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Short Communication

Pirmenol determination by high-performance liquid chromatography

GIFFORD L. HOYER

Cardiology Section, Department of Internal Medicine, College of Medicine, University of Arizona, Health Science Center, Tucson, AZ 85724 (U.S.A.)

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ABSTRACT

A method for the determination of pirmenol in serum is presented in this paper. The method consists of extraction of pirmenol and chlorodisopyramide (internal standard) from serum at an alkaline pH using methylene chloride. The organic extract was analysed using high-performance liquid chromatography. The mobile phase consisted of 0.01 *M* K_2HPO_4 (pH 2.4)–acetonitrile (94:6, v/v) delivered at ambient temperature and 2 ml/min through a 25 cm × 0.4 mm C_{18} reversed-phase column. Detection of the compounds of interest was achieved at 210 nm. The analytical method demonstrated low intra- and inter-assay variation. During the analysis of patient samples and a therapeutic drug mixture test serum, no substances that interfered with pirmenol detection were found. The method is shown to be stable, accurate, selective and sensitive enough to be utilized for the analysis of multiple samples such as may be encountered in clinical or research situations.

INTRODUCTION

Pirmenol is an antiarrhythmic drug [1] currently undergoing clinical testing to gain FDA approval. It has been shown to be effective *in vitro* [2] and in canine animal models [3,4]. The pharmacological profile of pirmenol is similar to other antiarrhythmics such as mexiletine, tocainide and cibenzoline [5]. The pharmacokinetics of pirmenol are consistent with a two-compartment model [5,6]. Pirmenol has a terminal half-life of 7–10 h when administered orally [3,5] or intravenously [7]. There is a wide range of plasma half-life; in one study it ranged from 4.2 to 16.9 h and was linear and dose-independent. Mean renal clearance of unchanged drug has been measured at between 32% [7] and 52% [5]. In tracer studies in animals renal elimination of a radioactive dose ranged from 40 to 50% with the remainder found in the feces after being excreted in the bile [8].

The antiarrhythmic efficacy of pirmenol is related to its plasma concentration [1], however, there is considerable inter-individual variability [9]. The mean (±

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S.D.) efficacious trough plasma concentration was 0.98 ± 0.29 μg/ml in one study [9]. In patients with sustained ventricular tachycardia, due to coronary ischemic heart disease, pirmenol has an overall efficacy of approximately 19% [10]. In addition it is well tolerated with a minimum of side-effects [11].

Inter-individual variability in clearance [7] and efficacy [9] suggest the necessity for clinical monitoring of blood levels, at least initially to better define the dosing regimen. The first assay method described was a fluorometric dye technique [2,3]. More recently high-performance liquid chromatographic (HPLC) assays for quantitating blood concentration of pirmenol have been described [12,13]. Both of these HPLC assays require chromatographic run times of close to 12 min and one method [12] requires double extraction. These methods are slow, therefore it is desirable to have an assay that is easier to perform and requires shorter chromatographic run times.

EXPERIMENTAL

Chemicals

Pirmenol hydrochloride was supplied by Parke Davis (Ann Arbor, MI, U.S.A.). The internal standard, chlorodisopyramide, was obtained from Searle (Chicago, IL, U.S.A.). Potassium phosphate dibasic (grade V) and nonylamine were purchased from Sigma (St. Louis, MO, U.S.A.) and acetonitrile (HPLC grade) was manufactured by Burdick and Jackson (Muskegon, MI, U.S.A.). Water for the aqueous component of the mobile phase was glass-distilled , treated by ion exchange, charcoal-filtered and subsequently filtered through a Nylon filter with 0.45-μm pores.

Instrumentation and chromatographic conditions

The liquid chromatographic system consisted of a Rheodyne (Reno, NV, U.S.A.) Model 7010 manual injection valve and a Hewlett Packard (Fullerton, CA, U.S.A.) Model 1090 liquid chromatograph with a diode-array detector. A Hewlett Packard Model 85-B personal computer controlled the chromatograph and detector in addition to recording and analyzing spectrographic data. Printed chromatograms and integration of absorbance data from the diode-array detector were obtained from the Hewlett Packard 3392A integrator. An Altex 25 cm × 0.4 cm I.D. reversed-phase column, packed with 5-μm octadecylsilane (ODS, C_{18}), was used for the chromatographic separation.

The mobile phase consisted of 0.01 *M* dibasic potassium phosphate buffer (K_2HPO_4) at pH 2.4 (using phosphoric acid) with 0.375 ml/l nonylamine–acetonitrile (94:6) delivered at a constant flow-rate of 2 ml/min. The column and mobile phase were maintained at ambient temperature. Mobile phase was pumped through the column for 30 min prior to analytical injections of standards or unknowns. The column used for this assay was utilized in several other assays under varying conditions. A conditioning injection, 4 μg of pirmenol and internal

standard, was applied to the column to ensure that any potentially active sites on the column packing would be occupied prior to sample analysis. The diode-array detector was set at 210 nm with a 4-nm wide window.

