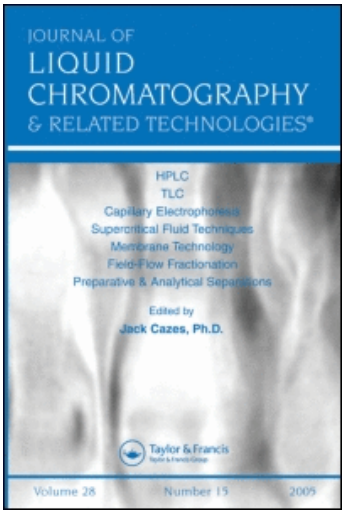

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DETERMINATION OF IBAFLOXACIN, A NEW QUINOLONE ANTIBACTERIAL, IN HUMAN AND DOG PLASMA AND URINE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A simple, selective and sensitive high performance liquid chromatographic method has been developed for quantifying plasma and urine ibafloxacin levels in humans and dogs.

Sample pretreatment is done by incorporation of an internal standard (IS) followed by a single step chloroform extraction. Samples are then chromatographed by reverse phase chromatography with UV detection. The lowest quantifiable concentration is 0.1 μg ibafloxacin/mL with a 1 mL sample. The assay was linear over the range of 0.1-50 μg/mL.

INTRODUCTION

Ibafloxacin (6, 7-Dihydro-9-flouro-5, 8-dimethyl-1-oxo-1H, 5H-benzo [i,j] quinolizine-2-carboxylic acid) is a tricyclic quinolone antibacterial drug which appears to act in a manner similar to flumiquine, a tricyclic quinolone antibacterial

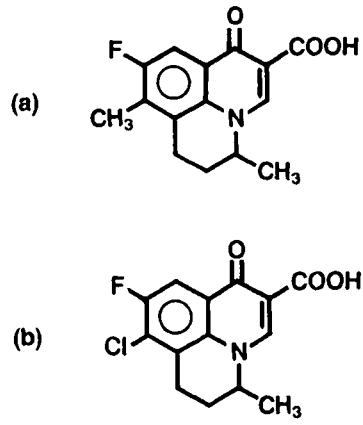

Figure 1. Chemical structures of: (a) ibafloxacin, and (b) internal standard

marketed by Laboratoires 3M Sante (France). Figure 1 shows the structure of Ibafloxacin and the IS.

This paper describes a high performance liquid chromatographic (HPLC) method for quantitation of ibafloxacin in plasma and urine. The method has been successfully applied for pharmacokinetic assessment of ibafloxacin in healthy human subjects and in laboratory beagle dogs.

MATERIALS AND METHODS

Reagents

Acetonitrile and chloroform were OmniSolve®. Sodium carbonate, hydrochloric acid and sodium hydroxide were ACS reagent grade. Deionized water was used.

Preparation of Standards

A solution containing 50 μg ibafloxacin/100 mL 0.1N NaOH was prepared as a dilution of a 1 mg ibafloxacin/mL 0.1N sodium hydroxide solution. The 0.1, 0.5, 1, 5, 10, and 25 μg ibafloxacin/100 μL standards were prepared by diluting the 50 μg ibafloxacin/100 μL solution with appropriate amounts of 0.1N sodium hydroxide.

The internal standard, 8-chloro-6,7-dihydro-9-flouro-5-methyl-1-oxo-IH, 5H-benzo [i,j] quinolizine-2-carboxylic acid, is an analog of ibafloxacin. A solution containing 7.5 μg/100 μL 0.1N sodium hydroxide was prepared as a dilution of a 1 mg IS/mL 0.1N sodium hydroxide solution.

Apparatus

The chromatographic analyses were performed on a modular liquid chromatograph equiped with a ConstaMetric® Metering III Pump (Laboratory Data Control, Riviera Beach, FL, U.S.A.), a WISP Model 710B automatic sample injector (Waters Assoc., Milford, MA, U.S.A.) and a Waters UV 440 detector (Waters Assoc., Milford, MA, U.S.A.) equipped with a 313 nm filter. The data were collected by a HP 3390 reporting integrator (Hewlett-Packard, Avondale, PA, U.S.A.).

