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Original Article

Simple High-Performance Liquid Chromatographic Method for Determination of Ciprofloxacin in Human Plasma

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Abstract

A rapid, simple and sensitive high-performance liquid chromatography method was developed for determination of ciprofloxacin in plasma by means of ultraviolet detection. Ofloxacin was used as an internal standard and separation carried on a Novapak C18 column using a mobile phase of 0.01 M phosphate buffer (pH =2.6): methanol (82:18 *v/v*). Extraction of drug was performed from plasma by liquid-liquid extraction and the average recovery was 78.2% The assay is precise, with inter-assay coefficient of variation of 6.70 % at 0.25-8 μg/ml (n=3). Using UV detection at 277 nm the detection limit for ciprofloxacin was 20 ng/ml of plasma and the mean extraction recovery was 78.2 %. Short elution time, using UV detector and usage of ofloxacin as internal standard are advantages of this method

Keywords: Ciprofloxacin; Determination; Plasma; HPLC.

Introduction

Ciprofloxacin is one of several new quinolone antimicrobial agents that show broad anti bacterial activity, low toxicity and potential for use as oral therapy in urinary tract as well as skin and soft infections ciprofloxacin is rapidly and well abosorbed from the gastro-intestinal tract. Oral bioavailability is approximately 70% and a peak concentration of a bout 2.5 μg/ml is achieved 1 to 2 h after a dose of 500 mg by mouth (1, 2). Several papers have been described for determination of ciprofloxacin in biological fluids by HPLC with UV (3, 4) or fluorescence (5, 6) detector or by microbiological methods (7). Poor reproducibility and accuracy for the last method has been reported (8). However, HPLC is the analytical method of choice for measuring ciprofloxacin (9). This paper describes an isocratic HPLC method using UV detection, which provides adequate sensitivity for routine use and diminishing the time of sampling and chromatographic analysis.

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Experimental

Standard and reagents. Ciprofloxacin was supplied by Bayer Pharma Research Center (Wuppertal-Elber-Feld, Germany) and ofloxacin was supplied by Akita Pharma Research Center (Japan). HPLC-grade methanol and all other analytical grade reagent (KH2PO4, H3PO4 and sodium chloride) were purchased from Merck Company (Darmstadt, Germany). HPLC grade water was obtained by double distillation in glass and purification through a Mill-Q water purification system (Millipore, Bedford, MA). Water was filtered through 0.45 μm filters and mobile phase were filtered through 0.22 μm filters (Millipore).

Apparatus

The waters HPLC system (Waters ASSOC., Milford, MA) employed consisted of a 510 pump, a Rheodyne injector (7105 model) with a 20 μl loop and a Waters 486 UV detector adjusted on 277 nm, connected to an advanced personal computer APICIV (NEC) with maxima 820 software and p 5200 NEC pin writer.

Chromatography condition

The separation was performed on an analytical 150×3.9 mm i.d. reversed-phase Novapak C18 (4 μm particle size) column. The mobile phase consisted of a mixture of methanol-phosphate buffer 0.01 M (18:82) which was adjusted to pH=2.6 with concentrated orthophosphoric acid. For adjusting ionic power of eluent, sodium chloride was added (1 g/lit) to mobile phase. The mobile phase was prepared daily and delivered at a flow rate of 2 ml/min.

Standard solutions

A stock solution of ciprofloxacin (50 μg/ml) and ofloxacin, internal standard (i.s.), (50 μg/ml) were prepared in methanol. 0.25 to 8 μg/ml solutions were sequentially prepared by serial dilution for plasma analysis. All the solutions were stable at least 4 weeks, when stored at -20 ºC.

