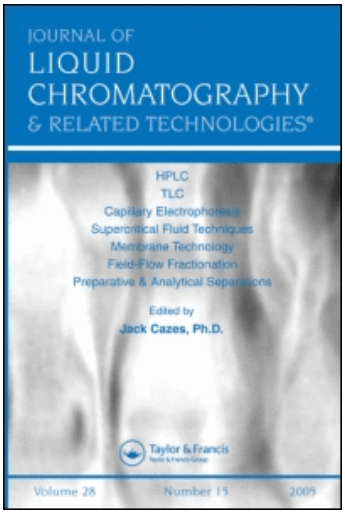

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Liquid Chromatographic Analysis of Ciprofloxacin and Ciprofloxacin Metabolites in Body Fluids

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LIQUID CHROMATOGRAPHIC ANALYSIS OF CIPROFLOXACIN AND CIPROFLOXACIN METABOLITES IN BODY FLUIDS

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ABSTRACT

An isocratic HPLC assay procedure for analysis of ciprofloxacin and three metabolites was developed. The procedure requires only dilution of bile, saliva, and urine samples prior to reverse-phase chromatography on a polystyrene-divinylbenzene (PSDVB) column; analysis of serum samples requires a cleanup step on a PSDVB cartridge prior to chromatography. The dependence of chromatographic efficiency on flow rate and temperature was investigated and the accuracy, precision, selectivity, and sensitivity of the procedure were evaluated. The developed procedure was also compared to a modified version of a published ciprofloxacin procedure that requires an octadecyl-silane (ODS) column for chromatographic separation. Similar efficiency, precision, and accuracy were observed with both procedures and

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both were used for analysis of clinical samples. However, the procedures were used for different purposes. The PSDVB procedure, because of more favorable column selectivity, was used to assay ciprofloxacin and its metabolites in bile, urine and saliva samples. The ODS procedure, because of a simpler serum preparation step, was used to assay ciprofloxacin in serum samples.

INTRODUCTION

Ciprofloxacin (1-cyclopropyl-6-fluoro-1,4,-dihydro-4-oxo-7 [1-piperazinyl] 3-quinolinecarboxylic acid) is a new quinolone carboxylic acid derivative with Gram-negative and Gram-positive bactericidal activity (1,2,3). Ciprofloxacin also exhibits a rapid onset of action and lacks cross-reactivity with penicillins, cephalosporins, and aminoglycosides (4). Since low (less than 0.1 mg/L) minimal inhibitory drug concentrations (MIC_{90}) were reported for most clinically relevant microorganisms (5), sensitive analytical procedures are necessary for assay of ciprofloxacin and ciprofloxacin metabolites in body fluids.

Since ciprofloxacin chromatographic (HPLC) and microbiological assay values obtained with serum samples showed a good statistical correlation (6,7), ciprofloxacin metabolites in serum samples are either present in relatively low concentrations or have low microbiological activity. However, discrepancies between microbiological and chromatographic assays of urine samples collected from subjects treated with ciprofloxacin have been observed (6,7). These discrepancies were attributed to the possible presence of microbiologically active metabolites. To date, four metabolites of ciprofloxacin have been identified in human urine and feces; these metabolites account for 10-18% of an administered dose (8,9).

Various HPLC procedures involving chromatographic separation of ciprofloxacin on an octadecylsilane (ODS) column were already published (6,7,10,11). However, the published procedures were developed primarily for assay of ciprofloxacin itself rather than ciprofloxacin metabolites. Since the relative polarity range of these metabolites is rather wide, isocratic elution of the ODS column does not yield a practical procedure for concurrent assay of ciprofloxacin and the less polar active metabolites. Consequently, a gradient HPLC procedure was developed for research purposes but because of its complexity the gradient elution was not used for routine analysis of clinical samples (12).

In the present study, we developed an isocratic elution HPLC procedure using a polystyrene divinylbenzene (PSDVB) column instead of the ODS column for chromatographic separation. The relative efficiency, selectivity, accuracy, and precision of the method were evaluated. The new procedure is suitable for routine assay of ciprofloxacin and three metabolites in clinical bile, saliva, and urine samples. Ciprofloxacin and its metabolites can also be analyzed in serum samples using the new method, but a cartridge cleanup step is essential for chromatography of serum samples. Since the serum cleanup step is time consuming, a modification of the previously published ODS procedure which does not require a cartridge cleanup of serum (11) was also investigated.

EXPERIMENTAL

Materials and Reagents

Glass distilled, HPLC grade acetonitrile, methanol, and water were used to prepare chromatographic solvents. Analytical reagent grade or ACS grade phosphoric acid, potassium dihydrogen

phosphate, sodium hydroxide, tetrabutylammonium bromide, and trichloroacetic acid were also required for preparation of chromatographic solvents.

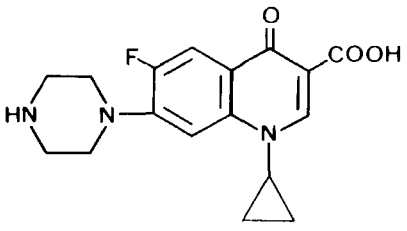
Ciprofloxacin, ciprofloxacin metabolites, and the N-ethyl and isopropyl analogs of ciprofloxacin were obtained from Bayer AG, Pharma Research Center (Wuppertal, FRG). Figure 1 illustrates the chemical structures of ciprofloxacin and its metabolites.

The polystyrene divinylbenzene (PSDVB-S) 5 micron beads were packed in 15 cm x 4.6 mm ID columns by Polymer Laboratories, Inc. (Amherst, MA). Chrom-Prep PRP-1 cartridges filled with polystyrene-divinylbenzene resin (320 µl capacity) were purchased from Hamilton Company (Reno, Nevada). The octadecylsilane (ODS) 5 micron spherical Nucleosil C_{18} particles packed in a 25 cm x 4.6 mm ID columns were obtained from Alltech Associates, Inc. (Deerfield, IL).