Chromatographic Procedure

The mobile phase consisted of 0.1 M sodium carbonate (pH 10) and acetonitrile (83:17), and is filtered through a 0.45 μm Nylon filter (Zetapor® Membrane, Neriden, CT, U.S.A.) before use.

The column used in the analysis was a PRP-1 150 x 4.1 mm ID column (Hamilton Company, Reno, Nevada). The analytical column was protected by a PRP-1 packed guard column. The mobile phase

flow rate was 1 mL/min. Under these conditions, ibafloxacin and the IS were eluted at 4.8 and 7.7 min, respectively.

Extraction Procedure

The following materials were pipetted into a 12 mL glass culture tube: 0.2 mL 0.1N sodium hydroxide (can be replaced with the calibration standard solution and IS), 1 mL plasma or urine, 0.3 mL 2N hydrochloric acid and 5 mL chloroform. The tubes were capped and shaken on a reciprocal shaker for 10 min. After shaking, the tubes were centrifuged at 1600 RPM for 10 min and the aqueous layer was then aspirated and discarded. The chloroform layer was transferred to a 12 mL glass culture tube and evaporated to dryness at 55°C under a stream of nitrogen. The residue was reconstituted in 1.0 mL mobile phase and 100 µL was injected into the HPLC system.

Calibration and Quantitation

The linear range of the method was 0.1 to 50 µg/mL for both plasma and urine samples from either dog or man. In practice, two ranges were used, 0.1 to 1 µg/mL and 1 to 50 µg/mL. This technique afforded more accurate measurements for the concentrations at the lower end of the range.

A least squares linear regression line of the peak height ratios (ibafloxacin/IS) versus ibafloxacin concentrations for each range was calculated. The slope and intercept of the line were used to calculate the concentration of ibaflaxicin in the set of unknown samples. The correlation coefficient of the least squares line was always >0.995.

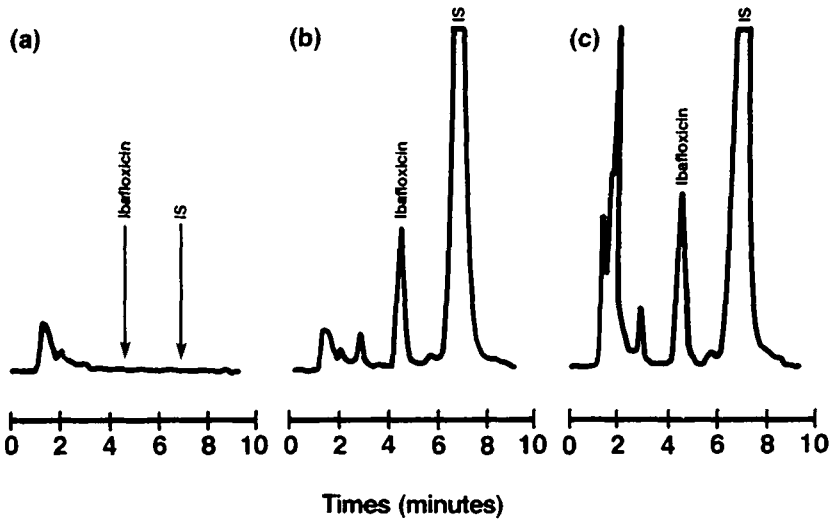

Figure 2. Typical chromatograms of human plasma: (a) blank plasma, (b) blank plasma spiked with 1 μg ibafloxacin/ml, (c) plasma of subject dosed with ibafloxacin (the peak represents 0.8 μg ibafloxacin/ml)

RESULTS AND DISCUSSION

Chromatography

Ibafloxacin and IS were well separated from human or dog plasma or urine by a simple sample pretreatment procedure. A single liquid-liquid extraction procedure, followed by evaporation of the organic phase and reconstitution of the residue in a basic mobile phase was used. The chromatograms of a blank plasma, blank plasma spiked with a standard solution of reference ibafloxacin, and a plasma sample from a subject after a single oral dose are shown in Fig 2. No chromatographic interference from the blank plasma was found for IS or ibafloxacin.

