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


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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.

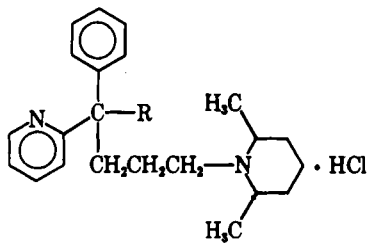


**Abstract** □ A sensitive, specific, and rapid high-performance liquid chromatographic procedure was developed for the determination of pirmenol in human biological fluids. Plasma or urine samples were alkalinized and extracted with cyclohexane. The organic extract was evaporated to dryness, reconstituted 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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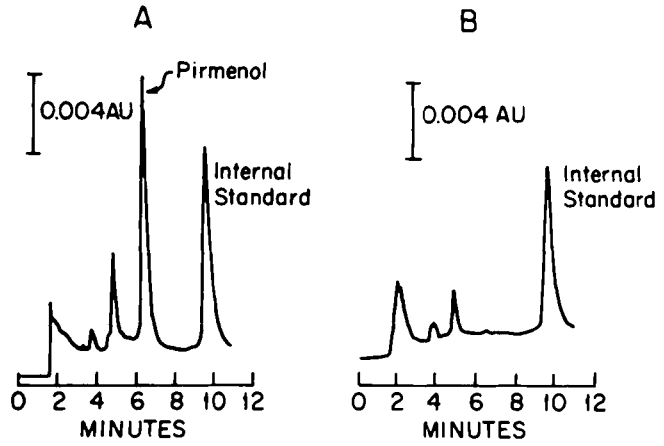


**Figure 1**—*Typical chromatograms for pirmenol and the internal standard in plasma. Key: (A) pirmenol in a 40-min plasma sample following infusion (150 mg iv at 5 mg/min) was estimated to be 1.7 μg/mL); (B) preinfusion plasma sample.*

chromatographic grade. Monobasic sodium phosphate[4], triethylamine[4], phosphoric acid[4], hydrochloric acid[5], and sodium hydroxide[5] were analytical reagent grade and were used as received.

**Apparatus**—The liquid chromatographic system was equipped with a constant-volume pump[6], valve-type injector[7], 254-nm fixed-wavelength detector[8], and a data acquisition system[9]. Separations were performed on a 25 cm × 4.6 mm i.d. stainless steel column containing 6-μm spherical trimethylsilane stationary phase[10].

**Mobile Phase**—The mobile phase was prepared by diluting 150 mL of acetonitrile to 1 L with phosphate buffer containing 0.5% triethylamine. The phosphate-triethylamine buffer was prepared by adding 6.9 g of monobasic sodium phosphate monohydrate and 5.0 mL of triethylamine to water, adjusting the pH to 2.6 with phosphoric acid, and then diluting to 1 L with water. The mobile phase was filtered through a 0.2-μm filter and deaerated by vacuum. The flow rate of the mobile phase was 1.5 mL/min, which produced a pressure of ~2700 psi. All separations were performed at ambient temperature. A 50-μL aliquot of extracted sample was injected into the chromatographic system.

**Standard and Stock Solutions**—Stock solutions of pirmenol (110.7 mg of pirmenol hydrochloride/10 mL of 0.05 M HCl) and the internal standard (II) (11.1 mg of II/100 mL of 0.05 HCl) were prepared. Standard solutions for plasma containing 0.25, 1.0, 2.0, 4.0, 6.0, 8.0, and 10 μg/mL and standard solutions for urine containing 4, 20, 40, 60, 80, 100, and 120 μg/mL were prepared by serial dilution of the stock pirmenol solution with 0.05 M HCl. The internal standard solution was further diluted 3:100.

**Pirmenol Plasma and Urine Seeded Control Pools**—One milliliter of 50-, 250-, or 400-μg/mL solutions of pirmenol (in 0.05 M HCl) was added to a 100-mL volumetric flask and brought to volume with control plasma to yield seeded control samples of 0.5, 2.5, and 4.0 μg/mL of pirmenol. Urine seeded controls were prepared by adding 0.5, 1.75, and 2.5 mL of a 1.0 mg/mL standard solution and diluting to 100 mL with control urine to yield 5.0-, 17.5-, and 25.0-μg/mL samples. The plasma and urine samples were then divided into 2.5-mL portions and frozen.