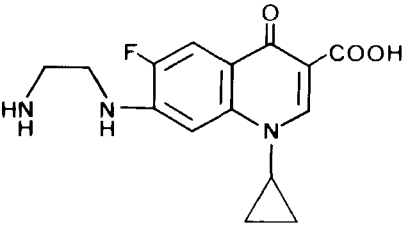
Instrumentation

The ODS column was monitored by a GM 970 Spectrofluorimetric detector which was purchased from Kratos, Inc. (Ramsey, NJ). The PSDVB column was connected to a Spectroflow 773 UV absorbance detector which was also obtained from Kratos, Inc.

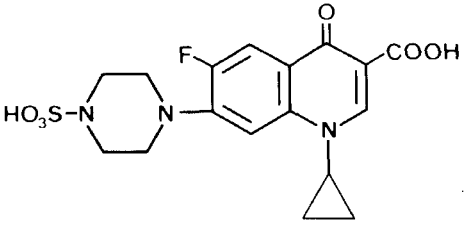
Both chromatographic columns were maintained at constant temperature with a CH-30 column heater and a TC-50 temperature controller which were purchased from Phenomenex (Rancho Palos Verdes, CA). The chromatographic solvents were pumped with either a Waters M-45 HPLC pump purchased from Millipore Inc. (Milford, MA) or a Rabbit-HP pump purchased from Rainin, Inc. (Woburn, MA). Both chromatographic procedures also used interchangeably ISS-100 Perkin Elmer (Norwalk, CT) and LC/9505 IBM (Danbury, CT) HPLC autoinjectors. Spectra-Physics (San Jose, CA) SP4100 and SP4270 Integrators were used to generate chromatographic data.



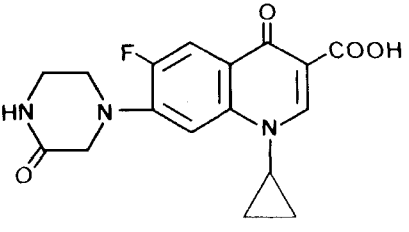
CIPROFLOXACIN



METABOLITE MI



METABOLITE M2



METABOLITE M3

FIGURE 1. Chemical structures and names of ciprofloxacin and ciprofloxacin metabolites;
ciprofloxacin: 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid
metabolite M1: 7-(2-aminoethylamino)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid
metabolite M2: 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-sulfo-1-piperazinyl)-3-quinolinecarboxylic acid
metabolite M3: 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(3-oxo-1-piperazinyl)-3-quinolinecarboxylic acid

Sample Preparation

Serum samples were diluted 1:1 with a 0.05 M potassium phosphate pH 3.0 buffer solution of the N-ethyl ciprofloxacin analog, which was used as internal standard. The concentration of the internal standard in phosphate buffer solution was 0.8 mg/L. For chromatography on the ODS column the diluted sample was filtered through a Gelman (Ann Arbor, MI) 0.45 μm pore, 25 mm diameter Acrodisc and a 10 μl aliquot of the filtered solution was injected onto the ODS column. Standard calibration curve samples were prepared from blank serum or plasma samples containing known concentrations of ciprofloxacin. The dilution factors and the concentration range of standard calibration curve samples matched those of the unknown serum samples.

Chromatography of serum or plasma samples on the PSDVB column required a cleanup step on a Chrom-Prep cartridge. Serum or plasma samples were diluted 1:1 with a 0.05 M potassium phosphate pH 3.0 solution of the isopropyl analog of ciprofloxacin; 1 ml of diluted solution was filtered through the cartridge. The charged cartridge was washed with 2.5 ml of the pH 3.0 phosphate solution which also contained 10% of methanol. The cartridge was then eluted with 1 ml of a 3:1:4 (v/v) mixture of acetonitrile, methanol, and aqueous 0.02 M trichloroacetic acid (TCA) solution adjusted to pH 3.0. The cartridge was also eluted with 1 ml of aqueous trichloroacetic acid solution (pH 3.0) and the two 1 ml aliquots were combined prior to chromatography.

Since the observed concentrations of ciprofloxacin and its metabolites in unknown urine samples were much higher than in serum, urine calibration curve samples and unknown urine samples were diluted at least 1:20 with 0.05 M potassium phosphate pH 3.0 buffer before the final 1:1 dilution with potassium phosphate buffer solution of internal standard. The internal standard used for assay of urine samples was the isopropyl analog of ciproflox-

acin; its concentration in the phosphate buffer solution was 4 mg/L. The more extensive dilution of the reference and unknown urine samples yielded clear solutions and thus eliminated the need to filter the diluted urine samples before injection.

Chromatography

Aliquots (10 µl) of the diluted and filtered reference and unknown serum samples were injected onto the 25 cm ODS column and eluted with a chromatographic solvent containing acetonitrile, methanol, and potassium phosphate pH 3.0 aqueous solution of 0.1 N tetrabutylammonium bromide. The volume-to-volume ratios of acetonitrile, methanol, 0.05 M potassium dihydrogen phosphate adjusted to pH 3.0 with phosphoric acid, and 0.1 M tetrabutyl ammonium bromide also adjusted to pH 3.0 with phosphoric acid, were 10:7:73:10. Chromatography was carried out at 40°C and a flow rate of 0.5 ml/min. The elution was monitored by the spectrofluorimetric detector at 277 nm excitation wavelength whereas the emission wavelength was above 360 nm (a 360 nm cutoff filter was used).

