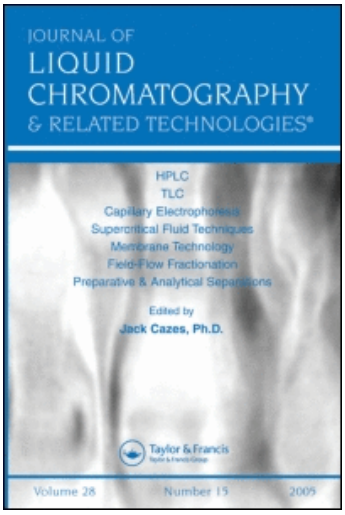


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Liquid Chromatographic Assay of Arbaprostil

G. W. Peng[a]; V. K. Sood[a]
[a] Pharmaceutical Research and Development The Upjohn Co., Kalamazoo, MI

To cite this Article Peng, G. W. and Sood, V. K.(1983) 'Liquid Chromatographic Assay of Arbaprostil', Journal of Liquid Chromatography & Related Technologies, 6: 8, 1499 — 1511
To link to this Article: DOI: 10.1080/01483918308064867
URL: http://dx.doi.org/10.1080/01483918308064867

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(8), 1499-1511 (1983)

LIQUID CHROMATOGRAPHIC ASSAY OF ARBAPROSTIL

G. W. Peng and V. K. Sood
Pharmaceutical Research and Development
The Upjohn Co., Kalamazoo, MI 49001

ABSTRACT

Arbaprostil was extracted from the solution formulations with ethyl ether-chloroform. The extraction efficiency was 94%. After evaporation of the extraction solvent, the samples were derivatized with p-nitrophenacyl bromide at 40°C for 30 minutes in the presence of N,N-diisopropylethyl amine. The prepared samples were dissolved in mobile phase and chromatographed on a silica gel column with acetonitrile, methylene chloride and water (150/350/2.5, v/v) as mobile phase. The chromatography separated the p-nitrophenacyl esters of arbaprostil, its 15-s-epimer and the degradation products derived from arbaprostil. When monitored by UV absorption, the degradation products could not be detected as they eluted near the solvent front under the peak of the derivatization reagent. The chromatographic responses were linear with the concentrations of arbaprostil. Assay results with good precision and accuracy were obtained.

INTRODUCTION

Most prostaglandins have small UV-extinction coefficients and derivatization with suitable chromophores to enhance UV absorbance is usually necessary for their quantitative determination. For prostaglandin free acids, various functionally substituted phenacyl bromide (1,2) and α-bromoacetonaphthone (3) are conveniently employed as derivatizing reagents to form the corresponding esters

 0148-3919/83/0608-1499$3.50/0

which can be separated by high pressure liquid chromatography (HPLC) and quantitated by monitoring the UV absorbance at 254 nm.

The derivatization of prostaglandin with p-nitrophenacyl bromide and the subsequent separation of the resulting esters by HPLC have been investigated (1). This procedure was utilized for the determination of the 15-epimer of dinoprost in bulk drug (4). The same reagent was also used to study the epimerization kinetics of arbaprostil (5). The present paper describes the application of p-nitrophenacyl bromide derivatization on a micro-scale for the quantitative determination of arbaprostil in aqueous formulations containing dextrose, alcohol, triacetin and polyoxyethylated vegetable oil.

Arbaprostil, like other C-15 alkyl substituted prostaglandins, is more resistant to enzymatic oxidation than the C-15 unsubstituted analog by 15-prostaglandin dehydrogenase (6,7). It is readily converted under acidic conditions into its biologically more active 15-s-epimer (5) and therefore serves as a prodrug of the s-epimer which inhibits gastric acid secretion (8) and promotes healing of duodenal and gastric ulcers (9,10). Like other E series prostaglandins, arbaprostil also dehydrates under acidic and basic conditions to form degradation products (5,11). These chemical and biological properties potentially make arbaprostil a highly potent but unstable drug. The facile epimerization and degradation also render the quantitative determination of arbaprostil difficult.

