

## High-Performance Liquid Chromatographic Procedure for the Simultaneous Determination of Norfloxacin, Fenbufen and Felbinac in Rat Plasma

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A high-performance liquid chromatographic method for the simultaneous determination of norfloxacin, fenbufen and felbinac extracted from 50  $\mu$ l of rat plasma is described. Chromatography was performed on a reversed-phase column with ultraviolet detection. By the present method, quantitative and reproducible determinations were possible for norfloxacin, fenbufen and felbinac over the concentration ranges of 0.2—20, 0.2—120 and 0.4—40  $\mu$ g/ml, respectively. The recoveries of norfloxacin, fenbufen and felbinac added to plasma were nearly 100% with a coefficient of variation of less than 8.0%. This method was found to be applicable to pharmacokinetic studies of each drug after the concomitant administration of norfloxacin and fenbufen.

**Keywords** HPLC; norfloxacin; fenbufen; felbinac; plasma; simultaneous determination; rat; coadministration

In a recent review article, it has been reported that severe convulsion is induced in some cases after the concomitant administration of a new quinolone antibacterial agent, enoxacin, and a non-steroidal anti-inflammatory agent, fenbufen (FNB).<sup>1)</sup> FNB is a pro-drug, which is readily metabolized to the active compound, 4-biphenylacetic acid (felbinac, FLB).<sup>2-4)</sup> Recently, norfloxacin (NFLX), which is also a quinolone antibacterial agent, has been used because of its broad spectrum of activity.<sup>5,6)</sup> It is presumed that convulsion similar to that encountered with enoxacin may be induced by the coadministration of NFLX and FNB. Therefore, it is important to clarify the pharmacokinetics of NFLX, FNB and FLB after the concomitant administration of NFLX and FNB.

We have previously described a high-performance liquid chromatographic (HPLC) procedure for the pharmacokinetic study of another new quinolone, ofloxacin, with FNB and FLB.<sup>7)</sup> This procedure was found to be a sensitive and selective assay method for these drugs in rat plasma and an adequate method to determine them simultaneously and readily.

In the present paper, we described the application of the previous method, with a slight modification, to the simultaneous determination of NFLX, FNB and FLB in rat plasma after the concomitant administration of NFLX and FNB.

### Experimental

**Chemicals** Norfloxacin was kindly supplied by Kyorin Yakuhin Co., Ltd. (Tokyo, Japan) and fenbufen and felbinac were provided by Lederle Japan, Ltd. (Tokyo, Japan). Nalidixic acid and *N*-phenylanthranilic acid (internal standards) of analytical grade were purchased from Nakarai Tesque Inc. (Kyoto, Japan). Each drug was dissolved in a small volume of 0.1N sodium hydroxide and diluted with distilled water to make the standard or internal standard solutions.

**Analytical Procedure** HPLC conditions were set up according to our previous method<sup>7)</sup> involving a reversed-phase ion-pair chromatography for the simultaneous determination of ofloxacin, FNB and FLB, except for the detection wavelength. In the previous method the detection wavelength from 0 to 8 min after injection was set at 300 nm, while it was shifted to 284 nm (the maximum absorbance wavelength of NFLX) in the present method.

The drugs along with added internal standards were extracted from 50  $\mu$ l of rat plasma by the same procedure as described in the previous paper.<sup>7)</sup>

**Calibration Curves** Known amounts of NFLX, FNB and FLB were added to the blank plasma in the ranges of 0.2—20, 0.2—120 and 0.4—40  $\mu$ g/ml (in plasma), respectively. These spiked plasma samples were analyzed and the peak-height ratio of the drug to internal standard (NFLX to nalidixic acid, FNB or FLB to *N*-phenylanthranilic acid) was

plotted against the plasma level of the drug.

**Drug Administration to Rats** Male Wistar rats (10 weeks old, 265—277 g) were cannulated in the right jugular vein and were administered a bolus intravenous dose of 5 mg/kg of NFLX and 10 mg/kg of FNB via the cannula. Blood (about 0.13 ml for the preliminary kinetic study or 0.5—1 ml for other purposes) was withdrawn from the cannula periodically into a heparinized tube. The plasma was immediately separated by centrifugation and kept frozen (–20°C) until the analysis.

**Reproducibility** Blood samples were taken from the rats at appropriate times after the concomitant administration of NFLX and FNB. Ten repeated analyses of plasma samples (50  $\mu$ l) were carried out.

**Recovery** The blank plasma was supplemented with known amounts of NFLX, FNB and FLB at three different concentrations. The determination of NFLX, FNB and FLB in these spiked plasma samples was then carried out according to the above procedure. The recovery of each drug was calculated by comparing the experimental value with the corresponding theoretical value.