The diluted reference and unknown urine samples were injected directly onto the 15 cm PSDVB column. New PSDVB columns were washed for 24 hours with chromatographic solvent before sample injection to eliminate initial peak tailing. The volume of sample injected onto the column was 20 µl. The chromatographic solvent was a mixture of acetonitrile, methanol, and 0.02 M trichloroacetic acid solution adjusted to pH 3.0 with 1 N sodium hydroxide. The volume-to-volume ratio of the three components was 22:4:74. The elution flow rate ranged from 0.5 to 1.0 ml/min. The column temperature was maintained at 30°C. The column eluate was monitored by a UV detector at 277 nm wavelength and 0.01 AUFS attenuation.

Quantitation

Peak height ratios were used to quantitate ciprofloxacin and its metabolites. Quantitation of ciprofloxacin with the ODS procedure was based on calibration curves obtained with reference standard samples containing different concentrations of ciprofloxacin and a fixed concentration of internal standard. The concentrations of ciprofloxacin ranged from 0.0125 mg/ml to 3.0 mg/L. The selected concentration range reflects the concentrations of ciprofloxacin observed in the clinical study serum samples.

Quantitation of ciprofloxacin and of metabolites M1, M2, and M3 with the PSDVB procedure was based on linear regression calibration lines obtained with serum and urine containing different concentrations of the four compounds and a fixed concentration of the internal standard.

RESULTS AND DISCUSSION

Chromatographic Conditions

Since the isocratic elution of ciprofloxacin and metabolites M1, M2, and M3 on the ODS column requires about one hour, we investigated an alternative reverse phase system based on polystyrene divinylbenzene (PSDVB) support. Preliminary investigation of the PSDVB column indicated that the aqueous phase containing phosphate buffer and tetrabutylammonium used in the previously published (8,11) ODS procedure for ciprofloxacin did not yield the necessary chromatographic efficiency and selectivity for separation of ciprofloxacin and its metabolites on the PSDVB column. Consequently, other buffers and counterions were investigated and a solvent consisting of a pH 3.0 aqueous solution of trichloroacetic acid, acetonitrile, and methanol was selected. The favorable chromatographic behavior of ciprofloxacin and its metabolites in this solvent may be attributed to

formation of the ciprofloxacin-trichloroacetic acid ion pair. It is of interest to note that the relative retention times of ciprofloxacin, its metabolites, and internal standard increase with decreasing polarity of the compound and thus reflect the reverse-phase character of the PSDVB support. Since we have observed that metabolite M2 exhibits a longer retention time in the absence of TCA, it is also possible that residual TCA trapped by the PSDVB matrix induces a net negative charge on the support, thereby reducing the retention time of this negatively charged metabolite.

The chromatographic efficiency of the selected PSDVB solvent was investigated as a function of flow rate and temperature. Figures 2 and 3 depict the observed relationships. It is apparent that 30°C yielded maximum column efficiency. Although we also observed increasing column efficiency with decreasing flow rate within the entire measured range, a 0.5 ml/min flow rate was used for routine analysis because of practical considerations.

The selected chromatographic conditions were used for routine assay of ciprofloxacin and its metabolites in clinical urine samples. Figures 4 and 5 illustrate the resolution of ciprofloxacin and its metabolites from blank urine constituents and urine of a patient treated with ciprofloxacin. Urine constituent peaks observed in the vicinity of metabolite M2 reduce the sensitivity and accuracy of metabolite M2 determination at low concentrations. However, metabolite M2 is usually present in relatively high (2 - 100 mg/L) concentrations in urine samples.

The above PSDVB chromatographic procedure was also applied to the assay of ciprofloxacin and its metabolites in saliva and serum samples. However, since serum samples were not diluted as much as urine and contained relatively high concentrations of proteins and lipids, shifting retention times and lower column efficiency were observed after sequential injections of serum

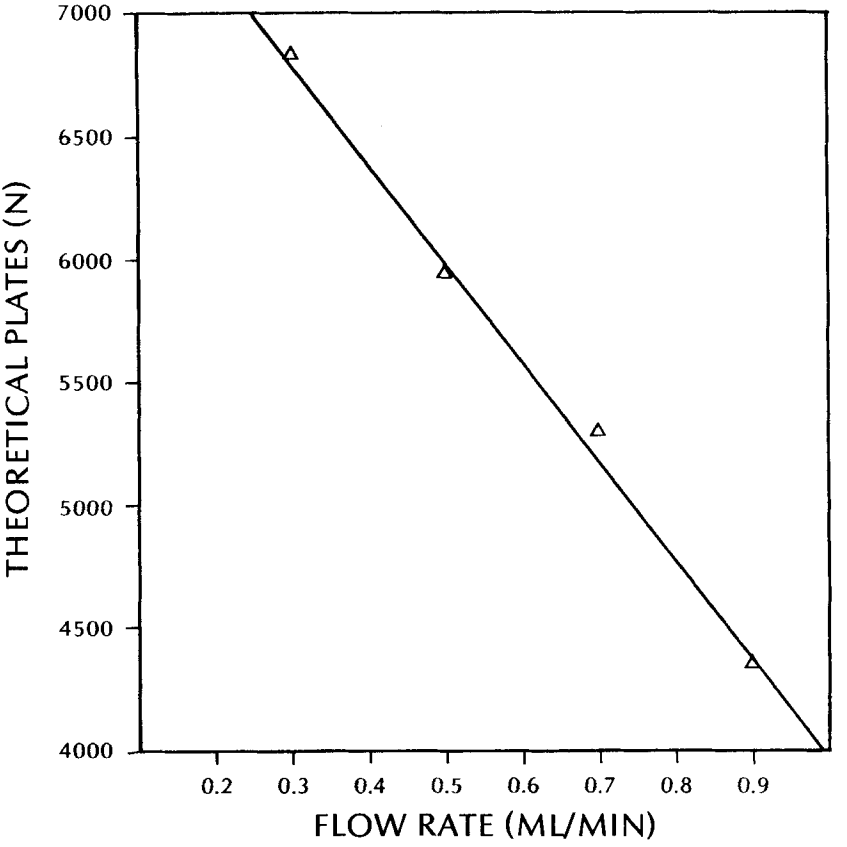


FIGURE 2. Dependence of PSDVB column efficiency on flow rate.

