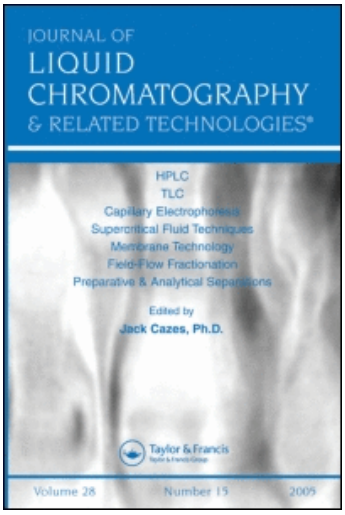

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Determination of Ciprofloxacin (Bay o 9867) in Biological Fluids by High-Performance Liquid Chromatography

W. Gau[a]; H. J. Ploschke[a]; K. Schmidt[a]; B. Weber[a]
[a] Bayer AG Sector Pharma Business Ethical Products Analytics and Quality Control Research Analytics, Wuppertal, Federal Republic of Germany

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DETERMINATION OF CIPROFLOXACIN (BAY o 9867) IN BIOLOGICAL FLUIDS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

W. Gau, H. J. Ploschke, K. Schmidt, and B. Weber
Bayer AG
Sector Pharma
Business Ethical Products Analytics and Quality Control
Research Analytics
D5600 Wuppertal, Federal Republic of Germany

ABSTRACT

A high-performance liquid chromatographic method for the analyses of ciprofloxacin (BAY o 9867) (1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid hydrochloride) in human serum, plasma and urine samples is described. Diluted serum, plasma, and urine samples are injected onto a RP-18 column without prior extraction or clean-up procedure. Ciprofloxacin is separated from the ballast by an eluent consisting of an 0.025M H_3PO_4 solution adjusted to pH=3 with tetrabutylammonium hydroxide and acetonitrile.

Ciprofloxacin is detected fluorimetrically giving a detection limit of 8ng/ml in plasma and serum and of 50ng/ml in urine. A statistical evaluation of the assay showed acceptable accuracy and precision for 10 to 500ng of BAY o 9867 per ml in serum and plasma and for 50ng to 600ng of BAY o 9867 per ml of diluted urine

Requests for reprints should be addressed to:
Dr. B. Weber, Bayer AG, Friedrich-Ebert-Straße 217-233, D5600 Wuppertal 1, FRG.

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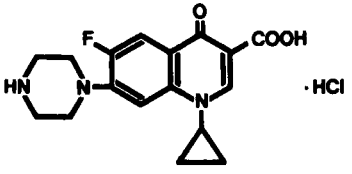
specimens. This method was used to monitor the concentrations of BAY o 9867 in serum, plasma and urine of volunteers after oral administration of ciprofloxacin.

INTRODUCTION

Ciprofloxacin (BAY o 9867, Fig. 1) is a new compound developed in the research laboratories of BAYER AG. This compound has a broad antimicrobial activity which encompasses grampositive and in particular, clinically important gram-negative pathogens (1-6). Up until now, the concentrations of BAY o 9867 in serum, plasma and urine of volunteers who had been given BAY o 9867 has been determined microbiologically. But the microbiological assay does not distinguish between BAY o 9867 and the active metabolites. Therefore it was necessary to develop a rapid, sensitive and specific high-performance liquid chromatographic method for the measurement of BAY o 9867 in body fluids.

MATERIALS AND METHODS

Chemicals. Ciprofloxacin (BAY o 9867) used in this study was produced by BAYER AG (batch 828193), HCl,



BAY o 9867
Ciprofloxacin

FIGURE 1: Structure of ciprofloxacin.

H_3PO_4, tetrabutylammonium hydroxide (40% in H_2O) and acetonitrile (Lichrosolve) were of reagent or higher grade and purchased from E. Merck, Darmstadt, FRG. The water used for chromatography was double distilled. All solvents were filtered prior to use through a 0.22µm filter membrane and degassed with helium.

Chromatography. All analyses were performed with a Spectra Physics (San Jose,Ca., USA) liquid chromatograph model SP8100 equipped with an integrator model SP4200. The drug substances were detected with the spectrofluorimeters model 3000 or model LS4 (λ ex =277nm, λ em =445nm) from Perkin Elmer. An analytical column (steel, 250x4mm i.d.) packed with Spherisorb ODS II, 5µm (Phase Separations, Queensferry, UK) was used for all analyses. The mobile phase consisted of 95% 0.025M H_3PO_4 solution adjusted to pH=3 with tetrabutylammonium hydroxide solution (40% aqueous) and 5% acetonitrile. The flow rate was 2ml/min. 10µl of samples were injected automatically onto the column via an injection loop.