### Results

Typical chromatograms resulting from the analysis of plasma blank, plasma blank spiked with NFLX, FNB and FLB, and a plasma sample obtained from a rat which was given NFLX and FNB are shown in Fig. 1. There was no peak of endogenous material in rat plasma. The retention times ( $t_R$ ) for NFLX, FNB and FLB were approximately 6.5, 9.0 and 10.0 min, respectively. The internal standards, nalidixic acid and *N*-phenylanthranilic acid, had  $t_R$  values of 5.0 and 16.0 min, respectively. These peaks were well separated from each other and also from a few small peaks which seemed to be derived from some minor metabolites of FNB.

Calibration curves were generated by least-squares linear regression analysis. Satisfactory linearity was observed in the ranges of 0.2—20  $\mu$ g/ml of NFLX, 0.2—120  $\mu$ g/ml of FNB and 0.4—40  $\mu$ g/ml of FLB. The regression equations of the calibration curves were  $y = 0.404x - 0.036$  ( $r = 1.000$ ) for NFLX,  $y = 0.419x + 0.099$  ( $r = 1.000$ ) for FNB and  $y = 0.197x - 0.007$  ( $r = 1.000$ ) for FLB, where  $y$  is the peak-height ratio of the drug to the internal standard,  $x$  is the concentration ( $\mu$ g/ml) of the drug in plasma, and  $r$  is the coefficient of correlation. The lower limits of the determination were 0.2  $\mu$ g/ml for NFLX, 0.2  $\mu$ g/ml for FNB and 0.4  $\mu$ g/ml for FLB. These calibration and sensitivity data for each drug were not affected by the presence of other drugs in the plasma.

Reproducibility was evaluated by ten replicate assays at each of three different concentrations of drugs in plasma.

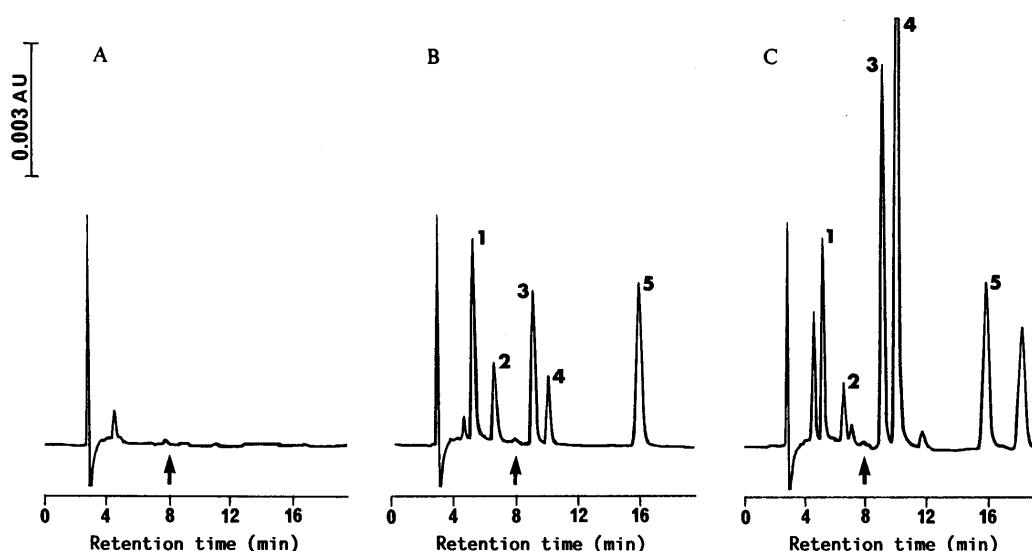


Fig. 1. Chromatograms of (A) a Plasma Blank, (B) a Plasma Blank Spiked with NFLX, FNB and FLB, (C) a Plasma Sample 1 h after Bolus Intravenous Administration of 5 mg/kg of NFLX and 10 mg/kg of FNB to a Rat

Peaks: 1, nalidixic acid (internal standard); 2, NFLX; 3, FNB; 4, FLB; 5, *N*-phenylanthranilic acid (internal standard). The arrow indicates the time-point for automatic change-over of the detection wavelength from 284 to 275 nm.

TABLE I. Reproducibility of the Determination of NFLX, FNB and FLB in Rat Plasma

Drug	Plasma level <sup>a)</sup> (μg/ml)	C.V. (%)
NFLX	0.34 ± 0.02	5.8
	1.50 ± 0.05	3.5
	3.30 ± 0.09	2.7
FNB	0.92 ± 0.01	1.0
	5.65 ± 0.04	0.7
	75.4 ± 0.8	0.9
FLB	1.19 ± 0.03	2.3
	6.61 ± 0.06	1.0
	27.6 ± 0.1	0.5

a) Mean ± S.D. of ten determinations.