EXPERIMENTAL

Chemical

Arbaprostil, 15-s-epimer of arbaprostil and 17β-hydroxy 17-methyl-4-androstene-3,11-dione (internal standard) were supplied by Pharmaceutical Research and Development Laboratories of The Upjohn Co. (Kalamazoo, MI). p-Nitrophenacyl bromide and N,N-diisopropylethyl amine were obtained from Pfaltz and Bauer, Inc.

(Stamford, CT) and Aldrich Chemical Co. (Milwaukee, WI), respectively, and used as received. Solvents for HPLC were from Burdick and Jackson Laboratory (Muskegon, MI). All other chemicals were reagent grade.

The aqueous formulations of arbaprostil contained 5% dextrose solution U.S.P., alcohol, triacetin and polyoxyethylated vegetable oil (Emulphor® EL-620, GAF Corp., N.Y.).

Extraction

Aliquots of 1 mL of the solution of arbaprostil in the formulations in 15-mL culture tubes fitted with teflon-lined screw caps were supplemented with internal standard, acidified with 0.5 mL 2% phosphoric acid and immediately extracted with 10 mL of ethyl ether-chloroform (4/1, v/v). After centrifugation, 8 mL of the organic extract was transferred to a new tube and the solvent was evaporated at 40°C under a stream of nitrogen. Aliquots of blank formulation vehicle were supplemented with arbaprostil and internal standard to prepare calibration standards for the quantitative determination of arbaprostil.

Derivatization

p-nitrophenacyl bromide, 2.5 mg in 1 mL acetonitrile, and N,N-diisopropylethyl amine, 6.25 µL in 0.5 mL acetonitrile, were added to the residues of the ether-chloroform extract of the samples. The mixtures were tightly capped with teflon-lined screw caps, vortexed briefly and placed in a water bath at 40°C. After 30 minutes, the solvent in the derivatization mixture was evaporated at 40°C under nitrogen. A 2 mL aliquot of HPLC mobile phase was added to each tube containing the derivatization residues. The samples were vortexed prior to chromatography.

Chromatography

The chromatographic analysis of arbaprostil was carried out using a model 6000 A solvent delivery pump (Waters Associates,

Milford, MA), a model 440 detector (Waters Associates) or a model LC 55-B detector (Perkin-Elmer Corp., Norwalk, CT) at 254 nm, a model 7120 sample valve injector (Rheodyne, Inc., Cotati, CA) and a variable span strip chart recorder.

The chromatographic separation was accomplished using a commercially prepared silica gel column (μ-Porasil, Waters Associates) with a mobile phase of acetonitrile, methylene chloride and water (150/350/2.5, v/v). The mobile phase flow rate was 1.5 mL/min. which generated a back pressure of about 1,000 psig.

The peak height ratios of arbaprostil p-nitrophenacyl ester/ internal standard were calculated from the peak height measurements. The concentrations of arbaprostil in the samples were calculated from the peak height ratios of the samples and the slope and intercept obtained by linear regression analysis of the calibration curve data.

RESULTS AND DISCUSSION

Extraction

Good recovery of arbaprostil from the formulations was obtained using ethyl ether-chloroform (4/1, v/v) as the extraction solvent. The extraction efficiency was found to be 94.2 ± 0.67% based on the total radioactivity recovery of tritium-labelled arbaprostil from the formulation.

The extraction of arbaprostil from the formulations was reproducible and simple with no emulsion formation and almost complete phase separation between the aqueous and the organic phases even though the formulation vehicle contained alcohol, triacetin and polyoxyethylated vegetable oil. However, some difficulties were encountered in our experience with the extraction of arbaprostil from its solutions in water. At low concentrations (e.g. 24 μg/mL), neither p-nitrophenacyl esters of arbaprostil nor its s-epimer was detected in the chromatograms of the prepared samples. At higher concentrations, the peak

height derived from arbaprostil was not reproducible among the samples and the peak derived from the s-epimer was often observed in the chromatograms.

