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Analysis of Oxprenolol in Formulations by High-Performance Liquid Chromatography

ADNAN EL-YAZIGI

Received December 6, 1982, from the *Department of Cancer and Radiation Biology Research, King Faisal Specialist Hospital and Research Centre, Riyadh 11211, Saudi Arabia.* Accepted for publication May 10, 1983.

Abstract □ A simple, accurate, and rapid high-performance liquid chromatographic method for the analysis of oxprenolol in commercial formulations is described. The analysis was performed on a cyano radial compression cartridge, with 0.0539 M, pH 3 phosphate buffer–acetonitrile–methanol (76:15.6:8.4) as the mobile phase. The flow rate was 5 mL/min, with detection at 272 nm; the mobile phase was employed for extraction. The assay was applied to the content uniformity test of three oxprenolol hydrochloride tablet formulations of different strengths and the contents of a 2-mg dry ampule for intravenous/intramuscular injection. The percent of label claim for each formulation tested was within 91.7–110%. The applicability of this assay to the analysis of some other β-blocking drugs was investigated. It was found that under the above conditions, atenolol, metoprolol, oxprenolol, and propranolol can be fully resolved in <3 min.

Keyphrases □ Oxprenolol—tablet and dry ampule formulations, assay alone or simultaneously with other β-adrenergic drugs, HPLC □ β-Adrenergic drugs—oxprenolol, propranolol, atenolol, and metoprolol, individual and simultaneous assay in formulations, HPLC

Oxprenolol, (±)-1-[*o*-(allyloxy)phenoxy]-3-(isopropylamino)-2-propanol (I), is a β-adrenergic drug frequently used in the treatment of hypertension (1), cardiac arrhythmias (2), and angina pectoris (3). The low dosage of I, particularly when administered intravenously, and its susceptibility to light and other storage conditions require assurance of potency and content uniformity of its dosage forms.

Several methods have been reported for the analysis of I in biological fluids including GC (4–8), TLC with fluorescence detection (9), and high-performance liquid chromatography (HPLC) (10, 11). While the GC assays involve prechromatography derivatization and multiple-step extraction, the TLC method includes derivatization of I to a fluorescing compound. The utilization of HPLC for the analysis of I provides a real advantage, since it does not involve lengthy derivatization steps and the compound is detected directly. However, the previously reported HPLC methods, in addition to lacking a high chromatographic efficiency, have not been applied to the analysis of I in formulations. No pharmacopeial assay of I is available.

The present report describes an expedient, accurate, and specific HPLC method for the analysis of I in formulations. The procedure can be applied for the chromatography of some other β-blocking drugs (atenolol, metoprolol, nadolol, and propranolol) under the same conditions employed for I.

EXPERIMENTAL

Materials—Methanol[1], acetonitrile[1], hexane[2], monobasic sodium phosphate[2], and 85% phosphoric acid[2] were either analytical or HPLC grade. Purified water[3] was used as obtained. The oxprenolol hydrochloride formulations[4] tested (20-, 40-, and 80-mg tablets and 2-mg dry ampules) were obtained in-house. Propranolol hydrochloride[5], nadolol[6], atenolol[5], and metoprolol tartrate[7] were used as received. Oxprenolol (I) was first extracted from a saturated solution of oxprenolol hydrochloride in 5 M NaOH with boiling hexane and was recrystallized several times (mp 77.1°C) from hexane.

Apparatus—The chromatograph used consisted of dual solvent delivery systems[8] with a mixing chamber, automatic sample injection processor[9], printer-plotter integrator data module[10], variable-wavelength UV detector[11], and radial compression module[12]. The analysis was performed on a 10-μm, 8-mm × 10-cm cartridge[13].

Chromatography Conditions—The mobile phase was methanol–acetonitrile–0.0539 M sodium phosphate buffer solution at pH 3 (8.4:15.6:76) filtered twice and deaerated before use. A flow rate of 5 mL/min was used throughout (pressure = 1100 psi). The UV detector was set at 272 nm, and the volume injected into the cyano cartridge was 40–60 μL.

[1] Burdick and Jackson Laboratories Inc., Muskegon, Mich.
[2] Fisher Scientific Company, Fair Lawn, N.J.
[3] Du Pont, Wilmington, Del.
[4] Ciba Laboratories, Horsham, Switzerland.
[5] ICI Limited, Macclesfield, Cheshire, U.K.
[6] E. R. Squibb & Sons, New Brunswick, N.J.
[7] Ciba-Geigy Co., Basle, Switzerland.
[8] Model M45; Waters Associates, Milford, Mass.
[9] Model 710B WISP; Waters Associates, Milford, Mass.
[10] Model 730 Data Module; Waters Associates, Milford, Mass.
[11] Model 480 Lambda; Waters Associates, Milford, Mass.
[12] Model RCM-100; Waters Associates, Milford, Mass.
[13] Radial Pak μ Bondapak CN; Waters Associates, Milford, Mass.

