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Report

Determination of Temafloxacin, Sarafloxacin, and Difloxacin in Bulk Drug and Dosage Forms by High-Performance Liquid Chromatography

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The fluoroquinolones, temafloxacin, sarafloxacin, and difloxacin, are determined in the bulk drug substances and in a variety of dosage forms using high-performance liquid chromatography (HPLC). The HPLC system used is also applicable for ciprofloxacin and norfloxacin. The procedure uses UV detection at 280 nm, which provides a linear response of the subject compounds to at least 20 μg/ml. Assay precision (RSD) values were ±1.2% or better for the bulk drugs and ranged from ±0.42 to ±2.3% for suspension, capsule, and tablet formulations. Drug recoveries were quantitative from the dosage forms tested. Sensitivity of the subject compounds is approximately 50 ng/ml (2.5 ng on column).

KEY WORDS: temafloxacin; sarafloxacin; difloxacin; ciprofloxacin; norfloxacin; high-performance liquid chromatography.

INTRODUCTION

The fluoroquinolones are synthetic broad-spectrum antibiotics which show activity against gram-positive and gram-negative organisms. These compounds are prepared either as free bases or as hydrochloride salts. The mechanism of action and antimicrobial spectrum for the fluoroquinolones have been reviewed in detail (1). For the quantitation of fluoroquinolones, a variety of techniques can be used. Determination of these compounds can be made using titrimetric or standard bioassay techniques, at the sacrifice of specificity and time, respectively. Gas–liquid chromatography (GLC) has been used for the determination of fluoroquinolones (2,3); however, derivatization is required to convert these materials into suitable volatile analogues.

A variety of procedures using high-performance liquid chromatography (HPLC) has been reported for the determination of fluoroquinolones and metabolites (4–11). Reverse-phase separations based on C_{18} columns are most frequently cited. Difloxacin and its metabolites have been determined in plasma and urine by HPLC using a C_{18} column and an ion-pair eluent (12).

In this report, we present a method which describes for the first time the determination of the fluoroquinolones temafloxacin, sarafloxacin, and difloxacin in the bulk drug substances and in different dosage forms. The method uses HPLC to provide rapid and reliable determination of these drugs with the same chromatographic conditions. Quantitation is achieved by the internal standard method and the chromatographic system is generally applicable for the determination of several other fluoroquinolones, including ciprofloxacin and norfloxacin. The chemical structures for these compounds are shown in Fig. 1.

MATERIALS AND METHODS

Instrumentation and Reagents

The HPLC system used in this work typically consisted of a ternary pump and autosampler, Model SP-8100 (Spectra-Physics, San Jose, CA), equipped with a UV detector, Model SF-769 (ABI Analytical, Kratos Division, Ramsey, NJ). Chromatograms were processed using a Model SP-4270 data handling system (Spectra-Physics, San Jose, CA). A Nucleosil C_{18} chromatographic column (5 μm, 4.6 mm × 15 cm, 100-Å pore size) was used (Alltech Associates, Deerfield, IL). The HPLC eluent was filtered through a 0.45-μm nylon membrane (Cuno, Inc., Meriden, CT). Chemicals and solvents were reagent grade and HPLC grade, respectively. The internal standard was *p*-nitroacetophenone (Aldrich, Milwaukee, WI), dissolved in acetonitrile/water (1:1) at a concentration of approximately 1.4 mg/ml. Bulk drugs and dosage forms of difloxacin, sarafloxacin, and temafloxacin hydrocholoride were produced at Abbott Laboratories, North Chicago, IL. Characterized in-house standards for

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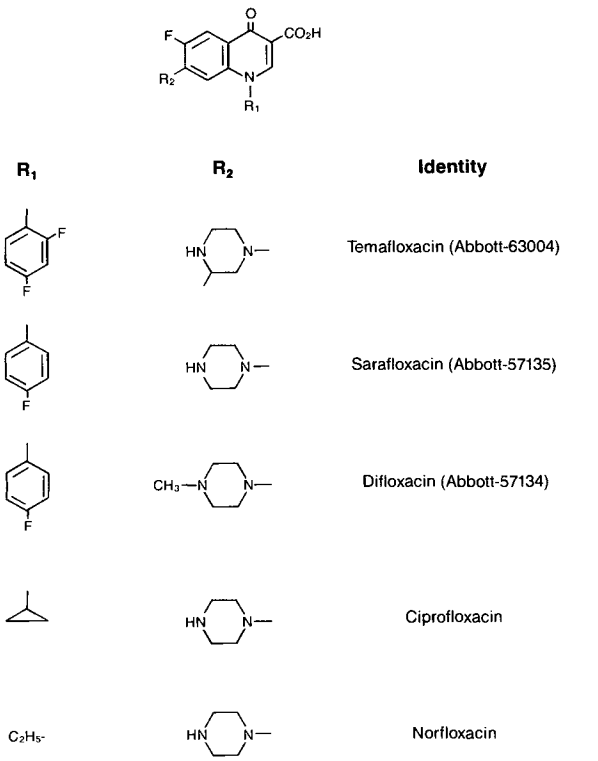