The difficulties encountered in the extraction of arbaprostil from water were not associated with poor extraction recovery, since the recovery of total radioactivity of tritium-labelled arbaprostil from water was essentially complete. The chromatography of samples prepared from tritium-labelled arbaprostil solution in water showed that at least 50% of the radioactivity was eluted from the column at early retention times and, when monitored by UV absorption, the radioactive peaks were near the solvent front and masked by the peak from the reagent. Most of the remainder of the radioactivity was eluted at the same retention times as the p-nitrophenacyl esters of arbaprostil and its s-epimer. Figure 1 shows the chromatograms of a sample prepared from labelled arbaprostil solution in water monitored by both UV absorption and radioactivity. The radioactivity chromatogram was obtained by counting the collected fractions of the column effluent. Similar experiments with a solution of tritium-labelled arbaprostil in the formulation vehicle showed that only very small quantities of the radioactivity were eluted at retention times different from that of arbaprostil ester. When the glassware was siliconized (Surfasil® Pierce Chemical Co., Rockford, IL) before use for the sample preparation, most of the difficulties encountered in the extraction of arbaprostil from water were eliminated and the extraction recovery became reproducible. Therefore, the extraction recovery of arbaprostil from water was complicated by rapid epimerization and degradation of arbaprostil in water and these processes were probably catalyzed by the surface of the glassware. The siliconization of the glassware had no obvious effect on the extraction recovery of arbaprostil from the formulation solutions.

Derivatization

The formation of p-nitrophenacyl esters of various prostaglandins for chromatography was reported previously (1). For the

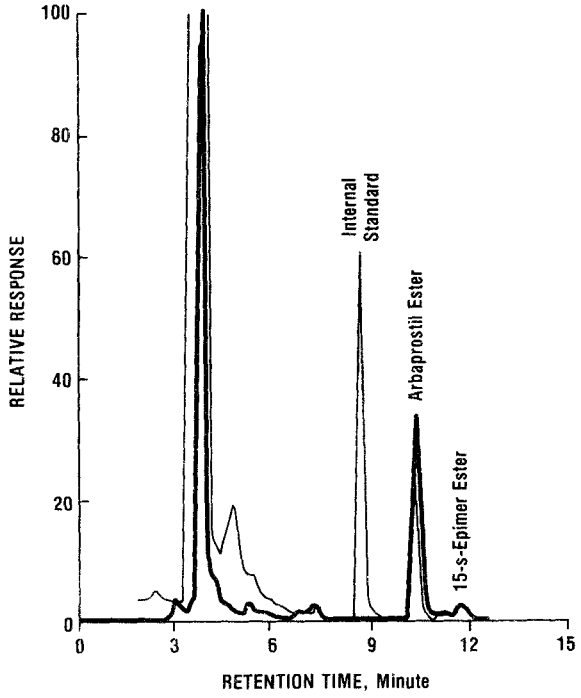


FIGURE 1. Chromatograms of a sample of ^{3}H-arbaprostil extracted from water. Mobile phase flow rate: 1 mL/min., UV absorption: ——, Radioactivity: ▬▬.

present application, we found that the reaction of the extract of 1 mL aliquots of the formulations containing about 20-800 µg (0.05-2.19 µmole) arbaprostil with 2.5 mg (10.25 µmole) of p-nitrophenacyl bromide and 6.25 µL (0.04 µmole) of N,N-diisopropylethyl amine at 40°C for 30 minutes produced the best results. These conditions resulted in minimal interferences in the chromatographic separation and a rapid and quantitative esterification of arbaprostil. Derivatization at lower temperatures (e.g., 22 and 30°C) required longer reaction times and derivatization at higher temperatures (e.g., 50°C) caused a decrease in arbaprostil peak heights after 15 minutes, indicating a potential stability problem. The conditions of p-nitrophenacyl esterification of arbaprostil at room tempera-

