slow to dissolve in the reaction solvent. Allowing the reaction to proceed for 60 min at 45°C, at which temperature the reaction medium is gently rfluxing on the sides of the reaction vessel, insured that all this material would dissolve and react eliminating these outliers. This phenomenon is illustrated in Table III.

The precision of this assay was determined by assaying five replicate samples of two lots of alprostadil on three days. Results of this study are given in Table IV. Within-day R.S.D. for the sets of five replicates ranged from 0.3% to 3.3%. The overall average R.S.D. was 1.6%.

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