Fig. 1. Chemical structures for fluoroquinolones determined. Structures shown represent the free base forms.

these materials were used for quantitation. Ciprofloxacin and norfloxacin were purchased from Miles Pharmaceutical Division, New Haven, CT, and Merck Sharpe and Dohme, West Point, PA, respectively.

Chromatographic Conditions

The HPLC eluent was an aqueous buffer (0.02 *M* citric acid and 0.02 *M* sodium citrate, adjusted to pH 2.4 with perchloric acid) mixed with acetonitrile at a ratio of 65 to 35 (v/v). The eluent was pumped at a flow rate of 1.0 ml/min with typical back pressures of 1200 to 1400 psi. The UV detector was operated at 280 nm at approximately 0.10 AUFS. Injection volumes were 50 μl.

Assay Procedure

Bulk drug substances of temafloxacin, sarafloxacin, and difloxacin were dissolved in acetonitrile/water (1:1) for stock solutions of approximately 1.2 mg/ml (base concentrations). The stock solutions were diluted serially in acetonitrile/water (1:1). All final dilutions were made in the HPLC eluent for drug concentrations of approximately 12 μg/ml. The internal standard preparation was added to yield approximately 14 μg/ml of *p*-nitroacetophenone in the final dilution. Each reference standard used for quantitation was prepared identically as described for the bulk drug substance. Three types of dosage forms were studied in this work: tablets, capsules, and hydroxypropylmethylcellulose-based suspensions. For tablets and capsules, a composite sample was prepared by grinding 20 tablets or by combining the contents of 20 capsules. An amount of sample equivalent to approximately 100 mg of drug was extracted into aceonitrile/water (1:1). This solution was diluted to 100 ml in acetonitrile/water (1:1), and the excipients were allowed to settle. The final sample preparation was then prepared as described for the bulk drug substance. Suspensions were sampled using 5-ml volumetric pipets while being stirred continuously on a magnetic stirrer. The drug was extracted into appropriate volumes of acetonitrile/water (1:1), then prepared as described for the bulk drug substance.

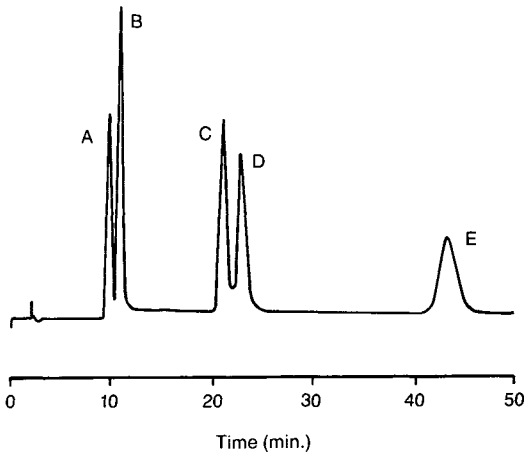

Fig. 2. Chromatogram for a synthetic mixture of fluorquinolones. Conditions are as stated in text, except the aqueous buffer/acetonitrile ratio is 90:10. Peak identities: A, norfloxacin; B, ciprofloxacin; C, sarafloxacin; D, difloxacin; E, temafloxacin.

Quantitation of the drug was performed versus a standard preparation using peak area ratios of drug to internal