samples. Presence of trichloroacetic acid in the solvent system and the relatively porous column support matrix may be conducive to precipitation and/or binding of proteins to column support and subsequent modification of chromatographic character. We did not observe this problem with saliva and urine samples even after sequential injection of hundreds of samples on the same column.

To circumvent the chromatographic complications observed with serum or plasma samples, a prechromatographic cleanup step

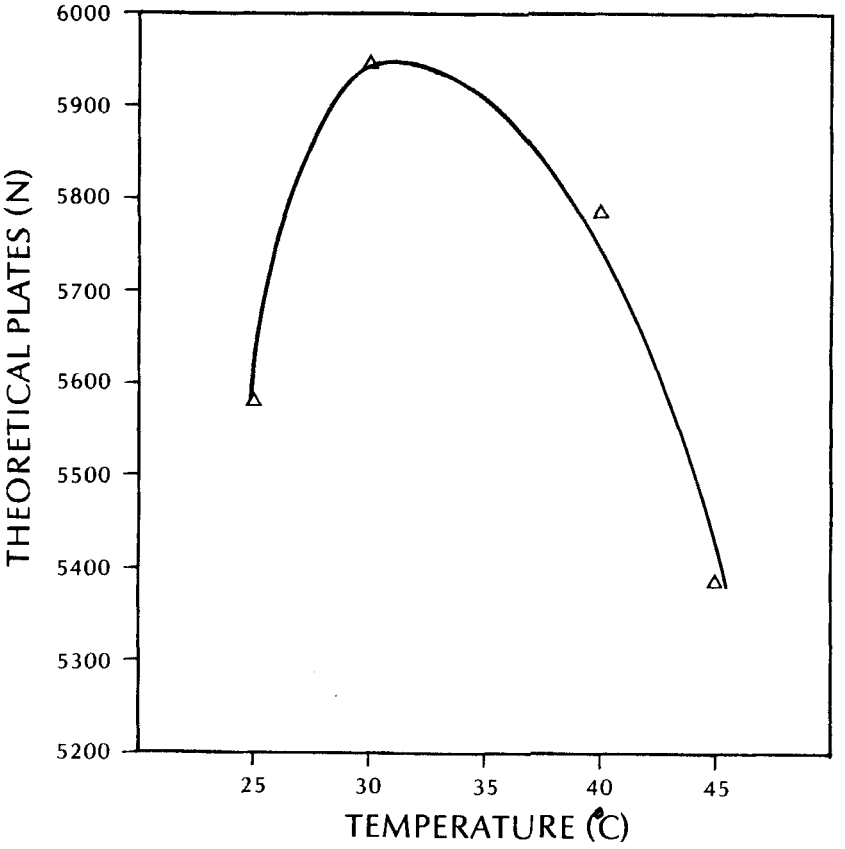


FIGURE 3. Dependence of PSDVB column efficiency on temperature.

involving the Chrom-Prep PRP-1 reverse-phase cartridge was investigated. The developed prechromatographic cleanup procedure extends considerably the effective life of the PSDVB column and facilitates concurrent analysis of metabolites M1, M2, and M3 as well as ciprofloxacin. Figure 6 illustrates representative chromatograms obtained with serum samples of a clinical study subject given ciprofloxacin. In contrast to urine, the serum of normal subjects contains relatively low (less than 0.2 mg/L)

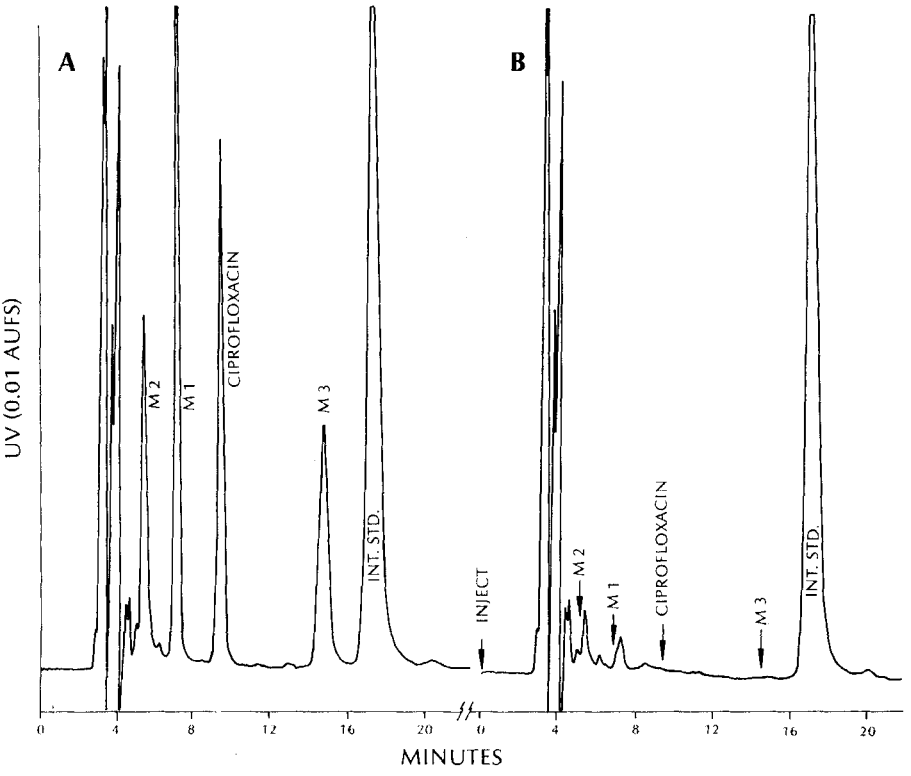


FIGURE 4. PSDVB column chromatograms of blank urine sample A containing known concentrations of internal standard, ciprofloxacin and its metabolites and blank urine sample B containing only internal standard; 10 ng of ciprofloxacin and metabolites was injected onto the column which was eluted at 0.5 ml/min flow rate.