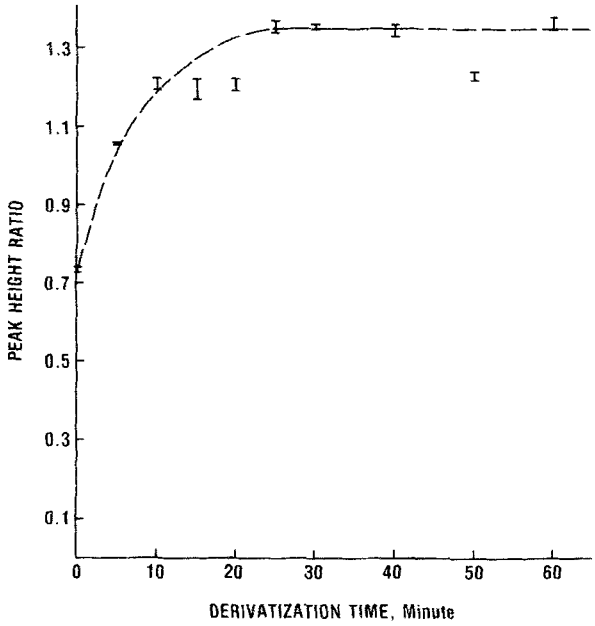


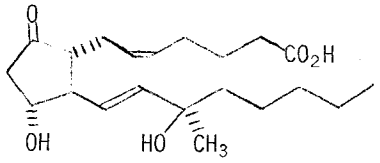
FIGURE 2. Time course of p-nitrophenacyl esterification of arbaprostil. Derivatization time vs. peak height ratio.

ture overnight were employed previously (5). Figure 2 shows the time course of p-nitrophenacyl esterification of arbaprostil under the optimal conditions. The arbaprostil ester/internal standard peak height ratios were measured to monitor the progress of the derivatization. The results shown in Figure 2 indicate that the reaction reached completion at about 25 minutes and the arbaprostil ester was stable in the reaction mixture at 40°C for at least 40 minutes. The internal standard was stable under the conditions of the derivatization.

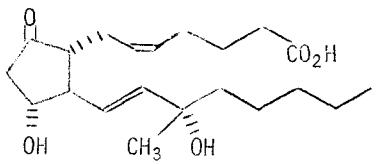
Chromatographic Separation and Linearity of Detection

Representative chromatograms of the p-nitrophenacyl ester of arbaprostil preparation from the solutions in formulation and in water as well as a sample of a blank formulation vehicle are

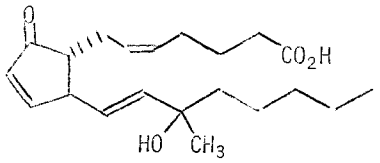
shown in Figure 3. The background interferences from the derivatization reagents and the extract of the formulation vehicle (Figure 3-a) were negligible and did not hinder the analysis of the formulation samples having arbaprostil concentrations of 20 μg/mL or higher. The internal standard and the p-nitrophenacyl esters of arbaprostil and s-epimer were eluted from the column with mobile phase flow of 1.5 mL/min. at the retention times of about 5.8, 6.8 and 7.7 minutes, respectively. The s-epimer was often present in arbarprostil solutions in water (Figure 3-d) but was very seldom observed in the formulations (Figures 3-b and -c).



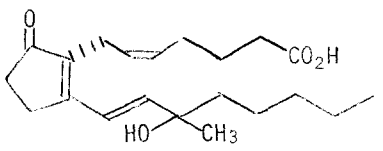
arbaprostil



15-s-epimer



15-methyl prostaglandin A_2



15-methyl prostaglandin B_2

As revealed by radioactivity, the early elution peaks near the solvent front derived from arbaprostil and masked by the peaks of reagent were probably from the A_2 and/or B_2 type prostaglandins (5,11) and other degradation products (5). Under the chromatographic conditions, the esters of 15-methyl prostaglandins A_2 and B_2 were eluted early. Under the conditions of gradient elution, using mobile