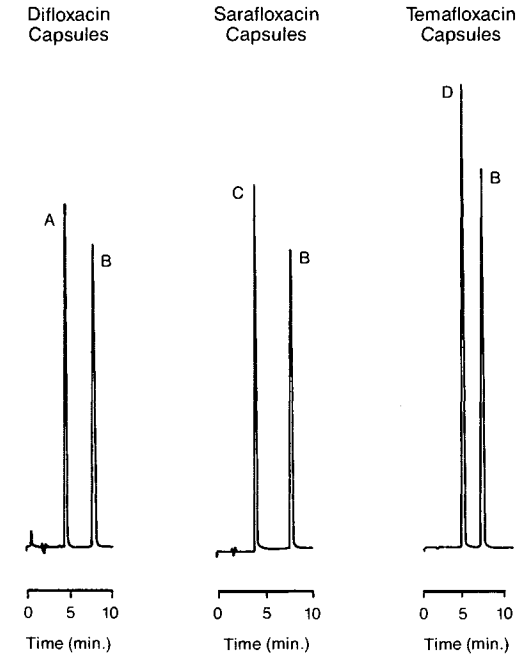

Fig. 3. Typical chromatograms for fluoroquinolone determinations in capsules. Peak identities: A, difloxacin; B, internal standard (*p*-nitroacetophenone); C, sarafloxacin; D, temafloxacin. Conditions stated in text.

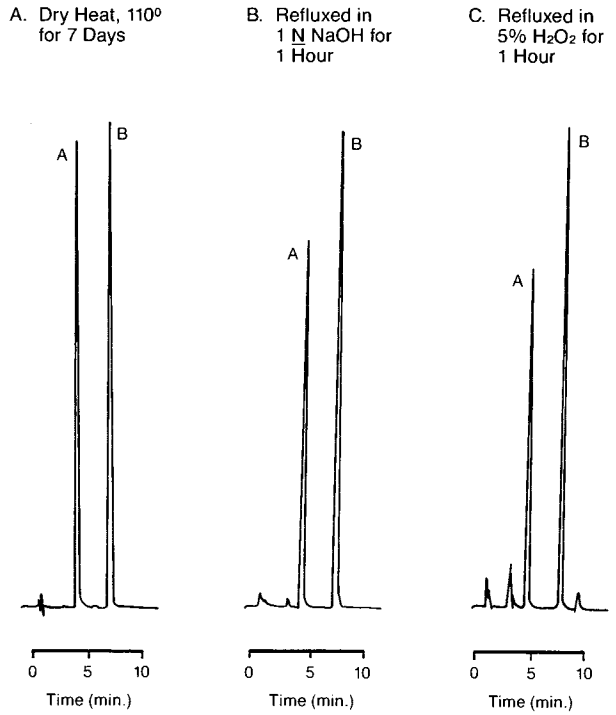

Fig. 4. Chromatograms for temafloxacin tablets stressed under various conditions. Peak identities: A, temafloxacin; B, internal standard (*p*-nitroacetophenone). Conditions stated in text.

standard and reported in terms of free base. Appropriate dilution factors were applied for the dosage forms.

RESULTS AND DISCUSSION

In this work, HPLC conditions were developed to determine several fluoroquinolones. Shown in Fig. 2 is a chromatogram for a synthetic mixture containing five fluoroquinolones. Use of the described method provides a convenient means of analysis and identification when multiple compounds are under development. The resolution shown in Fig. 2 is adequate for identification of the different drugs. For the more strongly retained Abbott compounds, temafloxacin, sarafloxacin, and difloxacin, a larger amount of acetonitrile was used in the eluent to increase the speed of analysis. The same internal standard is used for all bulk drugs and formulations. Shown in Fig. 3 are typical chromatograms for capsule formulations for these compounds. The assay procedure is stability-indicating, providing a separation of the degradation products, manufacturing impurities, and intact drugs. Shown in Fig. 4 are typical chromatograms for temafloxacin tablets after degradation under various conditions. The peak homogeneity of temafloxacin was confirmed by LC/MS techniques. Extraction of placebos of the formulations showed no interfering peaks even at a 10-fold increase in detector sensitivity. The sample and standard preparations for temafloxacin, sarafloxacin, and difloxacin are stable, which enables the use of automated sample injections.

Detector response for temafloxacin, sarafloxacin, and difloxacin was linear to at least 20 μg/ml. Plots of concentration (as free base, μg/ml) versus peak area ratios provided slopes of 0.108, 0.105, and 0.104, respectively. All three curves had correlation coefficients >0.999 and each curve essentially intersected the origin. With this behavior, one-point calibration routines were used for all quantitation.