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Table I—Calibration Curve Data [a] Obtained on Different Days

Day	Intercept	Slope	Correlation Coefficient
1	0.0193	3.031	0.99995
2	0.00945	3.043	0.99999
3	0.0138	3.031	0.99999
4	0.0159	3.027	0.99999
5	0.0139	3.033	0.99999

[a] Peak area ratio *versus* concentration plots obtained by linear regression analysis; mean of four determinations (two samples in duplicate injections).

Preparation of the Buffer and Standard Solutions—The buffer solution was prepared by adding dropwise (≅0.45 mL/drop) 85% phosphoric acid to 1 L of 0.05 M monobasic sodium phosphate to adjust the pH to 3. The final buffer concentration was ≅0.0539 M.

Fifty milligrams of I or propranolol hydrochloride (internal standard) was weighed and carefully transferred into a 50-mL volumetric flask with fresh mobile phase. The solution was shaken until a complete dissolution was obtained, then brought to volume with a fresh mobile phase.

Calibration Curves—Calibration curves were constructed by transferring 1, 3, 5, 10, and 20 mL of the I standard solution into 25-mL volumetric flasks, and 5 mL of the internal standard solution was added to each flask. The solutions were brought to volume with fresh mobile phase and thoroughly mixed. An aliquot (~3 mL) of each solution was transferred into an autosampler vial and placed on the tray. The automatic sample injection processor was programmed to inject 50 μL of each vial in duplicate. Standard curves were prepared on different days, to establish linearity, and prior to each assay, for the calculation of I in the samples analyzed.

Combined Tablet Sample Assay—Fifteen tablets of each strength, *i.e.*, 80, 40, and 20 mg, were weighed and pulverized into a fine powder. An aliquot equivalent to 20 mg of oxprenolol hydrochloride (17.58 mg of I) was accurately weighed and carefully transferred to a 100-mL volumetric flask with fresh mobile phase. The flask was then shaken for 2 min, and the fluid was brought to volume with fresh mobile phase. The suspension was filtered through a filtration unit[14], and ~20 mL of the filtrate was transferred to a 25-mL volumetric flask containing 5 mL of the internal standard solution. The solution was then shaken and brought to volume with an adequate aliquot of the above filtrate. An aliquot (~3 mL) of this solution (theoretical final concentration is 0.14 mg/mL of I) was transferred to an autosampler vial and analyzed as described earlier. This assay was performed on eight replicate samples in duplicate injections.

Combined 2-mg Dry Ampule Samples Assay—The contents of five dry ampules were carefully transferred to a 50-mL volumetric flask with a syringe after reconstitution with fresh mobile phase. The ampules and syringe were thoroughly rinsed with mobile phase, and the rinse was added to the flask.

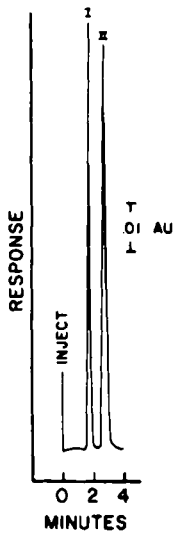

Figure 1—*Representative chromatogram of a calibration curve sample containing oxprenolol (I) with propranolol (II) as internal standard at a concentration of 0.2 mg/mL each. The mobile phase was pH 3 phosphate buffer–acetonitrile–methanol (76:15.6:8.4) at a flow rate of 5 mL/min; the stationary phase was a cyano cartridge.*

[14] Millipore.

Table II—Analyses of the Combined Dosage Unit Samples Showing the Reproducibility of the Assay

Sample	Percent of Label Claim[a] 80-mg Tablet	40-mg Tablet	20-mg Tablet	2-mg Dry Ampule
1	104.8	102.3	99.5	104.0
2	106.1	98.3	99.6	105.5
3	107.8	101.4	103.0	107.0
4	107.6	99.4	103.0	109.5
5	99.9	102.5	101.4	110.0
6	101.7	103.4	101.0	—
7	106.8	99.7	101.2	—
8	105.9	99.9	104.3	—
Mean	105.1	100.9	101.6	107.2
SD	2.85	1.79	1.70	2.56
CV	2.71	1.78	1.67	2.39

[a] Mean value of two injections.