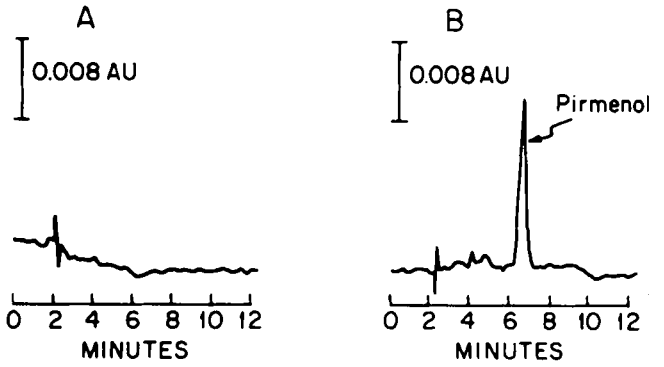


**Figure 2**—*Typical chromatograms for pirmenol in human urine. Key: (A) predrug urine sample; (B) pirmenol in an 18-h postdose urine sample of a patient administered 150 mg of pirmenol was estimated to be 4.0 μg/mL.*

[4] J. T. Baker, Phillipsburg, N.J.
[5] Acculute; Anachemia Chemical, Champlain, N.Y.
[6] Model-M-45; Waters Associates, Milford, Mass.
[7] Model U6K; Waters Associates, Milford, Mass.
[8] Model 440; Waters Associates, Milford, Mass.
[9] Sigma 10; Perkin Elmer, Norwalk, Conn.
[10] Zorbax TMS; DuPont, Wilmington, Del.

**Table I—Precision and Accuracy of the Pirmenol Calibration Curves During a 3-d Period**

| Pirmenol Conc. Added, μg/mL | N | Peak Area Ratio[a] | RSD, % | Relative Error, % |
|---|---|---|---|---|
| Plasma | | | | |
| 0.125 | 9 | 0.037 | 7.7% | −0.8 |
| 0.500 | 8 | 0.254 | 4.7% | 4.6 |
| 1.00 | 9 | 0.491 | 5.7% | −4.3 |
| 2.00 | 9 | 1.02 | 4.2% | −3.5 |
| 3.00 | 8 | 1.61 | 3.0% | 0 |
| 4.00 | 9 | 2.15 | 5.1% | 0.5 |
| 5.00 | 8 | 2.78 | 1.8% | 2.8 |
| Urine | | | | |
| 1.0 | 6 | 0.376 | 3.1% | 0 |
| 5.0 | 8 | 1.62 | 6.0% | −8 |
| 10.0 | 8 | 3.53 | 3.8% | −2 |
| 15.0 | 9 | 5.48 | 6.2% | 0 |
| 20.0 | 8 | 7.51 | 1.90% | 2 |
| 25.0 | 8 | 9.54 | 2.0% | 3.2 |
| 30.0 | 9 | 11.5 | 2.2% | 4.3 |

[a] Peak area used for urine samples.

**Assay for Pirmenol in Plasma and Urine**—To a 1-mL sample in a 10-mL screw-top test tube were added 0.5 mL (0.25 mL for urine samples) of either 0.05 M HCl or pirmenol standard solution, 0.5 mL of internal standard solution (no internal standard is used for urine samples), 4 mL of cyclohexane, and 0.15 mL (0.2 mL for urine samples) of 1 M NaOH. After shaking for 10 min on a horizontal shaker and centrifuging[11] at 206×*g* for 5 min, 3 mL of the organic phase was transferred to a clean tube and evaporated to dryness. The residue was reconstituted in 0.2 mL (1 mL for urine samples) of the mobile phase by vortexing, and 50 μL (100 μL for urine samples) was injected into the chromatograph.