Preparation of the standard solutions. The standard solution of BAY o 9867 are prepared by stepwise dilution of a stock solution (5.68mg BAY o 9867 dissolved in phosphate buffer 1/15M, adjusted with H_3PO_4 to pH=3) with the same solvent resulting in a final concentration of 11.4ng/ml.

Sample preparation. Urine is diluted with phosphate buffer 1/15M, adjusted with H_3PO_4 to pH=3, depending upon the collection periods after administration of the drug. For the collection periods 0-4h, 4-8h, and 8-12h the dilution factor is 1000. Collection periods 12-48h - dilution factor 50, 48-120h - dilution factor 2. An aliquot of the sample is passed through a filter membrane prior to injection.

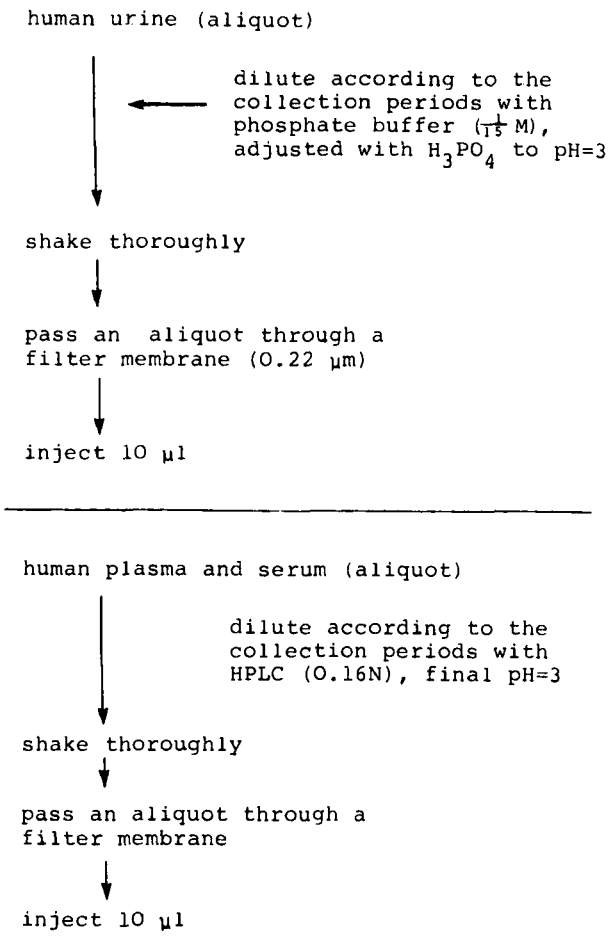


FIGURE 2: Sample preparation.

1ml serum or plasma sample (collection period 0.5-2h after the administration of the drug) is diluted with 2ml (3ml) HCl (0.16N) resulting in a pH-value of 3. 1ml portions of the samples taken at 3-24h after administration are diluted with 1ml HCl (0.16N). After filtration through a filter membrane (0.22µ) an aliquot is injected onto the chromatographic column. (Fig.2)

Calculation. The concentration of BAY o 9867 is determined by external standardization. The calibration curve is obtained by plotting the peak heights versus the concentrations of the standard solutions.

RESULTS AND DISCUSSION

HPLC methods have been developed for the closely related drugs norfloxacin (7,8) and pefloxacin (9). After extracting the drug substances with an organic solvent (CH_2Cl_2, $CHCl_3$) from the biological fluids the extract is chromatographed on RP- (8,9) or ion exchange material (7). The substances are detected by their UV-absorbance.

From our experiences with the analysis of β-lactam antibiotics (10), where the substances were analysed in untreated biological fluids, we decided to circumvent a time consuming clean-up procedure, and injected diluted serum, plasma- and urine-samples directly onto the chromatographic column. By lowering the percentage of the organic eluent in the mobile phase and adding tetrabutyl ammoniumhydroxide to the aqueous phase no precipitation of protein was observed during the analysis of serum and plasma samples. The lifetime of the chromatographic column is long. After 1000 injections the column must be discarded.

Figure 3 and figure 4 show chromatograms of plasma and urine samples. No interfering peaks in the blank sample disturb the detection of the target substance, whereas after application of ciprofloxacin a possible metabolite could appear in the retention window of the substance to be analysed. Therefore the known metabolites M-1 to M-3 (Fig. 5) were chromatographed under the same chromatographic conditions.