After the volume was brought to the mark with mobile phase, 8 mL of the solution was transferred to a 10-mL volumetric flask containing 2 mL of the internal standard solution. The flask was then shaken, and an aliquot (~3 mL) of this solution (theoretical final concentration 0.14 mg/mL of I) was transferred to an autosampler vial and analyzed as described earlier. This assay was performed on five replicate samples in duplicate injections.

Content Uniformity Test—*Tablet Formulations*—The tablet was pulverized into a fine powder and carefully transferred to a 100-mL volumetric flask with fresh mobile phase, and 20 mL of the internal standard solution was added. The suspension was then brought to volume with fresh mobile phase and shaken vigorously for 2 min. A sufficient aliquot of the suspension was filtered through a filtration unit[14], and the filtrate was transferred to an autosampler vial and analyzed in duplicate as described earlier.

Dry Ampule for Intravenous/Intramuscular Injection—The content of the dry ampule was reconstituted with 2-mL aliquots of fresh mobile phase and transferred with a syringe to a 10-mL volumetric flask containing 2 mL of the internal standard solution. The ampule and syringe were rinsed with small aliquots of the mobile phase, and the rinse was added to the 10-mL volumetric flask. The solution was then shaken and brought to volume with

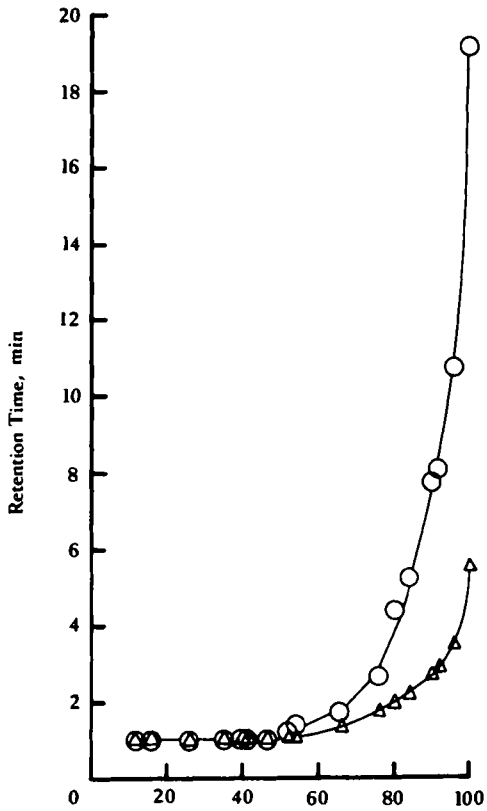

Figure 2—*Effect of the 0.0539 M phosphate buffer (pH 3) concentration in the mobile phase on the retention times of oxprenolol (Δ) and propranolol (○).*

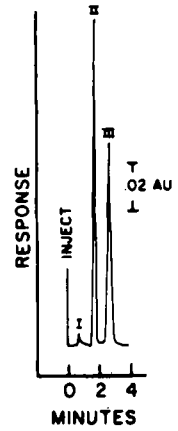

Figure 3—*Typical chromatogram obtained from the analysis of powdered 80-mg tablets. Key: (I) impurity; (II) oxprenolol; (III) propranolol.*

fresh mobile phase. An aliquot of this solution was transferred to an autosampler vial and analyzed in duplicate as described earlier.

Recovery of I from Tablets—Six accurately weighed 50-mg aliquots of a combined tablet powder (80-mg tablets) were transferred to 100-mL volumetric flasks. To five of these flasks, 5 mg of I was added; the remaining flask received no I. These samples were analyzed according to the procedure described under combined tablet sample assay using the sample which did not receive the additional 5 mg of I as a blank.

Chromatograph of Other β-Blockers—Dilute solutions of nadolol, atenolol, and metoprolol tartrate in fresh mobile phase were prepared and transferred to autosampler vials. The retention time of each drug was then determined under the same chromatographic conditions employed for I. A combined solution of atenolol, metoprolol tartrate, I, and propranolol hydrochloride in mobile phase was also prepared and chromatographed.

RESULTS AND DISCUSSION

A typical chromatogram of I and propranolol under the conditions employed is depicted in Fig. 1. As can be seen in this figure, a high efficiency (sharp, symmetrical peaks) and full resolution between I and propranolol are obtained in <3 min. Using C_{18} packing, the retention times under the conditions used were excessive, and although at high concentrations of methanol–acetonitrile in mobile phase the elution was quite rapid, tailing prevailed, suggesting strong binding of both compounds to silanol groups of the C_{18} packing. With the 0.0539 M phosphate buffer (pH 3)–acetonitrile–methanol mixture as the mobile phase, these compounds seemingly favor cyano packing, where both the normal- and reverse-phase modes of chromatography operate.