Standards

Pirmenol standard solutions (100 μl) containing 2, 4.05, 8.15, 24.5 and 41 μg/ml in water were pipetted singly into duplicate 12-ml screw-cap culture tubes. The total volume was brought up to 1 ml by adding 0.9 ml of pooled human serum to each standard tube. Solutions used for the standard curve were prepared fresh every 60 days to ensure accuracy. Between analytical procedures the standard solutions were stored at 4°C. A spiked control consisting of pooled human serum containing 1.06 μg pirmenol per ml serum was used to assess the inter- and intra-assay variation and the accuracy of individual assay runs. Spiked serum was stored at −20°C in 3-ml aliquots. Over a period of six months, no degeneration of pirmenol in frozen spiked serum was noted. Pooled human serum containing no detectable pirmenol was used as a blank (zero) control. Duplicate 1-ml samples of the spiked control, blank control and patient serum were pipetted into 12-ml screw cap culture tubes.

Serum extraction

Duplicate samples of the standards, spiked pooled serum, blank pooled serum or patient serum were extracted by liquid–liquid extraction. To each tube 150 μl of 1.0 *M* sodium hydroxide were added to adjust the serum to an alkaline pH, then 50 μl of chlorodisopyramide, 40 μg/ml in methanol (internal standard), was added to each tube. Methylene chloride (5 ml) was added to all tubes which were capped and shaken on a Labquake for 10 min. After shaking, the tubes were centrifuged at 1000 *g* for 10 min to separate the organic (lower) and aqueous (upper) phases. The aqueous layer was pipetted from the organic layer and discarded. The organic layer was pipetted to a conical centrifuge tube, taking care to avoid any remaining aqueous liquid, and dried under nitrogen in a water bath at 40°C. The dried extract was reconstituted in 200 μl of mobile phase, and 100 μl were injected onto the column.

Assay validation

The efficiency of the extraction method was measured by comparing the peak area of a known amount of pirmenol standard injected onto the column with the peak area of the extract of a known spiked serum. After correction for volume losses, the percentage pirmenol extracted from serum was determined. Several controls were utilized to assure the reproducibility and accuracy of the assay. Intra-assay variation was determined by analyzing seven aliquots of the same spiked serum on one day and determining the standard deviation and coefficient of variation. Inter-assay variation was determined by comparing the calculated

concentration of aliquots of spiked serum containing 1.06 μg/ml pirmenol on five different days.

In order to establish the accuracy and precision of the proposed assay we carried out studies using pooled serum spiked with a predetermined concentration of pirmenol. These samples were extracted, analyzed, and the calculated concentrations were established by determining the assayed values from the standard curve for the respective day of analysis. The difference between the nominal and calculated concentrations were then determined. Potential interference from several antiarrhythmics and other commonly prescribed drugs was tested by extracting and analyzing therapeutic drug monitoring (TDM) serum (Fischer Scientific, Phoenix, AZ, U.S.A.), a commercially prepared spiked serum.

RESULTS

Chromatograms of pirmenol standard and internal standard, extracted unspiked pooled serum, extracted spiked pooled serum and extracted patient serum are shown in Fig. 1. The peaks are identified as pirmenol and internal standard and have retention times of 2.9 and 3.6 min, respectively. In addition to the non-retained components, there is also an unidentified serum component with a retention time of 4.4 min.

A comparison of the chromatograms shows that compounds associated with pooled serum do not elute at times that interfere with the detection or quantitation of pirmenol or the internal standard. During the development of this assay and the analysis of 38 patient samples, it was found that contamination of the organic phase with aqueous phase, prior to the dry down, results in several potentially interfering peaks.

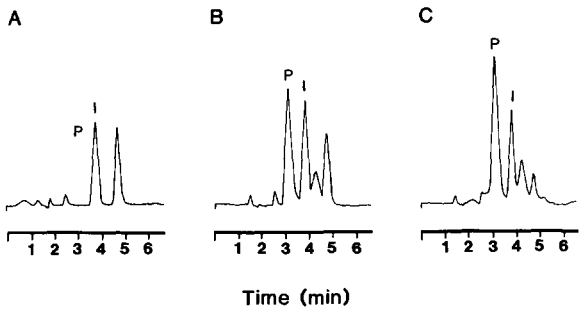

Fig. 1. Chromatograms of 1 ml extracted human serum. (A) Extracted pooled serum spiked with 2 μg of internal standard (I); (B) extracted pooled serum spiked with 2 μg of internal standard and 2.45 μg of pirmenol (P); (C) extracted patient serum containing 3.2 μg of pirmenol. Chromatographic conditions: mobile phase, 0.01 *M* dibasic potassium phosphate buffer (K_2HPO_4) pH 2.4 containing 0.375 ml/l nonylamine–acetonitrile (94:6); flow-rate, 2 ml/min; column, 5-μm ODS; detector, 210 nm with a 4-nm wide window.