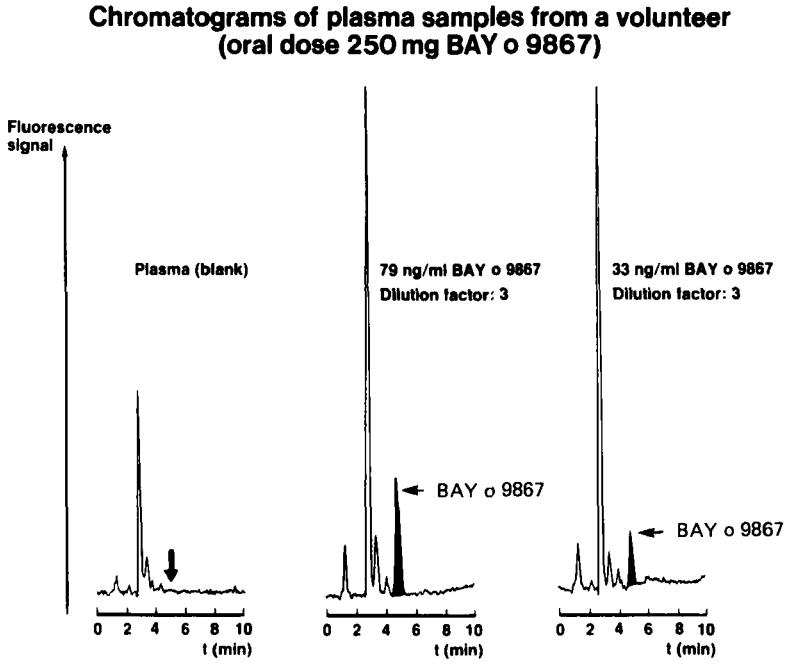


FIGURE 3: HPLC chromatograms of plasma samples.

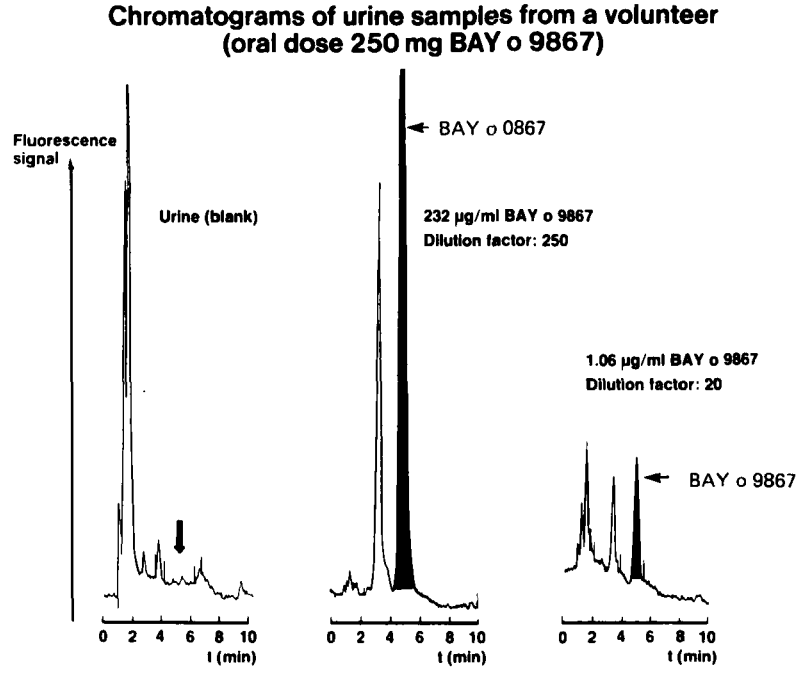


FIGURE 4: HPLC chromatograms of urine samples.

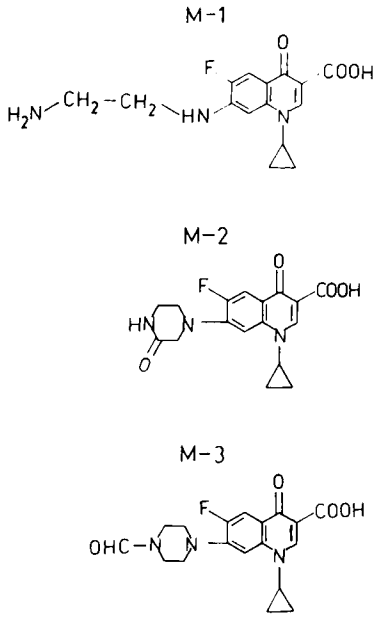


FIGURE 5: Structures of the metabolites of ciprofloxacin.

The k'-value of the metabolite M-1 is smaller then k'-value of ciprofloxacin while the metabolites M-2 and M-3 elute later than ciprofloxacin ($k'_{M_2,M_3} > k'$ BAY o 9867).