In an attempt to optimize the chromatography conditions of I and propranolol, the effect of the composition of the mobile phase on the retention times of these compounds was studied (Fig. 2). At zero concentration of the organic mixture (acetonitrile–methanol; 65:35) in the mobile phase the retention times of I and propranolol were 5.58 and 19.15 min, respectively, but the peaks were broad. As the concentration of the organic phase was increased, shorter retention times and sharper peaks were obtained; however, at an inorganic phase concentration of ≤46% (v/v), both compounds were eluted concomitantly without an appreciable change in retention times.

Based on these findings, it was concluded that the 76% phosphate buffer concentration in the mobile phase is an ideal composition for performing the chromatography, since it yields a high chromatographic efficiency and full resolution between I and propranolol in the shortest possible time; this was therefore selected for the analysis.

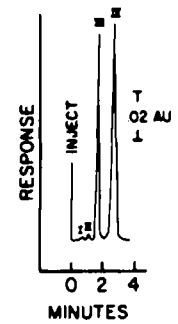

Figure 4—*Typical chromatogram obtained from the analysis of the contents of a 2-mg dry ampule. Key: (I, II) impurities; (III) oxprenolol; (IV) propranolol.*

Table III—Content Uniformity Test of Oxprenolol Hydrochloride Formulations According to the Described Assay

Sample	Percent of Label Claim[a] 20-mg Tablet	40-mg Tablet	80-mg Tablet	2-mg Dry Ampule
1	102.5	94.8	103.5	101.7
2	103.2	105.8	104.3	105.0
3	94.8	105.7	102.3	100.4
4	107.8	104.5	103.3	97.0
5	91.6	104.3	99.5	100.0
6	103.0	104.1	102.1	104.0
7	108.7	107.3	101.7	109.4
8	98.6	104.0	103.8	105.4
9	106.3	106.0	105.2	107.0
10	103.3	101.5	103.5	110.0
Mean	102.0	104.0	102.9	104.0
SD	5.50	3.60	1.60	4.22
Range	91.6–108.7	94.8–107.3	99.5–105.2	97.0–110.0

[a] Mean of four determinations (two samples in duplicate injections).

The peak area ratio (I/internal standard) *versus* concentration calibration curves were linear ($r > 0.9999$) in the concentration range employed in this study (0.04–0.8 mg/mL) and were utilized to calculate I concentrations in the samples analyzed. The day-to-day variations in the slope and intercept were very small (Table I), indicating excellent concentration–response reproducibility.

The chromatogram presented in Fig. 3 is representative of a combined 80-mg tablet sample assay. The small front peak (retention time = 0.7 min) is an impurity or tablet excipient peak and does not interfere with the assay, since it is fully resolved from the peaks in question. The two minor peaks (retention times 0.86 and 1.15 min) which appear in the chromatogram of a single dry ampule analysis (Fig. 4) were also fully resolved.

The recovery of I from tablets was determined by adding 5 mg of I to 50 mg of powdered 80-mg oxprenolol hydrochloride tablets, equivalent to ~14 mg of I. The analysis of five such samples yielded the following recoveries: 100.8, 98.5, 98.3, 99.2, and 99.1% with a mean of 99.2% and a coefficient of variation of 0.991%. These data indicate that the assay precision is excellent and that the extraction procedure (using mobile phase and 2 min of shaking) employed is adequate, yielding a full recovery of I from tablets.

The reproducibility of the assay to analyze I in commercial formulations is clearly evident from the results of the combined tablets or dry ampule assay (Table II) where eight replicate analyses of each tablet lot and five replicate analyses of the combined dry ampule were performed. At no instance did the coefficient of variation exceed 2.7%, which indicates excellent assay reproducibility.

The results of the content uniformity test of commercial oxprenolol hydrochloride formulations are provided in Table III. The data were obtained from the application of the assay to three different-strength tablet formulations and a dry ampule for injection. Ten samples of each lot were analyzed individually, and the percent of label claim for each was calculated using an oxprenolol hydrochloride to I conversion factor of 0.879. As demonstrated in

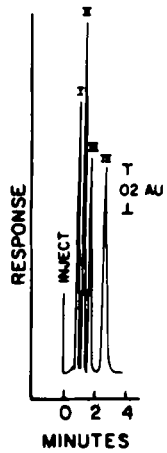

Figure 5—*Chromatogram obtained for a solution containing atenolol (I), metoprolol (II), oxprenolol (III), and propranolol (IV) in mobile phase.*

Table IV—Relative Retention Times[a] of Various β-Adrenergic Blocking Drugs

β-Blocking Drug	Relative Retention Time
Atenolol	0.340
Metoprolol	0.479
Nadolol	0.394
Oxprenolol	0.641
Propranolol	1.000

[a] Retention time of the β-blocking drug/retention time of propranolol.