metabolite concentrations. The corrected recovery of ciprofloxacin and its metabolites from serum or plasma after the cartridge cleanup ranged from 91.1% to 99.9%. The corrected recovery was based on 90.6% recovery of internal standard. Comparison of ciprofloxacin assay results obtained with a set of 48 serum samples assayed by the PSDVB column procedure with cartridge cleanup and by the ODS column

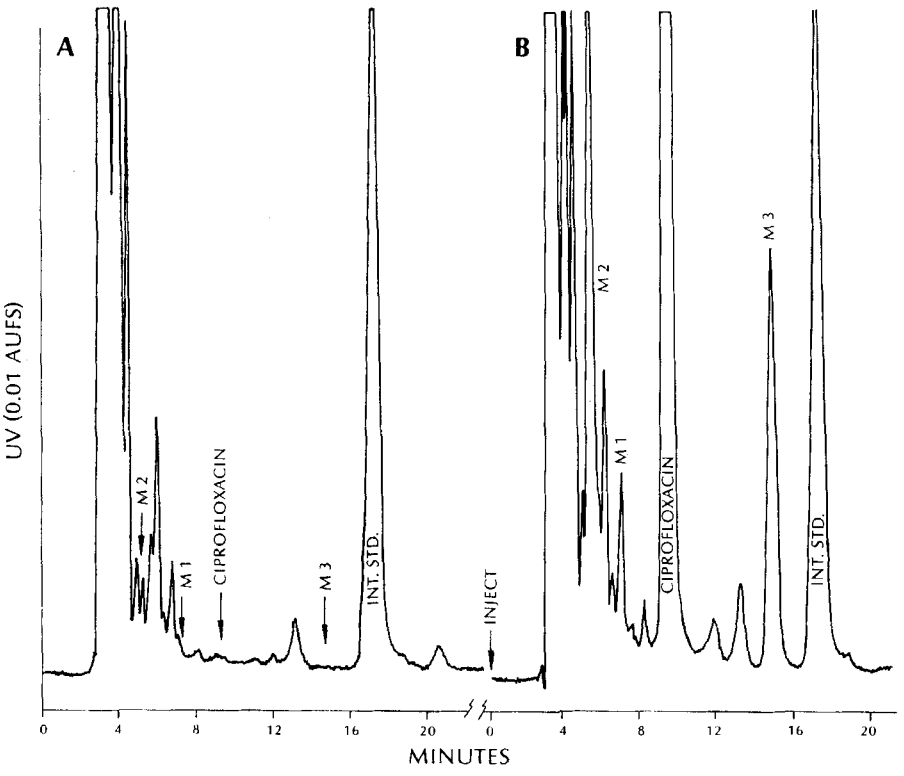


FIGURE 5. PSDVB column chromatograms of urine sample A collected from a patient prior to ciprofloxacin dose and urine sample B collected 0-2 hr after ciprofloxacin dose.

procedure without cartridge cleanup yielded no significant difference at the 95% confidence level. However, the cleanup step is time consuming and needs to be automated because many serum samples are collected during clinical studies.

The additional time required for cleanup of serum and plasma samples prior to chromatography on the PSDVB column and the indirect evidence that the concentration of active metabolites in serum is relatively low (7) prompted us to use a modified version of a previously published ODS procedure (6,11) for assay of serum

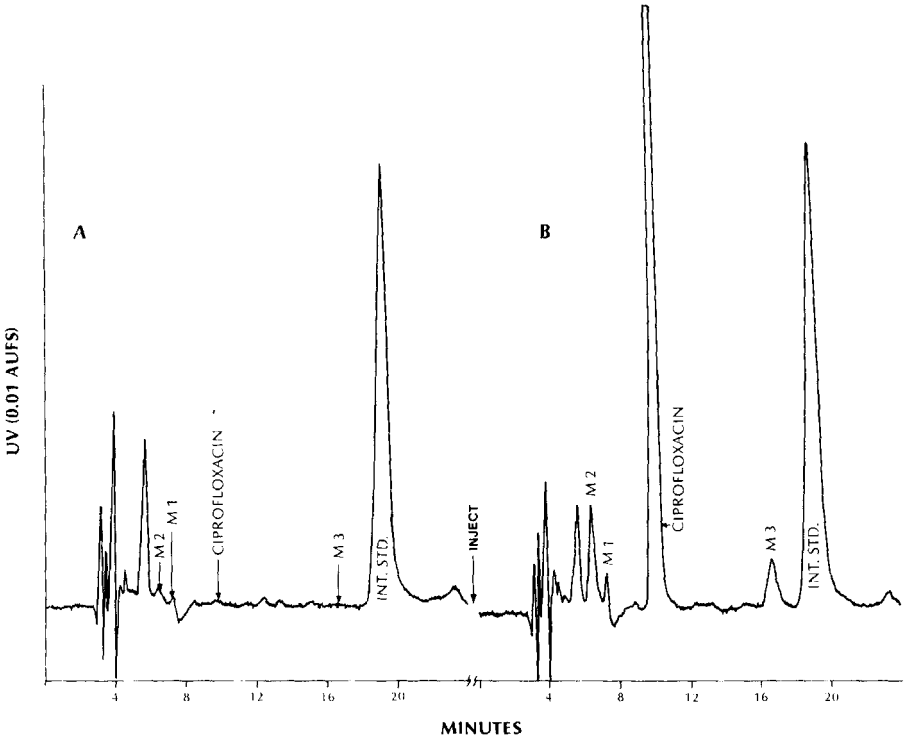


FIGURE 6. PSDVB column chromatograms of serum sample A collected from a patient prior to ciprofloxacin dose and sample B, 3 hr after ciprofloxacin dose.