Sample preparation

A modification of the method described by Jehl et al. (7) was used for extraction of drug from plasma. In a 10 ml glass screw-capped tube, 1 ml of plasma, 100 μl of i.s. and 3.5 ml of dichloro-methane were added. The contents were gently shaken for 10 min by rotation on a mixer and then centrifuged for 10 min at 1000 g. The upper aqueous layer was removed by aspiration. The organic phase was transferred to a second clean tube and evaporated at room temperature under N2 flow. To the dried residue, 1 ml of dichloromethane and 0.5 ml of ortho-phosphoric acid aqueous solution (pH=2) were added and mixed for 15 min. The tube was centrifuged at 1000 g for 15 min and 25 μl of upper aqueous layer was directly injected to HPLC.

Calibration curve

Stock solution was added to drug free human plasma to yield conentration ranging from 0.25 to 8 μg/ml of ciprofloxacin and 5 μg/ml of ofloxacin. Extraction and analytical procedure were performed as described method. Calibration curve was obtained by plotting peak area ratio of ciprofloxacin and i.s. against ciprofloxcain concentration.

Results and discussion

Under the chromatographic conditions described, ciprofloxacin and ofloxacin (internal standard) peaks were well resolved. Figure 1 shows typical chromatograms of blank plasma in comparison to spiked samples analyzed for a pharmacokinetic study. The average retention times of ciprofloxacin and ofloxacin were 8.3 and 10.6 min, respectively. The calibration curve for the determination of ciprofloxacin in plasma was linear over the range 0.25-8 μg/ml and the corresponding regression equation was y= 01.089 x + 0.044 (r= 0.9992), where y is the peak area ratio of ciprofloxacin to ofloxacin and x is the ciprofloxacin concentration (μg/ml) in plasma. The relative analytical recovery for plasma at three different concentrations of ciprofloxacin was determined. Known amounts of ciprofloxacin were added to drug-free plasma in concentrations ranging from 0.25-4 μg/ml. The internal standard was added and the relative recovery of ciprofloxacin was calculated by comparing the concentrations of the plasma to which drug had been added with the actual added concentration. The average recovery was 78%. The limit of detection was defined, as the ciprofloxacin concentration that produced a signal-to-noise ratio greater than 3. The limit of detection in plasma was 20 ng/ml based upon this criterion. This is sensitive enough for drug monitoring and other purposes such as pharmacokinetic studies. However, this method is more sensitive than some reported method (3, 4). We assessed the precision of the method by repeated analysis of plasma specimens containing known concentrations of ciprofloxacin. As shown in Table 1, coefficients

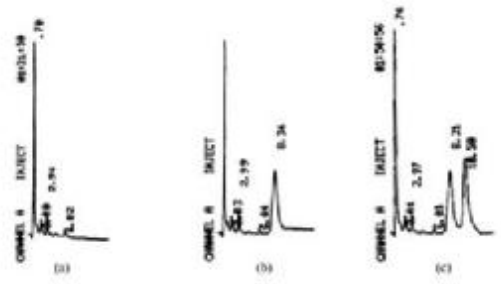

Figure 1. Representative chromatograms of (a) plasma blank, (b) plasma containing 4 μg/ml ofloxacin. (I. S.), (c) plasma containing 4 μg/ml I. S. and 4 μg/ml ciprofloxacin.

Table 1. Intra-day precision and reproducibility of the analysis of ciprofloxacin in human plasma.

Added amount of ciprofloxaci (μg/ml)	Observed Concentration Mean ± S.D. (n=3)	Coefficient of variation (%)
0.25	0.26 ± 0.017	6.45
0.50	0.51 ± 0.026	5.10
1.00	1.04 ± 0.014	1.35
4.00	3.89 ± 0.120	3.08
8.00	7.62 ± 0.510	6.69

of variation were less than 7%, which is acceptable for the routine measurement of ciprofloxacin. In order to decrease sampling and extraction errors, the internal standard was used. Therefore the coefficients of variation of our method is better than previous methods which did not use internal standard (10,11). The method described here provides a simple, reliable and reproducible HPLC assay of ciprofloxacin in plasma. The present technique is able to measure concentration of ciprofloxacin in pharmacokinetic studies.

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