Table III, the percent of label claim for all formulations tested were within the range of 91.6–110%. Unfortunately, no official USP or BP assay for I is available and, therefore, a comparison between the content uniformity test results obtained according to this method and those of an official assay was not possible. However, if this assay is to be used for such a test, it is clear that all the formulations investigated meet the USP content uniformity requirements (100 ± 15%).

The applicability of this assay for the analysis of some other β-blocking drugs was investigated, and the relative retention times (retention time of drug/retention time of propranolol) of these agents are listed in Table IV. It is clear that, with the exception of nadolol–atenolol (due to poor resolution), it is possible to analyze these drugs under the described chromatography conditions using one drug as the internal standard and the other as the unknown. Indeed, without changing a single chromatography condition, this assay can be utilized for the chromatography of atenolol, metoprolol, I, and propranolol simultaneously with a high efficiency and good resolution (Fig. 5) in <3.0 min.

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Improved Liquid Chromatographic Assay for the Analysis of Pirmenol in Plasma and Urine

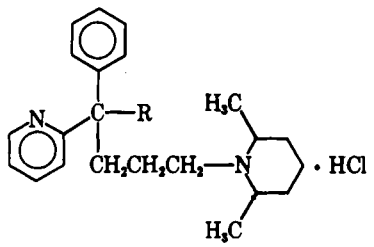
E. L. JOHNSON and L. A. PACHLA ˣ

Received September 7, 1982, from the *Department of Pharmacokinetics/Drug Metabolism, Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.* Accepted for publication April 22, 1983.

Abstract □ A sensitive, specific, and rapid high-performance liquid chromatographic procedure was developed for the determination of pirmenol in human biological fluids. Plasma or urine samples were alkalinized and extracted with cyclohexane. The organic extract was evaporated to dryness, reconstituted with the mobile phase, and then chromatographed on a microparticulate spherical trimethylsilane stationary phase with UV detection at 254 nm. The procedure for the assay of pirmenol in plasma was linear from 0.125 to 5.0 μg/mL. The reproducibility of the peak area ratios of the standard curves had relative standard deviations between 7.7 and 1.8% and a relative error of 0–4.6% over the linear range. The accuracy for the determination of pirmenol in human plasma containing 0.5, 2.5, and 4.0 μg/mL had relative errors of 9.0, 3.8, and 3.6%, respectively. Thirty compounds were tested and found not to interfere in the assay of the drug in plasma, and the method was found to be suitable for clinical samples. The urine procedure was linear between 1.0 and 30.0 μg/mL. The reproducibility of the peak areas of the standard curves had relative standard deviations that ranged from 1.9 to 6.2% over the linear range. The accuracy for the determination of pirmenol in human urine containing 5.0, 17.5, and 25.0 μg/mL had relative errors of 1.4, 0.5, and 2.8%, respectively.

Keyphrases □ Pirmenol—improved HPLC assay, plasma and urine, cyclohexane extraction □ HPLC—improved assay for pirmenol, plasma and urine, cyclohexane extraction

Pirmenol hydrochloride, (±)-*cis*-α-[3-(2,6-dimethyl-1-piperidinyl)propyl]-α-phenyl-2-pyridinemethanol monohydrochloride (I), a new antiarrhythmic drug currently being tested in Phase 1 clinical trials, is a promising agent because of its therapeutic response, lack of toxicity, and relatively long half-life (1). This report describes an improved procedure for the analysis of pirmenol when compared to a recently reported procedure (2). The present method is more accurate and precise due to the choice of extraction solvent and internal standard. The method has been validated for human biological samples, and various potential interfering compounds have been evaluated to demonstrate method specificity.



I: R = OH
II: R = H

EXPERIMENTAL

Reagents—Pirmenol hydrochloride[1] and the internal standard[1] (±)-*cis*-2-[4-(2,6-dimethyl-1-piperidinyl)-1-phenylbutyl]pyridine monohydrochloride (II) were used as received. Distilled water was further purified using an ion-exchange charcoal filtration system[2]. Cyclohexane[3] and acetonitrile[3] were

[1] Warner-Lambert/Parke-Davis, Ann Arbor, Mich.
[2] Water-I; Gelman Filtration Products, Ann Arbor, Mich.
[3] Omni-solv grade; MCB, Cincinnati, Ohio.

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