samples. The ODS column, being pellicular rather than porous, has a longer column life and does not require a cartridge cleanup step if chromatographic solvent containing tetrabutylammonium ion is used for elution. However, the ODS column does not have the selectivity suitable for isocratic analysis of ciprofloxacin and metabolites M1, M2, and M3.

Figure 7 illustrates representative chromatograms obtained with a blank serum and a serum sample of a patient treated with ciprofloxacin. Note that only metabolite M1 can be observed on

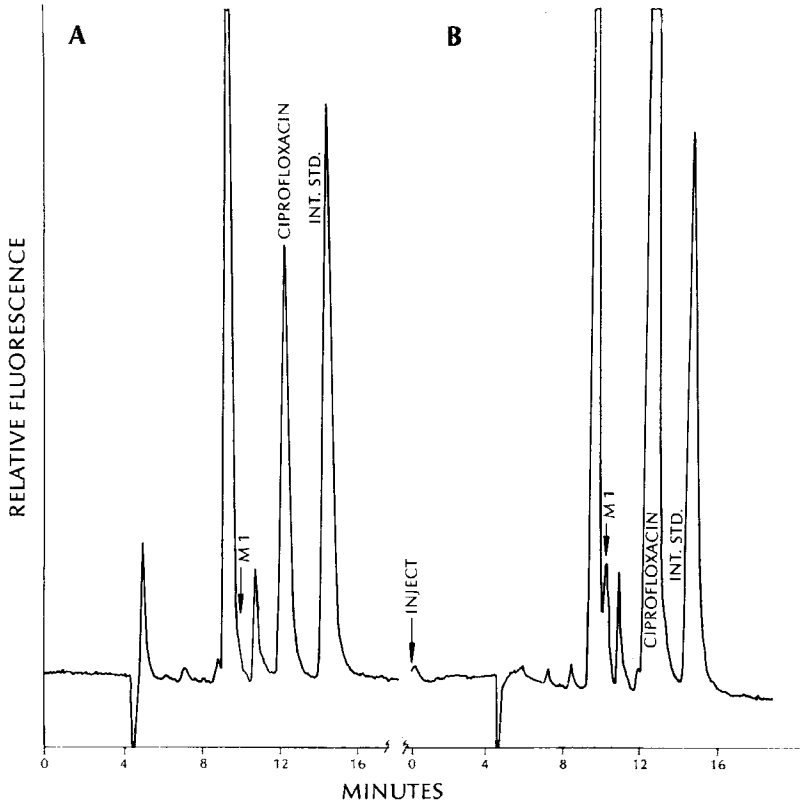


FIGURE 7. ODS column chromatograms of blank serum sample A containing known concentrations of ciprofloxacin and internal standard and serum sample B which was obtained from a patient treated with a 500 mg oral dose of ciprofloxacin. Ciprofloxacin peak observed on sample A chromatogram represents the injection signal observed with 3.75 ng on column.

this chromatogram and that metabolite M1 is relatively close to the solvent front. Other metabolites are either retained too long on the column or do not fluoresce with sufficient intensity.

Detection

The relative fluorimetric responses and UV absorbances of ciprofloxacin and ciprofloxacin metabolites depend on the structure of the compound. Thus, fluorescence of metabolite M1 is considerably more intense (more than tenfold) than the fluorescence of ciprofloxacin whereas metabolites M2 and M3 have very weak fluorescence (more than ten times weaker than ciprofloxacin). In contrast, the UV extinction coefficients of all four compounds are approximately the same.

Since the PSDVB procedure was used for assay of ciprofloxacin metabolites as well as ciprofloxacin whereas the ODS procedure was used only for assay of ciprofloxacin, the PSDVB column was monitored with the UV detector and the ODS column with the fluorimetric detector. Although fluorescence yields a lower detection limit for ciprofloxacin than UV absorbance, the concentration of ciprofloxacin and its metabolites was high enough for quantitative analysis of all four compounds even in diluted (1:20) urine samples. Furthermore, the UV detection limit can be enhanced if necessary by injection of more-concentrated urine samples. Table 1 summarizes the detection limits observed with the PSDVB and ODS procedures when 1:20 diluted urine and 1:1 diluted serum samples are assayed by the PSDVB (UV detection) and ODS (fluorimetric detection) procedures.