A linear relationship between the concentration of ciprofloxacin and the fluorescence signal is observed from 20 to 500ng/ml (Fig. 6).
The recoveries of ciprofloxacin from samples spiked with various amounts of ciprofloxacin (Fig. 7) is about 100%. The coefficient of variation for this determination lies within the range of 1% to 6%. Injecting a 10µl sample of plasma or serum the detection limit for ciprofloxacin is 7-8ng/ml resulting a limit of determination of 11ng/ml. At this concentration the confidence limit (0.95) is in the

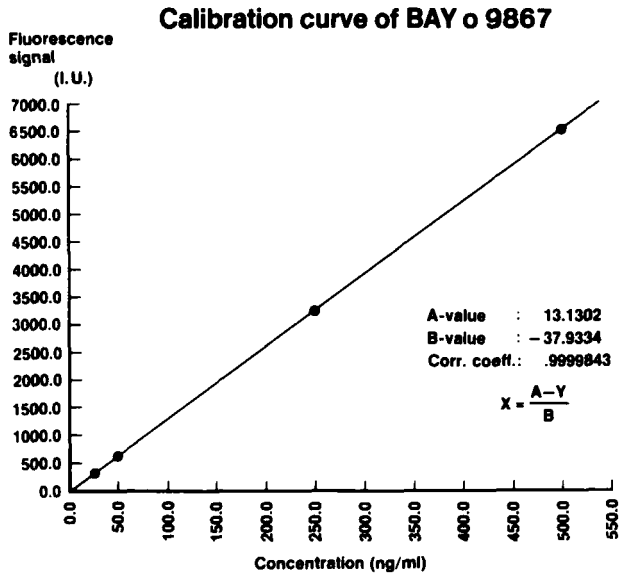


FIGURE 6: Calibration curve of the fluorescence assay.

Recovery study of BAY o 9867 with spiked serum-/plasma and urine samples

Sample	Theor. value	Actual value	C.V.	Percentage of the theor. value
Urine	36.2 µg/ml	35.0 ±0.37 µg/ml*	1.1	96.7
Urine	3.62 µg/ml	3.64±0.02 µg/ml*	0.5	100.6
Urine	0.362 µg/ml	0.37±0.02 µg/ml*	5.4	102.2
				$\bar{x}$ = 99.8 ± 2.8
Plasma	543.0 ng/ml	544.3 ±5.5 ng/ml*	1.0	100.2
Plasma	54.3 ng/ml	55.0 ±0.41 ng/ml*	0.7	101.3
Plasma	10.86 ng/ml	10.58±1.32 ng/ml*	12.5	97.4
				$\bar{x}$ = 99.6 ± 2.0
Serum	543.0 ng/ml	545.5 ±8.9 ng/ml*	1.6	100.5
Serum	54.3 ng/ml	53.13±0.9 ng/ml*	1.7	97.9
Serum	10.86 ng/ml	10.37±0.65 ng/ml*	6.3	95.5
				$\bar{x}$ = 98.0 ± 2.5

*n = 4, C.V. = Coefficient of variation

FIGURE 7: Recovery rates of ciprofloxacin from plasma, serum and urine.

