

## High-Performance Liquid Chromatographic Determination of Ciprofloxacin in Rat Brain and Cerebrospinal Fluid

Yoshihiro KATAGIRI,\* Kohji NAORA, Nobuhiro ICHIKAWA, Masakazu HAYASHIBARA and Kikuo IWAMOTO

Department of Pharmacy, Shimane Medical University Hospital, 89-1, Enya-cho, Izumo, Shimane 693, Japan. Received April 16, 1990

A novel high-performance liquid chromatographic method for the fluorometric determination of a newer quinolone, ciprofloxacin (CPFX), in rat brain and cerebrospinal fluid (CSF) was developed. CPFX in brain homogenate was extracted and injected onto a reversed-phase column without fluorescence derivatization. CSF was directly analyzed without the extraction procedure. Calibration curves were linear over the concentration ranges of 10 to 500 ng/g for brain and 5 to 500 ng/ml for CSF. The recoveries of CPFX added to brain were more than 97% with a coefficient of variation of less than 4%. The present method was sensitive and reliable enough to be utilized for detailed pharmacokinetic studies of CPFX in rat brain and CSF.

**Keywords** ciprofloxacin; HPLC; determination; brain; cerebrospinal fluid; rat

It has recently been reported that severe convulsions are induced when a new quinolone carboxylic acid (quinolones) and a non-steroidal anti-inflammatory agent, fenbufen, are administered concomitantly.<sup>1-3)</sup> Ciprofloxacin (CPFX) belongs to the quinolone group of antibiotics with an extended antibacterial spectrum. Our previous animal studies have shown that the elimination half-life of CPFX in plasma is prolonged and the total body clearance of CPFX is reduced by coadministration with fenbufen but the pharmacokinetics of fenbufen and its active metabolite, felbinac, were not affected by this quinolone in rats.<sup>4)</sup> Some quinolones are thought to penetrate the blood-brain barrier or blood-cerebrospinal fluid (CSF) barrier.<sup>5)</sup> Therefore, it is important to clarify the effects of the coadministration with fenbufen upon the kinetics of CPFX in the central nervous system (CNS). For this purpose, analytical methods which would enable a sensitive determination of CPFX concentrations in brain and CSF were required. Various high-performance liquid chromatographic (HPLC) methods have been reported for the analysis of CPFX in biological materials.<sup>6-16)</sup> Most of these methods concerned the determination of CPFX in serum (or plasma) and urine, and did not include the determination of CPFX in brain and CSF.

The present paper describes a highly sensitive analytical method for the determination of CPFX in rat brain and CSF using HPLC with fluorescence detection.

### Experimental

**Chemicals** Ciprofloxacin hydrochloride was kindly supplied by Bayer AG (Leverkusen, West Germany). Pipemidic acid (internal standard) of analytical grade was purchased from Sigma Chemical Company (St. Louis, MO, U.S.A.). Methanol, distilled water and sodium lauryl sulfate were of liquid chromatographic grade. All other reagents were of analytical grade.

**Animals and Drug Treatments** Male Wistar rats, 10 weeks old, cannulated into the right jugular vein, were used. A bolus dose of 10 mg/kg of CPFX was administered *via* the cannula to the rats. At designated times after the drug injection, each rat was lightly anesthetized with ether and CSF and blood were obtained by cisternal puncture<sup>17)</sup> and through the cannula, respectively. The serum was immediately separated by centrifugation with the serum separator, Fibrichin (Takazono Sangyo Co., Ltd., Osaka, Japan) and a portion of the serum was ultrafiltered by using MPS-3 (Amicon Corp., Danvers, MA, U.S.A.). Immediately after CSF and blood collection, the rat was sacrificed by microwave irradiation focused on its head for 0.8 to 1.0 s using a microwave applicator, Toshiba Model TMW-6402A (Tokyo, Japan). The brain was readily excised and weighed after careful removal of the dural and subarachnoidal vessels.

**Analytical Procedures** Each whole brain was homogenized in 2 volumes

(v/w) of ice-cold normal saline using a teflon homogenizer. To a portion (0.3 ml) of the homogenate, 0.5 ml of 0.2 M phosphate buffer (pH 7), 0.1 ml of the internal standard solution (0.5 µg/ml of pipemidic acid) and 3 ml of diethyl ether were added. The solution was shaken for 5 min and centrifuged at 3000 rpm for 5 min at 4°C. To the bottom aqueous phase, 1 g of ammonium sulfate and 5 ml of a mixture of organic solvent (chloroform-2-propanol, 9:1, v/v) were added. The mixture was shaken for 20 min and centrifuged at 3500 rpm for 5 min at room temperature. An aliquot (4 ml) of the organic phase was evaporated to dryness under a gentle stream of dry nitrogen at 40°C. The residue was reconstituted with 0.3 ml of a mixture of methanol-0.05 N sodium hydroxide (3:2, v/v) by vortexing and filtered through a membrane filter (0.45 µm). A 10 µl aliquot was used for injection onto the HPLC column. To 30 µl of CSF, 30 µl of 0.05 N sodium hydroxide containing 0.5 µg/ml of pipemidic acid (as the internal standard) was added and mixed. A 10 µl aliquot of the mixture was directly applied onto the column. CPFX concentrations in serum (total) and ultrafiltrate (free) were determined by our previous method.<sup>18)</sup>