Accuracy and Precision

The single-day accuracy and precision of the PSDVB procedure were determined with urine samples containing thirteen different concentration levels of ciprofloxacin and 7 different concentra-

TABLE 1
Fluorimetric (ODS column) and UV (PSDVB column) Detection Limits (mg/L) for Ciprofloxacin and Metabolites in Serum and Urine

Compound	FL.-ODS	UV-PSDVB	
	Serum	Serum	Urine
Ciprofloxacin	0.01	0.01	0.1
Metabolite M1	0.002[a]	0.005	0.05
Metabolite M2	ND[b]	0.05[d]	0.2
Metabolite M3	ND[c]	0.02	0.2

a - Theoretical detection limit; because of interference from serum constituent peaks, the actual detection limit ranged from 0.005 mg/L to 0.05 mg/L depending on degree of peak resolution.
b - Not determined due to interference from serum constituent peaks and weak fluorescence.
c - Not determined due to weak fluorescence and long retention time.
d - Detection limit in serum is not proportional to the detection limit in urine because of serum constituent peak interference.

tions levels of metabolites M1, M2, and M3. The precision at each concentration level was based on results obtained with five independent samples. Table 2 presents the calculated mean response ratios (RR), coefficients of variation (CV), and relative response factors (RF). It is apparent that nearly all CV values are below 5.0% and that linear calibration curves (Fig. 8) were obtained.

The within-day precision of the ODS procedure is presented in Table 3. It is apparent that, in general, both assays are more precise at higher concentration ranges and that linear calibration curves are observed above 0.1 mg/L concentrations (Figs. 7 and 8). However, at a lower concentration range (0.0125 - 0.1 mg/L) the ODS procedure yields variable response factors (Tab 3).

TABLE 2

Within-Day Accuracy and Precision Data
Obtained with the PSDVB Procedure

Conc. in Dil.(1:10) Urine (mg/L)	Ciprofloxacin			Metabolite M1			Metabolite M2			Metabolite M3		
	Mean RR	CV%	RF	Mean RR	CV%	RF	Mean RR	CV%	RF	Mean RR	CV%	RF
.05	0.041	4.4	1.225	0.062	4.3	0.806				0.024	11.4	2.119
.10	0.070	6.5	1.420	0.117	4.8	0.853	0.139	6.0	0.719*	0.045	10.2	2.242
.25	0.175	2.1	1.427	0.286	2.8	0.875	0.253	5.3	0.988	0.109	5.2	2.298
.50	0.359	3.7	1.391	0.587	3.0	0.851	0.457	4.5	1.094	0.221	3.2	2.258
.75	0.527	1.7	1.423	0.858	2.2	0.874	0.676	1.6	1.109	0.326	3.3	2.299
1.00	0.715	2.2	1.398	1.180	2.0	0.848	0.887	2.2	1.127	0.439	2.7	2.276
1.50	1.087	1.6	1.380	1.776	1.5	0.845	1.318	2.7	1.138	0.655	2.9	2.291
2.00	1.441	1.7	1.387	2.370	1.5	0.844	1.765	1.6	1.133	0.859	2.5	2.328
3.00	2.126	2.1	1.411									
4.00	2.819	1.2	1.419									
6.00	4.240	1.2	1.415									
8.00	5.600	1.3	1.428									
10.00	7.161	2.0	1.396									
Mean RF			1.394			0.849			1.098			2.264
SD			0.053			0.021			0.056			0.064
CV			3.8			2.5			5.1			2.8

* Outlier; not included in the mean value.

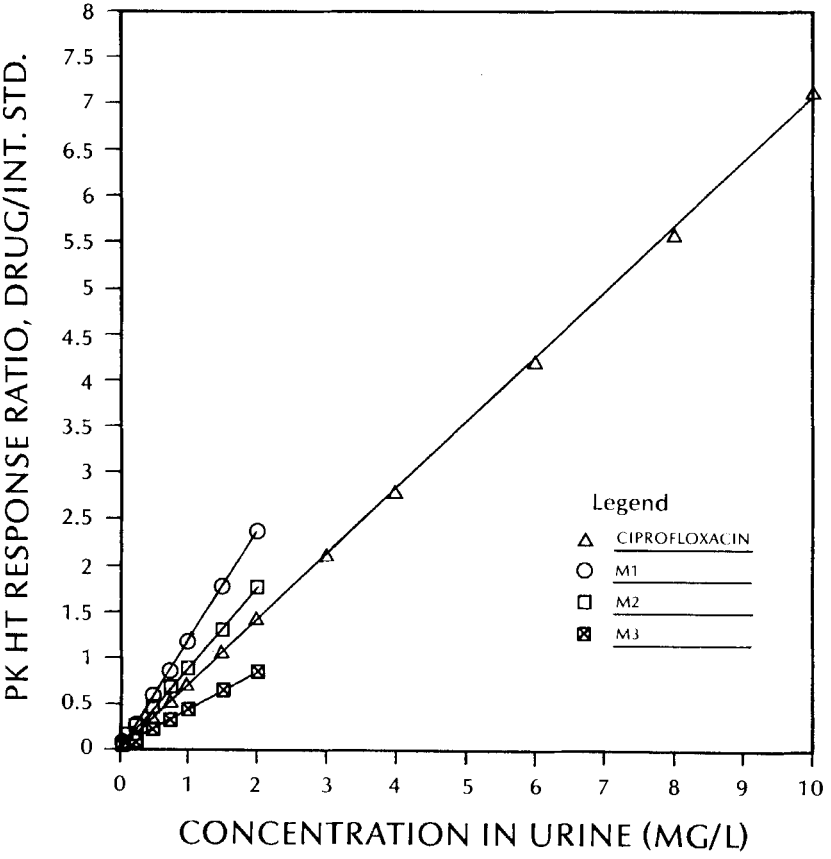


FIGURE 8. PSDVB procedure calibration curves obtained with urine containing different concentration levels of ciprofloxacin and ciprofloxacin metabolites.