**Interference Study**—The specificity of the plasma procedure was assessed by direct injection of a number of potentially interfering compounds. If a compound eluted in the void volume or had a retention time >12 min, then the compound was deemed not to interfere in the assay. Those compounds having a retention time similar to either pirmenol or the internal standard were further tested in the following manner. Plasma containing 1 μg/mL of pirmenol was spiked with 100 μg/mL of the potentially interfering compounds. These samples were then assayed for pirmenol. If the pirmenol concentration deviated >±5% of the established value, than the compound was considered to interfere in the assay.

**Method Application**—To test the suitability of the procedure, samples from a clinical trial were assayed. In this trial, patients received a 150-mg dose of pirmenol intravenously or orally. For the intravenous dosing, patients were infused over a 30-min period with the drug dissolved in 5% dextrose. Blood samples were drawn at 15, 30, 32, 35, 40, 50, 60, 75, 90 min, and 2, 3, 5, 7, 9,

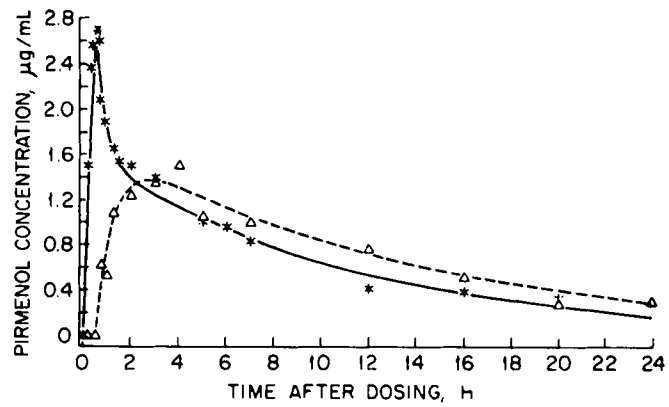


**Figure 3**—*Plasma concentration-time curve in a patient given 150 mg of pirmenol. Key: (*) intravenous; (Δ) oral.*

[11] Centra 7R, International Equipment Company, Needham Hts, Mass.

Table II—Extraction Efficiency of Tritiated Pirmenol in Plasma and Urine Determined by Liquid Scintillation Counting

| | Plasma | | | Urine | | |
|---|---|---|---|---|---|---|
| [$^3$H]Pirmenol Added, ($\mu$g/mL) | 0.1 | 1.0 | 5.0 | 1.0 | 10.0 | 25.0 |
| *N* | 3 | 4 | 3 | 4 | 4 | 4 |
| Mean Recovery (%) | 84.8 | 85.2 | 87.4 | 82.5 | 89.4 | 90.3 |
| *RSD* (%) | 7.6 | 8.8 | 1.6 | 4.4 | 1.1 | 1.2 |

Table III—Determination of Pirmenol Concentrations in Seeded Plasma and Urine Control Pooled Samples Assayed During a 3-d Period

| | Plasma | | | Urine | | |
|---|---|---|---|---|---|---|
| Pirmenol Conc. Added, ($\mu$g/mL) | 0.500 | 2.500 | 4.000 | 5.0 | 17.5 | 25.0 |
| *N*]9 | 9 | 8 | 9 | 9 | 9 | 9 |
| Mean Conc. Found, ($\mu$g/mL) | 0.545 | 2.594 | 4.144 | 4.9 | 17.6 | 25.7 |
| *RSD* (%) | 5.0 | 3.4 | 3.6 | 5.2 | 1.5 | 1.8 |
| Relative Error (%) | 9.0 | 3.8 | 3.6 | −1.4 | 0.5 | 2.8 |

12, 16, 20, and 24 h into heparinized tubes, and the plasma was harvested following centrifugation. For the oral study, the same patients were administered 150-mg capsules 48 h after the intravenous dose. Blood samples were taken at 0, 0.25, 0.50, 0.75, 1, 1.25, 1.5, 2, 3, 4, 5, 7, 12, 16, 20, and 24 h following drug administration. The plasma was harvested following centrifugation. Timed urine collections of 2 h were collected for the first 6-h period followed by 6-h collections for 48 h postdose.