**Apparatus and HPLC Conditions** The HPLC system consisted of a Shimadzu LC-4A high-performance liquid chromatograph (Kyoto, Japan) equipped with a Rheodyne 7125 syringe-loading sample injector (Cotati, CA, U.S.A.) and a Shimadzu RF-530 fluorescence spectrophotometer operating at an emission wavelength of 445 nm and an excitation wavelength of 277 nm. A reversed-phase column (150 × 4.6 mm i.d.) packed with Wakosil 5C18 (Wako Pure Chemical Industries Ltd., Osaka, Japan) was used. The mobile phase was CH<sub>3</sub>OH-0.02 M KH<sub>2</sub>PO<sub>4</sub> (3:2, v/v) containing 2 mM sodium lauryl sulfate adjusted to pH 2.5 with phosphoric acid. The flow-rate was 0.8 ml/min and separation was performed at 40°C. The chromatographic data were calculated with a Shimadzu C-R2AX data module.

**Calibration Graphs** The homogenates of drug-free brain (from untreated rats) were spiked with increasing amounts of CPFX in the concentration range of 10–500 ng/g wet tissue. The calibration curve for CSF was prepared by using distilled water spiked with CPFX standard solution to make the concentration range of 5–500 ng/ml. These samples were then assayed according to the analytical procedure described above. Calibration graphs were constructed of the peak-area ratio of CPFX to an internal standard against drug concentration.

**Precision and Accuracy** Brain samples were obtained from rats at 10 min and 3 h after the administration of 10 mg/kg of CPFX, whereas the CSF sample was obtained at 90 min. The precision for the assay of CPFX was examined by performing ten replicate analyses in brain and CSF. In order to evaluate the accuracy of the present method for determination of CPFX concentration in brain, the drug-free homogenates of brain were spiked with 30 or 300 ng/g of CPFX. Recovery was determined by comparing the known added amount of the drug with its amount measured by calculating from an adequate standard.

### Results

Typical chromatograms resulting from HPLC analysis of CPFX in brain and CSF obtained from the rat are shown in Fig. 1. The chromatograms depicted in Fig. 1 are representative for the analysis of blank samples from the

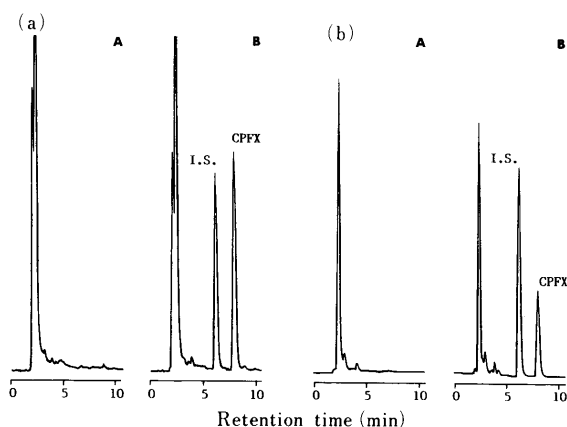


Fig. 1. Typical Chromatograms Obtained from Rat Brain (a) and CSF (b)

A, blank sample; B, sample obtained from a rat 30 min after intravenous administration of 10 mg/kg of CPFX. I.S., internal standard (pipemidic acid). Detector range was set at 2 or 4 for brain or CSF, respectively.

TABLE I. Precision of the Assay of CPFX in Rat Brain and CSF

Sample (Unit)	CPFX level <sup>a)</sup>	C.V. (%)
Brain (ng/g)	25.9 ± 1.2	4.4
	231 ± 5	2.3
CSF (ng/ml)	50.0 ± 0.7	1.5

C.V., coefficient of variation. a) Mean ± S.D. of 10 determinations.

TABLE II. Accuracy of the Assay of CPFX in Rat Brain

Added (ng/g)	Found <sup>a)</sup> (ng/g)	Recovery <sup>a)</sup> (%)	C.V. (%)
30	29.3 ± 1.1	97.7 ± 3.8	3.8
300	292 ± 4	97.4 ± 1.2	1.3

a) Mean ± S.D. of 6 determinations.

drug-free rats (Fig. 1A) or brain and CSF samples taken from rats after 30 min of the intravenous administration of CPFX (Fig. 1B). Pipemidic acid (internal standard) and CPFX were eluted at retention times of about 6.1 and 8.0 min, respectively, as well resolved peaks. No interfering endogenous peaks could be detected in the chromatogram of either drug-free brain or CSF. Furthermore, it was confirmed that the peaks for fenbufen and its metabolites were not detected on the chromatogram in the present conditions.