Table 4 presents the long-term (day-to-day) precision data obtained with both procedures. The presented data are based on mean response factors (RF) determined on different days of assay. The CV of the mean ciprofloxacin response factors observed with the PSDVB procedure on eight different urine assay days was 5.5%. The corresponding CV values of mean metabolite M1, M2, and M3 response factors were 4.8%, 5.5%, and 15.5%. The relatively high

TABLE 3
Within-Day Precision Data
Obtained with the ODS Procedure

Conc. (mg/L)	Response Ratios (RR) 1	2	3	4	Mean RR	SD	CV%	RF
0.0125	0.028	0.027	0.027	-	0.027	0.0006	2.1	455
0.025	0.035	0.039	0.034	-	0.036	0.002	7.3	676
0.050	0.064	0.068	0.064	0.059	0.064	0.0037	5.8	781
0.100	0.107	0.113	0.114	0.116	0.113	0.0039	3.4	885
0.250	0.287	0.275	0.293	0.290	0.286	0.0079	2.8	874
0.500	0.540	0.574	0.577	0.541	0.558	0.0202	3.6	896
0.750	0.823	0.862	0.897	0.877	0.865	0.0313	3.6	867
1.000	1.172	1.164	1.180	1.176	1.173	0.0068	0.6	853
1.250	1.396	1.400	1.468	1.499	1.441	0.0510	3.5	867
1.500	1.695	1.735	1.708	1.793	1.733	0.0432	2.5	866
2.000	2.263	2.266	2.264	2.279	2.268	0.0074	0.3	882
3.000	3.230	3.450	3.451	3.491	3.406	0.118	3.5	881

TABLE 4

Long Term (Day-to-Day) Mean Response Factors (RF)
Obtained on Different Days by the
PSDVB AND ODS Assay Procedures

	PDSVB Procedure				ODS Procedure
		Metabolites			
	Cipro	M1	M2	M3	Cipro
	1.27	0.879	2.291	3.49	965
	1.41	0.865	2.146	2.23	920
	1.44	0.937	2.216	2.75	928
	1.45	0.952	2.046	3.06	894
	1.45	0.933	1.962	3.16	1045
	1.41	0.949	-	3.11	815
	1.52	1.000	-	2.43	958
	1.52	0.970	-	2.43	1057
Mean RF	1.43	0.936	2.132	2.83	947
CV(%)	5.5	4.8	5.5	15.5	8.3

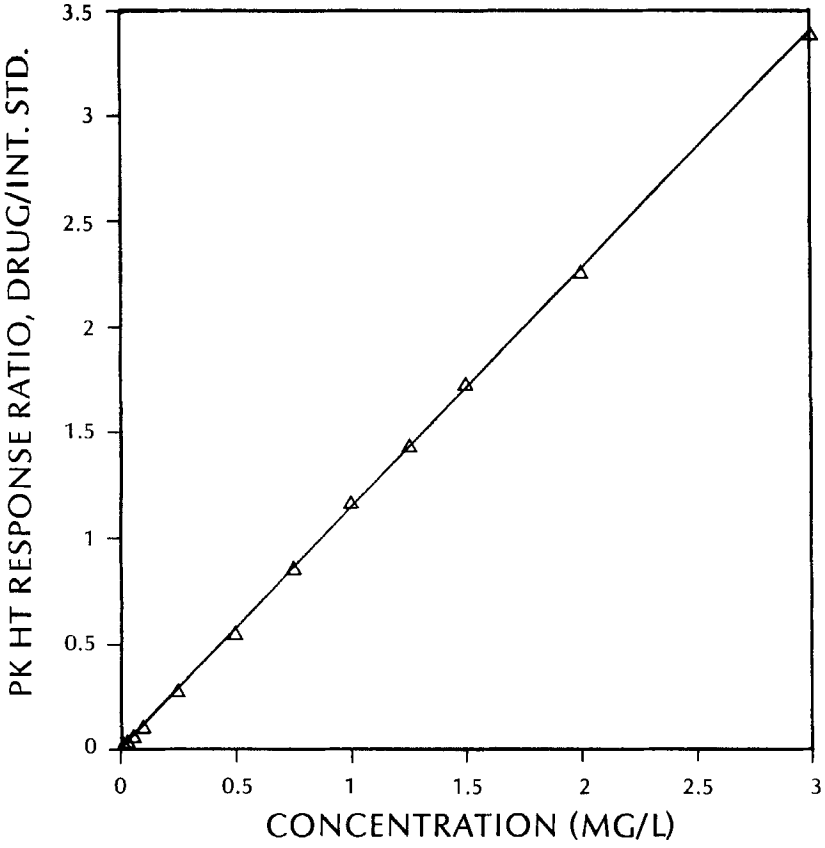


FIGURE 9. ODS procedure calibration curve obtained with serum containing different concentration levels of ciprofloxacin.

CV of metabolite M3 mean response factor is due to the relatively greater metabolite M3 chromatographic retention time and peak height variations with different solvent batches and columns. The CV obtained with mean ODS procedure ciprofloxacin RF values was higher (8.3% vs 5.5%) because, in general, UV detectors give more reproducible and stable responses than fluorimetric detectors. However, the observed day-to-day CV values do not

compromise the precision and accuracy of either procedure since concentrations of each set of unknown samples were calculated by reference to standard calibration samples prepared and analyzed each time the unknown samples were assayed.

CONCLUSIONS

The PSDVB procedure described above is sufficiently specific, sensitive, accurate, and precise for assay of ciprofloxacin and three metabolites in clinical bile, urine, and saliva samples. The procedure is also relatively efficient since the sample preparation step requires only dilution. However, at present, this procedure is not suitable for high volume routine assay of ciprofloxacin and its metabolites in clinical serum samples. Consequently, serum samples can be assayed more efficiently using a modified version of a previously developed ODS column procedure. Since the ODS procedure is suitable only for analysis of ciprofloxacin itself, the cartridge cleanup of serum for the PSDVB procedure needs to be automated to make the procedure suitable for high volume assay of serum (and plasma) samples.

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