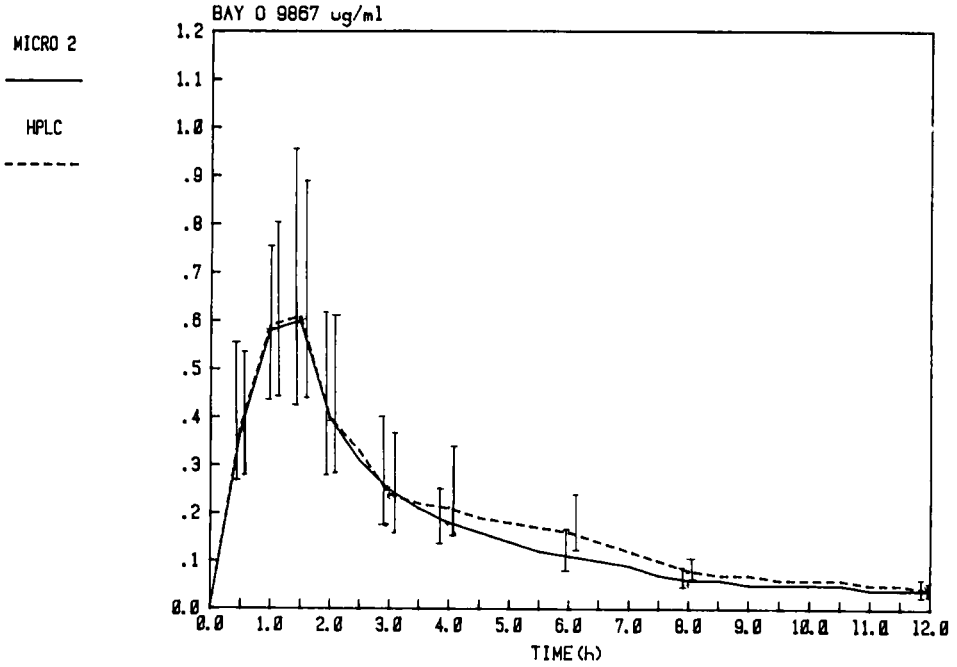


FIGURE 8: Plasma levels of ciprofloxacin determined by HPLC and bioassay.

range of 7.8 - 15.5ng/ml. The limit of determination for the substances in urine is 50ng/ml. A pharmacokinetic analysis of plasma obtained from six volunteers after oral administration of 250mg ciprofloxacin (tablet) is shown in figure 8. In this graph the values obtained by HPLC are depicted together with the concentrations measured by the bioassay (agar well technique, E. coli 4004). The corresponding concentrations of ciprofloxacin in the urine are shown in figure 9. The statistical evaluation of this comparative study demonstrate table 1 and 2. The results reveal good agreement with respect to plasma concentrations while the urinary excretion measured

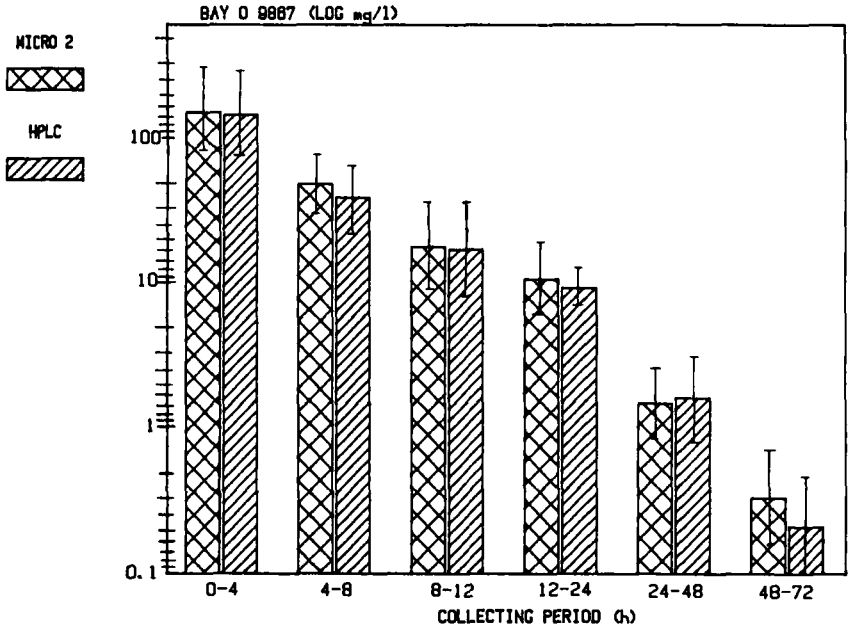


FIGURE 9: Levels of ciprofloxacin in urine determined by HPLC and bioassay.

Table 1: Plasma levels and urinary excretion, geometric means and geometric sd of absolute values.

			mean	sd	percent of dose
Plasma	AUC (h*mg/l)	bioassay	2.056	1.730	
		HPLC	2.258	1.693	
	Cmax (mg/l)	bioassay	0.711	1.623	
		HPLC	1.693	0.827	
Urine	(mg)	bioassay	61.25	1.439	25
		HPLC	55.18	1.415	22

Table 2: Analysis of variance of log. transformed values (degrees of freedom of F-tests: 1/5).

effect of the methods	F	P
plasma AUC	1.19	0.32
plasma Cmax	1.19	0.32
urinary excretion	7.99	0.04

by bioassay is about 15% higher (statistically significant) than the amount measured by HPLC. This difference is due to the microbiologically active metabolites which are separated from ciprofloxacin during chromatography while in the bioassay the metabolites are measured together with the parent compound.

In conclusion, we have described a highly sensitive, specific and fast method for the analysis of ciprofloxacin (BAY o 9867) in biological fluids.

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The present address of Dr. Gau is BAYER AG, Business Sector Agrochemicals, Chemical Research, Pflanzenschutzzentrum Monheim, 5090 Leverkusen, Bayerwerk.

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