TABLE 1
Intra-day Precision and Accuracy (n=5)

Spiked (μg/mL)	Coefficient of Variation (%)	Relative Error (%)
0.1	0	+30.0
0.5	1.4	-2.0
1.0	0.7	-4.0
5.0	1.1	-0.8
10.0	0.6	-0.4
25.0	1.9	+0.4
50.0	1.2	+2.3

TABLE 2
Inter-day Precision (n=8)

Concentration (μg/mL)	Mean ± SD (μg/mL)	Coefficient of Variation (%)
.1	.14±0.02	12.9
0.5	.49±0.02	4.4
1.0	.95±0.02	2.4
5.0	4.9 ±0.12	2.6
10.0	10.1 ±0.07	0.7

Precision and Accuracy

The precision and accuracy for plasma were determined by analyzing five replicates at each of seven concentration levels (0.1, 0.5, 1, 5, 10, 25 and 50 μg/mL). The precision, expressed as the coefficient of variation, was less than 2% over the entire concentration range. The accuracy, expressed as the relative error, was between -4% to +3% for all concentration levels except the lowest. At 0.1 μg/mL, the relative error was +30%. Table 1 shows the detailed intra-day precision and accuracy results.

The inter-day precision was determined by analyzing replicate samples over a 2 week period. The coefficient of variation ranged

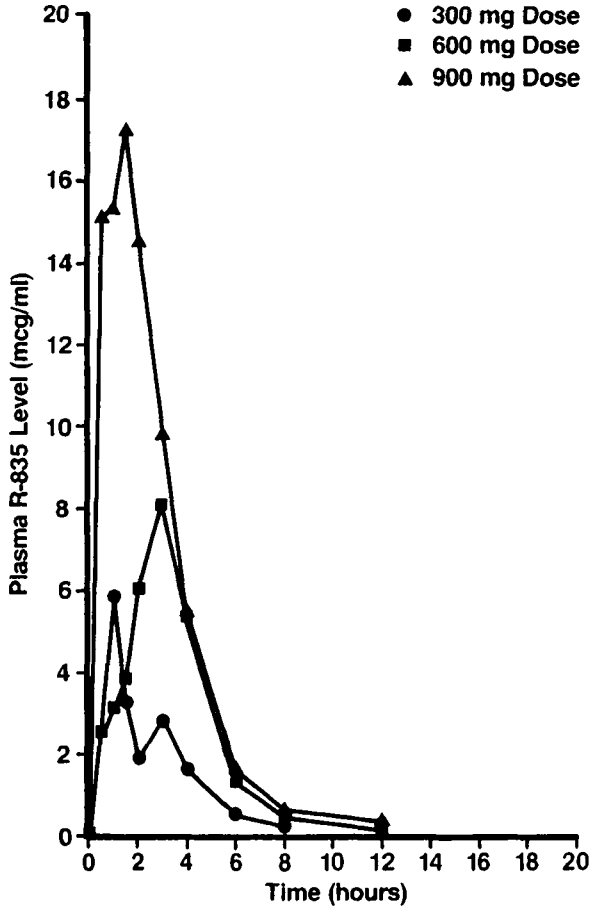

Figure 3. Plasma concentrations of R-835 after oral administration of single 300, 600, and 900 mg doses to a healthy male subject

from 0.7% - 12.9% over a concentration range of 0.1 - 10 μg/mL ibafloxacin (Table 2).

Application

Plasma and urine levels of unchanged ibafloxacin have been determined in healthy human volunteers after single escalating oral doses of ibafloxacin sodium salt. Oral absorption of ibafloxacin in human volunteers appeared to be reasonably prompt (peak plasma levels of ibafloxacin were usually achieved between 1 and 2 hours postdose). Plasma levels of ibafloxacin were reasonably proportional to dose level in these human volunteers. A representative plot of unchanged ibafloxacin in plasma versus time for three dose levels of oral ibafloxacin sodium salt is depicted in Fig 3. Little unchanged ibafloxacin was excreted in human urine; on the average, slightly less than 3% of an oral dose could be accounted for in urine.

SUMMARY

The newly developed HPLC method for ibafloxacin using UV detection described in this paper is simple, fast, sensitive,and selective. It can be used to measure clinically relevant concentrations of ibafloxacin in both plasma and urine from laboratory animals and from humans. This HPLC method should be useful for determining ibafloxacin pharmacokinetics as well as for clinical management of patients.