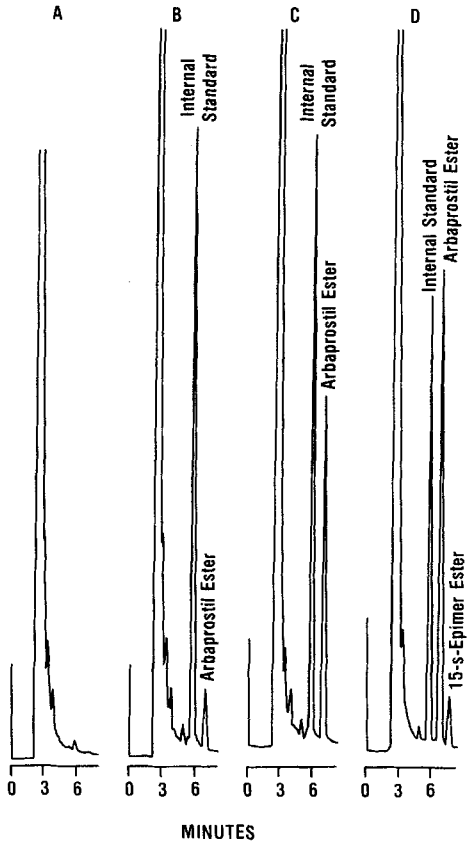


FIGURE 3. Chromatograms of samples of (A) blank formulation, (B) formulation with 24 µg/mL arbaprostil, (C) formulation with 150 µg/mL arbaprostil, and (D) solution in water with 301 µg/ml arbaprostil. Samples B, C, and D contained internal standard.

phase of methylene chloride, acetonitrile and 2-propanol from 350/50/5 (v/v) to 350/150/5 (v/v) and a silica gel column (µ-Porasil), these degradation products were separated into 4 peaks. Two of these peaks had retention times similar to those of the epimers of 15-methyl prostaglandins A_2 and B_2. The other two peaks had different retention times. From these preliminary results, the identity of these components could not be established.

The esterified arbaprostil and the internal standard were stable in the mobile phase solution at ambient temperature (22°C) for at least 72 hours. The peak heights of arbaprostil ester and the internal standard showed no change when the sample was chromatographed during this period of time. The chromatography of the calibration standards immediately after preparation and 72 hours thereafter also showed no significant change in the slopes of the resulting calibration curves. For example, the calibration curves with slopes of 0.00396 and 0.00401 and the intercepts of 0.00890 and -0.00653 were obtained by repeated chromatography of a set of calibration standards immediately and 72 hours, respectively, after their preparation.

When the derivatized samples were evaporated, redissolved in mobile phase and chromatographed, the arbaprostil ester/internal standard peak height ratios were proportional to the arbaprostil concentration in the range from about 20 to at least 600 μg/mL in formulation solution. This proportionality was indicated by the constant peak height ratios obtained by the chromatography of a 600 μg/mL sample and its dilutions and by the linearity of the calibration curves. The correlation coefficients of the calibration curves were usually better than 0.999 with negligible intercepts. The slopes did not vary significantly from day to day. For example, 21 calibration curves prepared over a span of 7 weeks had an average slope of 0.003994 ± 0.000141 (mean ± S.D., RSD = 3.5%, range = 0.003756 to 0.004284).

When the derivatization mixture in acetonitrile was directly chromatographed, the peak height ratios were found to vary with the injection volume. Replacing acetonitrile (by evaporation) with the mobile phase as the solvent for injection of the derivatization mixture, identical peak height ratios were obtained independent of the injection volume (5-20 μL). These observations were reproducible simply by switching the solvent. Table 1 shows some typical peak height ratios obtained by injection of the acetonitrile solution of a sample. The peak height ratios

TABLE 1

Effect of Injection Volume on Peak Height Ratio of Arbaprostil (200 μg) p-Nitrophenacyl Ester and Internal Standard (75 μg) in Acetonitrile (2 mL).