The calibration curves exhibited linear relationships over the concentration ranges of 10–500 ng/g in brain and 5–500 ng/ml in CSF. The regression equations by the method of least-squares were  $y = 10.46x - 0.021$  ( $r = 1.000$ ) for brain and  $y = 9.73x - 0.0019$  ( $r = 1.000$ ) for CSF, where  $y$  is the peak-area ratio of the drug to the internal standard,  $x$  is the concentration in brain (ng/g) or CSF (ng/ml) and  $r$  is the coefficient of the correlation. The limits of determination were established in brain at 10 ng/g and in CSF at 5 ng/ml.

As shown in Table I, the brain assays had coefficients of variation below 5% at the concentration tested and assays in CSF had coefficient of variation below 2%. Table II shows the recovery data for CPFX spiked to brain tissue homogenates at two different concentrations. The recoveries

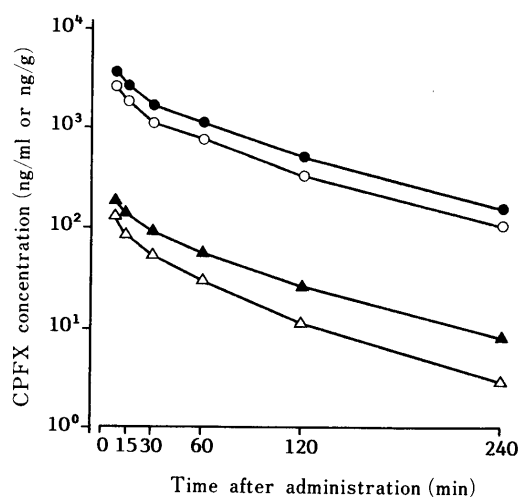


Fig. 2. CPFX Concentrations in Serum, Brain and CSF after Bolus Intravenous Administration (10 mg/kg) to Rats

●, serum total; ○, serum free; ▲, brain; △, CSF. Each point represents the mean of three rats.

for CPFX were 97.7 and 97.4% at the concentrations of 30 and 300 ng/g, respectively, with the coefficients of variation less than 4%.

The present method was then used in the preliminary study of CPFX pharmacokinetics in the CNS. Figure 2 shows the concentration *versus* time data of CPFX after bolus intravenous administration of 10 mg/kg of CPFX to rats. The serum total concentrations of CPFX showed a bi-exponential decline with time. Serum free concentration of CPFX was found to decline in proportion to its serum total concentration. CPFX concentrations in brain and CSF already showed the maximum value 7.5 min after the drug injection and then declined with serum free concentration.

## Discussion

We have previously described the HPLC methods for simultaneous determination of CPFX and fenbufen in rat plasma using ultraviolet (UV) detection.<sup>18)</sup> However, this method is not sensitive enough to determine CPFX in rat brain or CSF. CPFX is one of the relatively few drugs that exhibit a high magnitude of fluorescence without any derivatization, leading to good sensitivity.<sup>13)</sup> Therefore, we attempted to utilize the fluorescence detection of CPFX. This method involves the solvent extraction procedure for the brain samples and the direct injection of CSF samples after dilution with an equal volume of the internal standard solution. No appreciable interferences of endogenous substances were detected and the detection sensitivity was sufficient to determine CPFX without fluorescence derivatization. CPFX concentration as low as 10 ng/g in brain or 5 ng/ml in CSF can be measured using this method. Since the present method is able to detect considerably low concentrations, the assay is expected to be applicable to the detailed study of CPFX disposition in brain and CSF. The replicate analyses of rat brain and CSF indicated very low coefficients of variation. In the accuracy test, the measured amounts of CPFX indicated more than 97% of the known amount spiked to the brain tissue homogenate. Both reproducibility and recovery in the determination of CPFX were satisfactory over a wide range of concentration.

As is obvious from Fig. 2, CPFX concentration in either brain or CSF was found to rise shortly after the drug injection and to be well correlated with its serum free concentration. However, both brain/serum free and CSF/serum free concentration ratios were less than 0.1. These findings suggest that CPFX may instantaneously penetrate into the brain and CSF with a change in its serum free concentration but cross the blood-brain and blood-CSF barriers to a relatively small extent.

In conclusion, the HPLC method for the determination of CPFX in rat brain and CSF has been developed and provides a highly sensitive, accurate and reproducible analytical procedure. This method has been used successfully in kinetic studies of CPFX in the CNS.

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