Each fluoroquinolone studied was freely soluble in acetonitrile/water (1:1). While providing good solubility of the drug, this mixed solvent provided poor solubility for some of the excipients used in the capsule and tablet formulations. To determine the recovery of the fluoroquinolones from the dosage forms, standard addition and recovery ex-

Table I. Standard Addition and Recovery Data for Fluoroquinolones in Various Dosage Forms

Capsules		Tablets		HPMC Suspensions	
Addn. level (%)[a]	Recovery (%)	Addn. level (%)[a]	Recovery (%)	Addn. level (mg/ml)	Recovery (%)
		Difloxacin			
80.4	102.0	82.6	100.6	6.06	99.4
99.6	101.0	101.4	99.8	30.2	102.1
122.9	99.5	124.0	101.8	91.3	103.0
				120.5	102.6
		Sarafloxacin			
80.5	100.4	80.8	100.4	6.03	99.3
100.9	100.8	100.8	99.9	30.0	100.2
120.2	100.0	120.2	100.0	90.1	100.0
				120.0	103.2
		Temafloxacin			
54.1	101.3	50.9	100.2	37.3	100.8
103.3	100.4	100.1	99.2	75.0	99.8
151.2	99.5	151.7	98.5	149.8	99.9
				599.9	100.1

[a] Additions are based on 100-mg sample size. Where multiple dose formulations were prepared, the lowest strength (highest excipient to drug) is used.

Table II. Precision Data for Fluoroquinolone Determinations in Bulk Drug and Dosage Forms

Compound	Dosage form	Mean (% theory)	*N*	% RSD
Difloxacin	Bulk drug	100.0[a]	10	±0.91
	100-mg capsules	100.3	8	±1.2
	200-mg tablets	102.7	18	±1.4
	6-mg/ml suspension	100.5	6	±2.3
	120-mg/ml suspension	102.6	6	±0.42
Sarafloxacin	Bulk drug	100.2[a]	10	±1.2
	200-mg capsules	101.3	11	±1.7
	250-mg tablets	100.3	12	±1.3
	6-mg/ml suspension	100.2	8	±1.8
	120-mg/ml suspension	101.3	8	±1.6
Temafloxacin	Bulk drug	99.1[a]	9	±0.61
	100-mg capsules	99.7	10	±0.82
	100-mg tablets	98.2	10	±1.1
	7.5-mg/ml suspension	100.3	9	±0.86
	30-mg/ml suspension	98.0	9	±0.67
	120-mg/ml suspension	91.6	9	±0.99

[a] Calculated on the anhydrous solvent-free basis.

periments were performed. For each type of formulation, the drug was added to the placebo. The spiked placebos were prepared as described in the method and the recovery of each drug was determined. Where multiple strengths of the same dosage form occurred, the formulation having the lowest strength was used, which provided the highest ratio of excipient to drug. Shown in Table I are standard addition and recovery data for difloxacin, sarafloxacin, and temafloxacin from capsule, tablet, and suspension formulations. As shown the recovery ranged from 99.4 to 103.0% for difloxacin. The recovery for sarafloxacin was 99.3 to 103.2% and the recovery for temafloxacin was 98.5 to 101.3%. As shown in Table I, the largest range of recovery values was obtained from HPMC suspensions. These data were evaluated statistically (13) by plotting the regression line of concentration of drug added (x) versus concentration of drug found (y) for each of the three fluoroquinolones. For difloxacin, sarafloxacin, and temafloxacin, slopes were, respectively, 1.029, 1.027, and 1.001, and intercepts were, respectively, −0.203, −0.673, and −0.106. At the 95% confidence level, the slope values were not significantly different than 1.0 and the intercept values were not significantly different from 0. These findings, in combination with the established precision of the method, constitute quantitative recovery of the drugs.

To determine precision of the assays, the described method was performed on both bulk drug and dosage forms. At least two different analysts performed the determinations on separate days. Precision data for temafloxacin, sarafloxacin, and difloxacin are presented in Table II. As shown, relative standard deviation (RSD) values ranged from ±0.42 to ±2.3% for formulations. Bulk drug determinations had RSD values of ±0.61 to ±1.2%.

In this work, the drugs studied were quantitated after dilution to the low microgram per milliliter range. UV detection provided adequate sensitivity for determining the drugs in formulation and bulk substances. With increased detector gain, the subject compounds are determinable to approximately 50 ng/ml (2.5 ng on column). Significantly improved sensitivity can be achieved with the stated HPLC conditions in combination with fluorescence detection.

In conclusion, a general HPLC method is reported for determining temafloxacin, sarafloxacin, and difloxacin in bulk drug and dosage forms. The separation can be used to identify the different compounds, as well as other fluoroquinolones. Dosage forms and bulk drugs are prepared in a mixed acetonitrile/water solvent and finally diluted in the HPLC eluent. The method is stability-indicating and is valid with respect to detector linearity, precision and quantitative recovery of the drug from placebos.

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