Injection Volume, μL	2.5	5	10	15
Peak Height Ratio	2.1987	2.5370	3.4528	4.3243

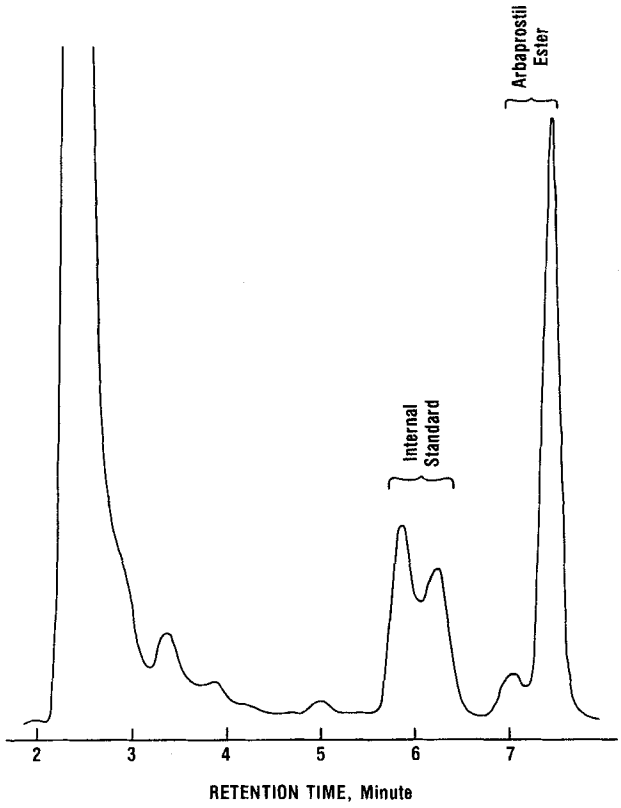


FIGURE 4. Chromatograms of acetonitrile solution of the derivatization mixture of arbaprostil and internal standard. Injection volume 25 μL.

increased from 2.1987 for a 2.5 µL injection to 4.3243 for a 15 µL. For injection volumes of 20 and 25 µL, the formation of double peaks was obvious for both the internal standard and the arbaprostil ester as shown in Figure 4.

The above phenomena of changing peak height ratios with injection volume were related to the peak broadening which led to the double peak formation (Figure 4). Injection of acetonitrile probably disturbed the equilibrium between the stationary and the mobile phases with respect to water present in the system. This disturbance influenced the adsorption interaction between the solutes and the silica stationary phase and changed the chromatographic separation. When small quantities of water (5-15 µL) were added to the acetonitrile solution (2 mL) of the derivatization mixture, the chromatographic peak broadening and splitting were effectively suppressed. Methylene chloride and ethyl ether did not completely dissolve the derivatization mixtures. However, the supernates resulting from centrifugation of the mixtures in methylene chloride and in ethyl ether behaved like the mobile phase and the water supplemented acetonitrile solutions in that there was no peak broadening and that the chromatographic peak height ratios did not vary with the injection volume. The proportionality between peak area and concentration was not investigated.

TABLE 2

Assay Accuracy and Precision

Label Conc. µg/mL	No. of Sample	Concentration found Mean ± S.D., µg/mL	% RSD	% of Label
24.1	4	23.95 ± 0.25	1.05	99.4
301.6	4	295.10 ± 1.58	0.54	97.9
626.8	4	615.60 ± 1.69	0.28	98.2

Assay Accuracy and Precision

Table 2 shows some data indicating the accuracy and precision of this analytical method. At arbaprostil levels of 24.1, 301.6 and 626.8 μg/mL in the formulations, the concentrations found were within 3% of the corresponding theoretical values and the relative standard deviations were less than 2%. These represented the excellent assay accuracy and the intra-assay reproducibility. The reproducible slopes of the 21 calibration curves with a relative standard deviations of 3.5% as mentioned earlier were an indication of the excellent inter-assay reproducibility.

ACKNOWLEDGEMENTS

F. A. Fitzpatrick and M. A. Wynalda are acknowledged for their helpful suggestions.

REFERENCES

1. W. Morozowich and S.L. Douglas, Prostaglandins, 10, 19 (1975).
2. F.A. Fitzpatrick, Anal. Chem., 48, 499 (1976).
3. R.W. Stotz and D.H. Hassing, Anal. Chem., 54, 345 (1982).
4. T.J. Roseman, S.S. Butler and S.L. Douglas, J. Pharm. Sci., 65, 673 (1976).
5. M.V. Merritt and G.E. Bronson, J. Am. Chem. Soc., 100, 1891 (1978).
6 E.W. Yankee and G.L. Bundy, J. Am. Chem. Soc., 94, 3651 (1972).
7. E.W. Yankee, U.F. Axen and G.L. Bundy, J. Am. Chem. Soc. 96, 5865 (1974).
8. W. Peterson, W. Feldman, I. Taylor and M. Bremer, Dig. Dis. Sci., 24, 381 (1979).
9. W.P. Fong and S.M.M. Karim, Med. J. Australia, 2, 127 (1976).
10. G. VanTrappen, Gastroenterology, 78, 1283 (1980).
11. R.G. Stehle and T.O. Oesterling, J. Pharm. Sci., 66, 1590 (1977).