## RESULTS AND DISCUSSION

The present procedure for the analysis of pirmenol in human plasma and urine involves a single-step alkaline extraction with cyclohexane. The supernatant is evaporated to dryness and reconstituted with the mobile phase. Cyclohexane was found to give improved extraction recoveries and cleaner sample extracts compared with ether (2). An internal standard was found not to be necessary in the urine procedure due to the high pirmenol concentration and simple sample preparation (3). The validity of the assay was established through a careful study of linearity of response, reproducibility of standard curve, extraction recovery, accuracy, precision, and potential interference studies.

The retention times for pirmenol and the internal standard were 6.4 and 9.5 min, respectively. Figure 1 illustrates typical chromatograms for control human plasma and that from the plasma of a patient who received an intravenous dose of pirmenol. Figure 2 illustrates typical chromatograms for blank urine and an 18-h postdose urine sample. The total time required for the plasma and urine assays was 12 and 7 min, respectively.

The linearity of response of the amount of pirmenol added to control human plasma and urine was found to be linear over a range of 0.125–5.00 $\mu$g/mL for plasma and 1–30 $\mu$g/mL for urine. These ranges cover the projected therapeutic plasma and urine concentration ranges. The best-fit line was obtained using linear regression analysis with weighting factors of 1/concentration squared (4). The results of a typical regression analysis were: peak area ratio = 0.563 × plasma concentration + 0.0357 ($r$ = 0.9987) and peak area = 0.367 × urine concentration + 0.0067 ($r$ = 0.9948).

The reproducibility of the calibration curves was assessed by assaying triplicates of each plasma and urine standard over a 3-d period. The relative standard deviations of the peak area ratios for plasma and peak areas for urine between the lowest and highest concentrations were found to range from 7.7 to 1.8% and from 1.9 to 6.2%, respectively. The accuracy for these standards had relative errors of 0–4.6% and 0–4.3% for plasma and urine, respectively. These data are summarized in Table I.

The extraction efficiency for tritium-labeled pirmenol was determined using liquid scintillation counting[12] and is given in Table II. These data show that the extraction efficiency of pirmenol from plasma and urine ranges from 85 to 87% and 82 to 90%, respectively.

The relative error of the plasma procedure was determined by assaying seeded control plasma pooled samples containing 0.5, 2.5, and 4.0 $\mu$g of pirmenol/mL over a 3-d interval and was found to be 9.0, 3.8, and 3.6%, respectively. The relative error of the urine procedure was assessed by assaying seeded control urine pooled samples containing 5.0, 17.5, and 25.0 $\mu$g/mL and was found to be 1.4, 0.5, and 2.8%, respectively (Table III).

The specificity of the plasma assay was assessed by determining the chromatographic behavior of a number of potentially interfering compounds. The following compounds were found not to interfere with the quantification of pirmenol based on retention time: acetaminophen, clonidine hydrochloride, diazepam, disopyramide, ethacrynic acid, fenoprofen sodium, furosemide, hydralazine hydrochloride, hydrochlorothiazide, ibuprofen, methotrimeprazine, methyldopa, prazepam, prazosin hydrochloride, procainamide, propranolol hydrochloride, propoxyphene hydrochloride, reserpine, and tolmetin sodium. Compounds that had a similar retention time as primenol, but were found not to interfere were: aspirin, bretylium tosylate, chlorthalidone, naproxen, oxazepam, quinidine, salicylate, triamterene, and zomepirac sodium. To date, over 650 human plasma samples and 200 urine samples have been assayed for pirmenol using this methodology, and no apparent interfering compounds have been detected. Figure 3 illustrates typical plasma time courses for pirmenol after intravenous and oral dosing.

The method provides several improvements over a previous procedure (2). The improvements include cleaner sample extracts, improvement in chromatographic baseline due to choice of reconstitution solvent, an internal standard for plasma that is structurally similar to pirmenol, and increased sample throughput and ability to reinject samples if required. The data for the procedure are in agreement with the predicted precision expected for the internal or external calibration technique (3).

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[12] Tricarb Model No. 3375; Packard Instruments, Downers Grove, Ill.