TABLE II. Recoveries of NFLX, FNB and FLB Added to Rat Plasma

Drug	Added (μg/ml)	Recovery <sup>a)</sup> (%)	C.V. (%)
NFLX	0.20	104.2 ± 7.9	7.6
	1.0	100.2 ± 1.3	1.3
	10.0	99.1 ± 2.1	2.1
FNB	0.20	101.4 ± 4.0	3.9
	2.0	100.1 ± 0.7	0.7
	20.0	101.3 ± 0.8	0.8
FLB	0.40	99.1 ± 3.8	3.8
	2.0	100.3 ± 1.4	1.3
	20.0	99.9 ± 0.2	0.2

a) Mean ± S.D. of five determinations.

Coefficients of variation (C.V.) for NFLX, FNB and FLB ranged from 2.7 to 5.8%, 0.7 to 1.0% and 0.5 to 2.3%, respectively, as shown in Table I.

Table II shows the recovery data for NFLX, FNB and FLB when the rat plasma was supplemented with each drug at three different concentrations. The recoveries from plasma spiked with NFLX, FNB and FLB were 99.1–104.2%, 100.1–101.4% and 99.1–100.3%, respectively.

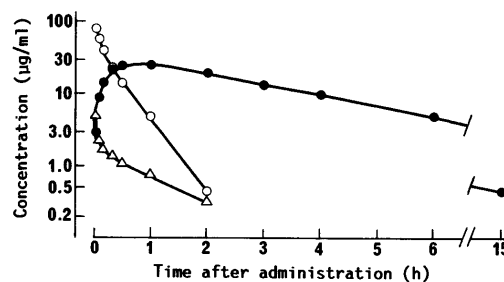


Fig. 2. Plasma Concentrations of NFLX, FNB and FLB after Bolus Intravenous Administration of 5 mg/kg of NFLX and 10 mg/kg of FNB to a Rat

△, NFLX; ○, FNB; ●, FLB.

The coefficients of variation were less than 8.0% for each drug.

Plasma concentrations of NFLX, FNB and FLB after bolus intravenous administration of 5 mg/kg of NFLX and 10 mg/kg of FNB to a rat were determined, and the time-courses of these plasma levels are shown in Fig. 2. Both NFLX and FNB disappeared from rat plasma bi-exponentially with time. The biotransformation of FNB to its active metabolite, FLB, was found to be quite rapid and the elimination of FLB was relatively slow, occurring in a mono-exponential fashion.

## Discussion

The HPLC technique is widely used for the determination of drugs in biological samples. HPLC methods have been developed to determine FNB and FLB separately.<sup>8,9)</sup> NFLX has also been determined by HPLC in biological fluids.<sup>10–12)</sup> These methods, however, were not necessarily suitable for the simultaneous assay of NFLX, FNB and FLB in plasma. We have previously described a novel HPLC procedure for the simultaneous determination of ofloxacin, FNB and FLB in rat plasma.<sup>7)</sup> Based on the chemical similarity of NFLX to ofloxacin, it was considered

that NFLX, FNB and FLB might be determined simultaneously and separately by this HPLC procedure under identical analytical conditions. In fact, the previous conditions were found to give a suitable sensitivity for FNB and FLB. However, the sensitivity for NFLX was too low to be suitable for detailed pharmacokinetic studies. Therefore, we modified the wavelength of ultraviolet detection for NFLX from 300 to 284 nm and were able to establish a simple and reliable method to determine NFLX, FNB and FLB simultaneously. A comparison of the blank and sample plasma clearly indicates that there is no interference from other plasma components, and this procedure is very specific for simultaneous determination of these drugs (Fig. 1).

In addition, the required sample size is very small, only 50  $\mu$ l of plasma. In spite of this small volume of the plasma sample, the lower limits of determination of NFLX, FNB and FLB by the present method were 0.2, 0.2 and 0.4  $\mu$ g/ml, respectively. All of these levels are far below the therapeutic range. In contrast, the published HPLC methods required 1 or 2 ml of plasma for the determination of FNB<sup>8,9)</sup> and 0.2 to 1 ml of plasma or serum for the determination of NFLX.<sup>10-13)</sup> The smaller volume of the plasma sample is advantageous for time course assay in pharmacokinetic studies in individual rats. Thus, the present method has a significant advantage in both sensitivity and selectivity over the previous methods, which were developed independently for the determination of NFLX, FNB and FLB.

As is clear from the present results, both reproducibility and recovery in the determination of each drug were

satisfactory over a wide concentration range. The present method which we have developed for the simultaneous determination of NFLX, FNB and FLB in rat plasma is simple, sensitive, precise and accurate enough to utilize for detailed pharmacokinetic studies of these drugs, as exemplified in Fig. 2. It was found that the present HPLC method could be applied to the analysis of plasma samples from individual rats given NFLX and FNB intravenously.

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