TABLE I

PIRMENOL ASSAY VALIDATION DATA

	Mean (μg/ml)	Standard deviation	Coefficient of variation (%)
Intra-assay variation (n = 7)	1.15	0.094	8.17
Inter-assay variation (n = 5)	1.126	0.027	2.4

Linear regression parameters (n = 6): mean correlation coefficient, 0.99966; mean slope, 0.0029; mean y-intercept, 0.515.

The assay is linear between concentrations of 0.20 and 4.1 μg/ml. The average correlation coefficient of six standard curves run over a two-month period was 0.99966 with a standard deviation of 0.00033 and a coefficient of variation of 0.033%; the average y-intercept was 0.0029 and the average slope was 0.515.

Intra-assay variation was determined by analyzing seven samples containing 1.06 μg/ml pirmenol on the same day. It was found that the intra-assay variation was 8.17%. The average calculated concentration was 1.15 μg/ml with a standard deviation of 0.094 and an accuracy of 8.5% as judged by the difference from the nominal concentration. Inter-assay variation was gauged by comparing the calculated concentration of spiked serum containing 1.06 μg/ml pirmenol as determined over five days. The average calculated concentration was 1.126 μg/ml with a standard deviation of 0.027 and a coefficient of variation of 2.4%. The average inter-assay accuracy was 6.3% with a range from 5.7 to 9.4%. The accuracy of the assay over the range of the standard curve varied from 6.1% at 0.20 μg/ml to 0.5% at 4.1 μg/ml. The coefficient of variation ranged from 9.8% at 0.20 μg/ml to 1.1% at 4.1 μg/ml.

During the analysis of 38 patient samples no interfering compounds were noted. During the analysis of extracted TDM serum no interfering peaks were noticed (Table I). The efficiency of the extraction procedure for pirmenol (n = 6) was determined to be 83% at a concentration of 1 μg/ml.

DISCUSSION

We have developed an HPLC assay for pirmenol that is accurate, precise and selective. The assay is more sensitive than the previously published assays. In addition, this method requires only a single extraction step, and has a total chromatographic run time of 7 min. The retention times of pirmenol and internal standard are 2.9 and 3.6 min, respectively. Due to the short retention times, the peak shape of both pirmenol and the internal standard are sharp, resulting in baseline separation of the two compounds. The run time is almost twice the

retention time of the internal standard in order to eliminate interference with subsequent injections by a late-eluting component having a retention time of 6.9 min. During the initial development of this assay it was found that increasing the column temperature above ambient temperature caused an increase in pirmenol retention time which resulted in band broadening and decreased separation from the internal standard chlorodisopyramide.

As shown in Table I, the intra- and inter-assay coefficients of variation for independently prepared spiked serum (1.06 μg/ml) are consistently less that 10%. The range of the inter-assay coefficient of variation for the standard is less than 10%, although as the pirmenol concentration declines the variation increases. Although the lowest standard used in this assay was 0.205 μg/ml it is not the lowest quantifiable amount. The lowest detectable concentration, defined as a peak with a height three times the baseline noise, after extraction from serum was 0.005 μg/ml. The range of the standard curve was selected to cover the potential range of clinical trough blood concentrations. A study of the pharmacokinetics of pirmenol would require an expanded range of concentrations.

No interfering compounds or drugs, as listed in Table II, were found in 18 patient samples, spiked TDM serum or pooled human serum. A compound that is associated with the aqueous layer elutes at 2.45 min. During normal extraction procedures this compound is a minor contaminant with baseline separation from pirmenol. However, if the organic extraction layer is contaminated by a small amount of the aqueous layer this contaminant may become a major component of the chromatogram and possibly override low concentrations of pirmenol.

In conclusion, the analytical method presented should be of use in clinical laboratories for measuring therapeutic drug levels and in research laboratories for investigating the pharmacokinetics of pirmenol.

TABLE II

DRUGS TESTED FOR INTERFERENCE WITH PIRMENOL

Antiarrhythmic drugs	Other drugs	
Digoxin	Acetominophen	Methotrexate
Disopyramide	Amikacin	Netilmicin
Lidocaine	Amitriptyline	Nortriptyline
N-Acetylprocainamide	Cardamazepine	Phenobarbital
Phenytoin	Chloramphenicol	Primidone
Procainamide	Desipramine	Salicylate
Propranolol	Ethosuximide	Theophylline
Quinidine	Gentamicin	Tobramycin
	Imipramine	Valproic acid
	Lithium	Vancomycin

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